Development of a Novel Class of Pharmacodynamic Hybrids/*NO* Donors that Targets COX-2 Selectively and a Machine-Assisted Flow Procedure for the Synthesis of Meclinertant, a Neurotensin Receptor Probe

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Abstract

The work described below is divided into two parts.

In the first chapter, the development of a novel class of pharmacodynamic hybrids is discussed. These compounds are endowed with analgesic/anti-inflammatory properties along with their ability to release nitric oxide which makes them an important contribution to the development of effective COX-Inhibiting Nitric Oxide Donors.

In the second chapter, the synthesis of an important neurotensin receptor antagonist (SR 48692, Meclinertant) is described. The synthesis of Meclinertant is achieved with the use of machine-assisted flow protocols through a robust, reliable and scalable route.

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Table of contents

Development of a Novel Class of Pharmacodynamic Hybrids/NO Donors that Targets COX-2 Selectively **16**

Introduction 17

Non-Steroidal Anti-inflammatory Drugs. 17

Molecular hybridisation and nitric oxide. 21

Generation of exogenous nitric oxide. 23

CINODs and Naproxcinod. 24

Rationale and aims. 26

Selective COX-Inhibiting Nitric Oxide Donors. 27

Stepwise approach to solubility and metabolic stability. 28

Acetic esters derivatives: the first generation 31

Chemistry. 31

In vitro assay. 32

Stability and solubility assessment. 34

Vasorelaxing activity. 34

Whole human blood assay. 36

In vivo testing. 37

Conclusions. 39

Glycine esters derivatives: improving solubility 40

Chemistry. 43

Solubility assessment. 44

In vitro testing. 45

In vivo testing. 46

Vasorelaxing effect. 48

Stability assessment. 49

Conclusions. 50

Three generations of amides: improving stability whilst keeping solubility 51

Chemistry. 52

Solubility assessment. 56

Stability in simulated gastric fluid (SGF-without pepsin), phosphate buffered solution (PBS) and rat plasma. 57

In vitro COX-2 inhibition. 58

Writhes reduction in the acetic acid-induced abdominal constrictions in mice. 59

Reduction of hyperalgesia in the carrageenan induced inflammation and edema reduction.. 61
Ex vivo nitric oxide releasing properties. 62
Human whole blood assay. 64
Conclusions. 66
Conclusions 67
Experimental procedures 68
Chemistry. 68
1-[4-(Methylthio)phenyl]pentane-1,4-dione (12). 69
1-[4-(Methylsulfonyl)phenyl]pentane-1,4-dione (13). 69
1-Phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrole (14a). 70
1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrole (14b). 70
1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrole (14c). 70
1-[(4-Methylthio)phenyl]-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrole (14d). 71
Ethyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15a). 71
Ethyl 2-[1-[(3-fluoro)phenyl]-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15b). 72
Ethyl 2-[1-[(4-fluoro)phenyl]-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15c). 72
Ethyl 2-[1-[(4-methylthio)phenyl]-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15d). 72
Ethyl 2-[1-[(4-methoxy)phenyl]-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15e). 72
Ethyl 2-[1-pheny-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (16a). .. 73
Ethyl-2-methyl-1-[3-(fluoro)-phenyl]-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-acetate (16b). 73
Ethyl-2-methyl-1-[4-(fluoro)-phenyl]-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-acetate (16c). 73
Ethyl-2-methyl-1-[4-(methylthio)-phenyl]-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-acetate (16d). 74
Ethyl-2-methyl-1-[4-(methoxy)-phenyl]-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-acetate (16e). 74
2-[1-Phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17a). 74
2-[1-(3-fluoro)phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17b). 75
2-[1-(4-fluoro)phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17c). 75

2-[1-(4-methylthio)phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17d). 75

2-[1-(4-methoxy)phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17e). 75

2-(Nitroxy)ethyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1a). 75

3-(Nitroxy)propyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1b). 76

3-(Nitroxy)butyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1c). 76

2-(Nitroxy)ethyl 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1d). 77

3-(Nitroxy)propyl 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1e). 77

4-(Nitroxy)butyl 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1f). 77

2-(Nitroxy)ethyl 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1g). 78

3-(Nitroxy)propyl 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1h). 78

4-(Nitroxy)butyl 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1i). 78

2-(Nitroxy)ethyl 2-[1-(4-methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1j). 79

3-(Nitroxy)propyl 2-[1-(4-methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1k). 79

4-(Nitroxy)butyl 2-[1-(4-methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1l). 79

2-(Nitroxy)ethyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (1m). 80

3-(Nitroxy)propyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (1n). 80

4-(Nitroxy)butyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (1o). 80

2-Hydroxyethyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2a). 81

3-Hydroxypropyl 2-[1-Phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2b). 81

4-Hydroxybutyl 2-[1-Phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2c). 81

2-Hydroxyethyl 2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3 yl]acetate (2d). 82

3-Hydroxypropyl 2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2e). 82

3-Hydroxypropyl 2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2f). 82

2-Hydroxyethyl 2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2g). 82

3-Hydroxypropyl 2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2h). 83

3-Hydroxypropyl 2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2i). 83

2-Hydroxyethyl 2-[1-(4-Methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (2j). 83

3-Hydroxypropyl 2-[1-(4-Methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2k). 84

3-Hydroxybutyl 2-[1-(4-methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2l). 84

2-Hydroxyethyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (2m). 84

3-Hydroxypropyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (2n). 84

3-Hydroxypropyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (2o). 85

2-Nitroxyethanol (19a). 85

3-Nitroxypropanol (19b). 86

4-Nitroxybutanol (19c). 86

2-(Nitroxy)ethyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3a). 86

3-(Nitroxy)propyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3b). 87

4-(Nitroxy)butyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3c). 87

2-(Nitroxy)ethyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3d). 88

3-(Nitroxy)propyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3e). 88

4-(Nitroxy)butyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3f). 88

2-Hydroxyethyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4a). 89

3-Hydroxypropyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4b). 90

4-Hydroxybutyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4c)..... 90

2-Hydroxyethyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4d). 90

3-Hydroxypropyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4e). 91

4-Hydroxybutyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4f). 91

Ethyl 2-(hydroxyimino)-2-[2-methyl-5-[4-(methylsulfonyl)phenyl]-1-(3-fluorophenyl)-1H-pyrrol-3-yl]acetate (20a). 92

Ethyl 2-(hydroxyimino)-2-[2-methyl-5-[4-(methylsulfonyl)phenyl]-1-(4-fluorophenyl)-1H-pyrrol-3-yl]acetate (20b). 92

Ethyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (21a). 93

Ethyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (21b). 94

tert-Butyl (ethoxycarbonyl)-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]methyl carbamate (22a). 94

tert-Butyl (ethoxycarbonyl)-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]methyl carbamate (22b). 95

N-tert-Butyl-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-*1H*-pyrrol-3-yl]-2-amino-acetic acid (23a)..... 95

N-tert-Butyl-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]-2-amino-acetic acid (23b)..... 96

2-(Nitroxy)ethyl N-tert-butyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24a)..... 96

3-(Nitroxy)propyl] N-tert-butyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24b). 96

4-(Nitroxy)butyl N-tert-butyl-(ethoxycarbonyl)-2-[1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]-2-amino-acetate (24c). 97

2-(Nitroxy)ethyl N-tert-butyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24d). 97

3-(Nitroxy)propyl N-tert-butyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24e)..... 98

4-(Nitroxy)butyl N-tert-butyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24f). 98

N-Benzyl-(ethoxycarbonyl)-[1-(3-fluorophenyl)-2-methyl-5-[4 (methylsulfonyl)phenyl]-1H-pyrrol-3-yl] methylcarbamate (25a). 99

N-Benzyl-(ethoxycarbonyl)-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]methylcarbamate (25b). 100

N-(Benzyloxy)carbonyl-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]-2-amino-acetic acid (26a)..... 100

N-(Benzyloxy)carbonyl-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]-2-amino-acetic acid (26b)..... 100

2-(Nitroxy)ethyl N-benzyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino acetate (27a). 101

3-(Nitroxy)propyl N-benzyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino acetate (27b)..... 101

4-(Nitroxy)butyl N-benzyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino acetate (27c). 101

2-(Nitroxy)ethyl N-benzyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (27d). 102

3-(Nitroxy)propyl N-benzyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (27e)..... 102

4-(Nitroxy)butyl N-benzyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino acetate (27f)..... 102

N-[2-(Nitroxy)ethyl] 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-*1H*-pyrrol-3yl]acetamide (5a)..... 103

N-[3-(Nitroxy)propyl] 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (5b). 104

N-[2-(Nitroxy)ethyl] 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (5c)..... 104

N-[3-(Nitroxy)propyl] 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (5d). 104

N-[2-(Hydroxy)ethyl] 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (6a)..... 105

N-[3-(Hydroxy)propyl] 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (6b). 106

N-[2-(Hydroxy)ethyl] 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (6c)..... 106

N-[3-(Hydroxy)propyl] 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (6d). 106

(*R,S*)-2-[2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-nitroxypropanoic acid (7a). 107

(R,S)-2-[2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-nitroxybutanoic acid (7b). 107

(R,S)-2-[2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-nitroxypropanoic acid (7c). 108

(R,S)-2-[2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-nitroxybutanoic acid (7d). 108

(R,S)-2-[2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-hydroxypropanoic acid (8a)..... 108

(R,S)-2-[2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-hydroxybutanoic acid (8b)..... 109

(R,S)-2-[2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-hydroxypropanoic acid (8c)..... 109

(R,S)-2-[2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-hydroxybutanoic acid (8d)..... 109

2-(Nitroxy)ethylamine nitrate salt (29a)..... 110

2-(Nitroxy)propylamonium nitrate (29b). 110

2-Amino-3-(nitroxy)propanoic acid nitric salt (31a). 110

2-Amino-4-(nitroxy)butanoic acid nitric salt (31b). 110

N-[2-(Nitroxy)ethyl] (R,S)-2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide (9a). 110

N-[3-(Nitroxy)propyl] (R,S)-2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide (9b). 111

N-[2-(Hydroxy)ethyl] (R,S)-2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide (10a). 111

N-[3-(Hydroxy)propyl] (R,S)-2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide (10b). 111

N-[2-(Nitroxy)ethyl] (R,S)-2-[(tert-butyl)oxycarbonyl]amino-[2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)]acetamide (32a)..... 112

N-[3-(Nitroxy)propyl] (R,S)-2-[(tert-butyl)oxycarbonyl]amino-[2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)]acetamide (32b). 112

N-[2-(Nitroxy)ethyl] (R,S)-[(benzyloxy)carbonyl]amino-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)acetamide (33a). 113

N-[3-(Nitroxy)propyl] (R,S)-[(benzyloxy)carbonyl]amino-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)acetamide (33b). 113

Biology and Pharmacology. 113

In Vitro Anti-Inflammatory Study. 113
Ex Vivo Vasorelaxing Activity. 114
Ex vivo determination of the formation of nitrites and nitrates, in rat liver homogenate. 116
Ex vivo Human Whole Blood (HWB) Assay. 117
In Vivo Analgesic and Anti-Inflammatory Study 117
Solubility and stability assessment. 118
Solubility assessment in phosphate buffered saline (PBS) and simulated gastric fluid (SGF). 118
Stability assessment in phosphate buffer saline (PBS), simulated gastric fluid (SGF) and rat plasma 118
References 120
***A Machine-Assisted Flow Procedure for Meclinertant, a Neurotensin Receptor Probe* 125**
Introduction 126
Flow chemistry. 126
Neurotensin 128
Meclinertant. 129
The synthesis of 2-aminoadamantane-2-carboxylic acid 132
Meclinertant in flow 148
Synthesis of 2,6-dimethoxyacetophenone. 148
The pyrazole core. 157
The final coupling. 161
Conclusions 162
Experimental procedures 163
Chemistry. 163
2-Ethynyl adamantan-2-ol (11). 164
2-Ethynyl-2-acetamido adamantane (12a). 165
2-Ethynyl-2-benzamido adamantane (12b). 167
2-Methyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (13a). 169
2-Phenyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (13b). 171
2-Methyl-4-(adamantane-2'-spiro)-oxazolin-5-one (14a). 173
2-Phenyl-4-(adamantane-2'-spiro)-oxazolin-5-one (14b). 174
2-Aminoadamantane-2-carboxylic acid hydrochloride (8). 176
2-Acetylamido adamantane-2-carboxylic acid (15a). 178
2-Benzamido adamantane-2-carboxylic acid (15b). 179
3-Acetyloxy 2-cyclohexen-1-one (22). 180

2-Acetyl 1,3-cyclohexandione (23). 180
2'-Hydroxy-6'-methoxy-acetophenone (24). 181
2',6'-Dimethoxyacetophenone (3). 182
Ethyl 4-(2,6-dimethoxyphenyl)-2,4-dioxobutanoate (4). 183
Ethyl 1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carboxylate (5). 184
1-(7-Chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carboxylic acid (6). 186
2-[1-(7-Chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carboxamido]adamantane-2-carboxylic acid (2) 187
N-(4-vinylbenzyl)-N-methylpyridin-4-amine (26). 188
Dimethylaminopyridine monolith 189
References 190

Abbreviations

AA	Arachidonic acid
ACHC	Aza-bis(cyclohexanecarbonitrile)
AcOH	Acetic acid
ASA	Acetyl salicylic acid
Boc	Tertbutyloxy carbonyl
BPR	Back pressure regulator
CBz	Benzyloxy carbonyl
CFC	Continuous flow coil
CINODs	COX Inhibiting Nitric Oxide Donors
CMC	Carboxymethyl cellulose
cNOS	Constitutive Nitric Oxide Synthase
COX-1	Cyclooxygenase-1
COX-2	Cycloxygenase-2
CV	Cardiovascular
DCM	Dichloromethane
DIPEA	Diisopropyl ethyl amine
DMAP	Dimethylamino pyridine
DMC	Dimethyl carbonate
DMF	Dimethyl formamide
DMI	Dimethyl imidazole
DMSO	Dimethylsulfoxide
DSC	Differential scanning calorimetry
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et2O	Diethyl ether
FBS	Fetal bovine serum
GAG	Glycosaminoglycans
GC	Guanilate cyclase
GI	Gastrointestinal
GTN	Glyceryl Trinitrate
HOBt	*1H*-Hydroxybenzotriazole
HRMS	High resolution mass spectrometry
HWB	Human Whole Blood

IL-β	Interleukin-β
iNOS	Inducible Nitric Oxide Synthase
ISDN	Isosorbide nitrate
LC-MS	Liquid chromatography-mass spectrometry
LPS	Lipopolysaccharide
MeOH	Methanol
Na_2EDTA	Edetate disodium
NIS	N-iodosuccinimide
NMR	Nuclear magnetic resonance
NO	Nitric Oxide
NOBA	4-Nitroxybutanol
Nox	Nitrates and nitrites
NSAIDs	Non Steroidal Anti-inflammatory Drugs
OA	Osteoarthritis
ODQ	1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one
PBS	Phosphate buffer saline
PFA	Perfluoroalkoxy polymer
PG	Prostaglandin
PGE2	Prostaglandin E2
PTC	Phase transfer catalyst
PTSA	Para-Toluene sulfonic acid
PyBop	Benzotriazol-1-yl-oxy tripoyrrolidinophosphonium hexafluorophosphate
SGF	Simulated gastric fluid
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin liquid chromatography
TMS	Tetramethyl silane
tNSAIDs	traditional Non Steroidal Anti-inflammatory Drugs

Flow chemistry symbols

Polymer tubing

Switching valve or T-piece

Pump

Reactor (coiled polymer tubing)

Pressure chamber

Column

Backpressure regulator (related to specified pressure)

Functionalised monolithic reactor

Polyme supported reagent

Chapter 1

Development of a Novel Class of Pharmacodynamic Hybrids/NO Donors that Targets COX-2 Selectively

Introduction

Non-Steroidal Anti-inflammatory Drugs. Acetyl salicylic acid (ASA) was first synthesised in 1853 by the French chemist Charles Frédéric Gerhardt (**Figure 1**).[1] However, years later (1899) a German chemist named Felix Hoffmann, who worked for Bayer, rediscovered Gerhardt's molecule. Hoffmann then convinced Bayer to market the new wonder drug under the name of Aspirin which was patented on February 27, 1900.[2] Since then, Aspirin has been one of the most popular over-the-counter *non-steroidal anti-inflammatory drugs* (NSAIDs) used as pain reliever and anti-inflammatory drug for fever, headache, migraine and acute pain.

Figure 1. Charles Fréderic Gerhardt, Felix Hoffmann and Aspirin (its chemical structure and products marketed by Bayer at the beginning of the 20th century)

NSAIDs are a chemically heterogeneous class of molecules sharing the biochemical feature of blocking the synthesis of prostanoids (prostaglandins, thromboxanes and prostacyclins) (**Figure 2**). Prostanoids are important biologically active autacoids generated through the arachidonic acid (AA) cascade by a class of enzymes defined prostaglandin-H synthases which are most popularly known as Cyclooxygenases-1 (COX-1) and Cyclooxygenases-2 (COX-2). These autacoids play an important role in maintaining homeostatic functions in the human body as they are involved in cytoprotection of gastric mucosa, renal physiology, blood flow

regulation, gestation. Additionally they are implicated in a number of pathological conditions such as inflammation, cardiovascular diseases and cancer.[3,4]

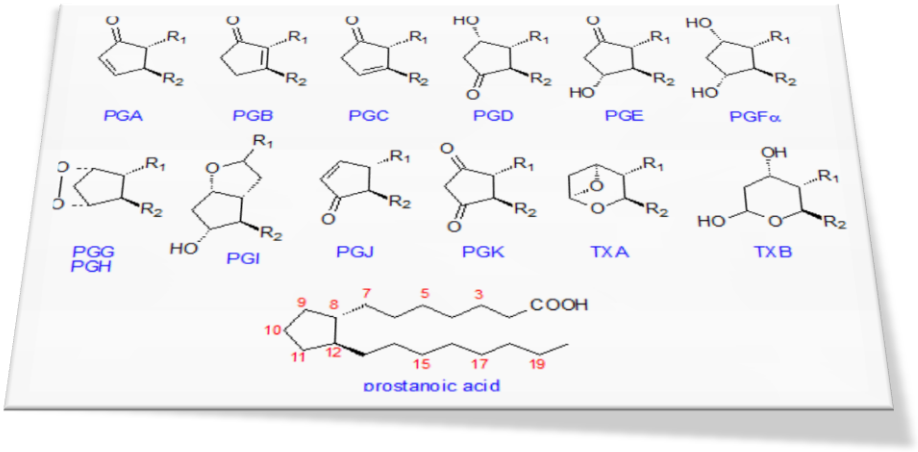


Figure 2. Structures of prostaglandins, prostaclyclins and tromboxanes

Though COX-1 and COX-2 both exhibit cyclooxygenase and peroxidase activities, different catalytic and transcriptional pathways are involved in their regulation in the human body (**Figure 3**).[5, 6]

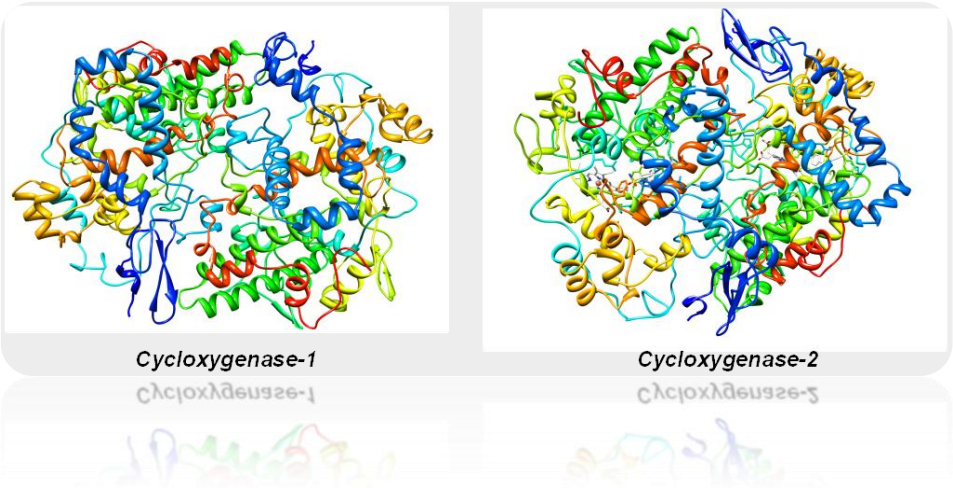


Figure 3. Crystallographic structures of COX-1/flurbiprufen complex and COX-2/DuP-697 complex

Remarkably, COX-2 requires considerably lower levels of hydroperoxides to initiate cyclooxygenase catalysis than those required by COX-1 and COX-2 exerts its activity at lower levels of free AA than COX-1. Another important difference is related on the regulation of the expression of the two isoenzyme genes. COX-1 has the structural features of an "housekeeping" gene while COX-2 appears as an immediate early gene.[7, 8] Moreover, both mRNA and protein of COX-1 are more stable than those of COX-2.[9] Thus, COX-1 is constitutively expressed in almost all tissues and is responsible for physiological functions such as gastrointestinal cytoprotection, platelet aggregation and vascular smooth muscle tone modulation.[10] On the other hand, COX-2, the second isoform, was identified as an inducible enzyme, highly expressed in response to interleukin-1β (IL-1β) and other inflammatory stimuli.[10] However, this strict separation of the function of COX-1 and COX-2, as constitutive and inducible, respectively, is now considered naïve. In-depth knowledge of COX-isoform biology has shown that COX-1 can be up-regulated in some circumstances (such as cell differentiation) and COX-2 can be constitutively expressed in physiologic conditions.[11] The only thing that appears clear is that COX-2 is the isoform directly involved in mediating and enhancing the inflammatory/hyperalgesic stimulus.

According to US National Center for Health Statistics, over 50 % of prescriptions for NSAIDs in the last decade were related to diseases of the musculoskeletal system and connective tissue, including arthritis. In addition, NSAIDs are widely used as analgesics in various pathologies for the treatment of mild to moderate pain and may be combined with opioids to treat moderate to severe pain (**Figure 4**).

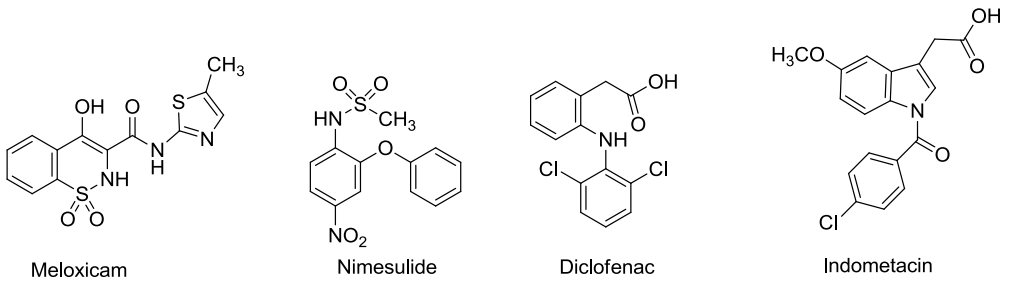


Figure 4. Structures of some NSAIDs

Most of the "Big Pharmas" are leaving the area of research for new pain treatments, since they consider this area too challenging, and despite a substantial investment there has been minimal progress in developing new efficacious and safe analgesics.[12] Indeed, the use of NSAIDs is hampered by side-effects which include gastrointestinal (GI) disorders ranging from minor dyspepsia to major ulcers, kidney effects and cardiovascular (CV) effects.[13] The GI side-effects (known as NSAID-induced gastropathy) are the most relevant problem in long-term NSAIDs administration.[12,13] NSAIDs are widely used worldwide and since gastroduodenal erosions and ulcerations are estimated to affect up to half of chronic NSAIDs users, this GI adverse effect has a major world health implication. The development of COX-2 selective NSAIDs or coxibs was meant to circumvent these side effects, by selectively inhibiting the isoenzyme involved in the production of pro-inflammatory mediators (**Figure 5**).[14]

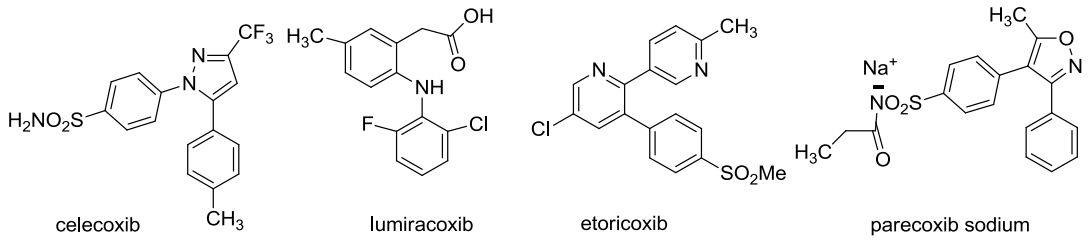


Figure 5. Structures of some coxibs

Though clinical trials have shown a reduction of gastrointestinal and renal side effects, an unexpected CV toxicity emerged during the coxibs post marketing outcome studies. Many subsequent studies were carried out to define the CV risks associated with coxibs and traditional NSAIDs (tNSAIDs). Though the increased cardiovascular morbidity was firstly

highlighted in large clinical trials investigating the safety of coxibs, later it appeared in studies involving tNSAIDs as well.[15]

Renal side-effects of NSAIDs are less common, sometimes transient and often reversible upon drug withdrawal. However these side-effects, both in terms of incidence and severity, increase in patients with risk factors such as diabetes, heart failure, renal dysfunction and in the elderly. These side-effects seem to be common to coxibs and tNSAIDs and range from electrolytes retention/reduced glomerulal filtration to nephritic syndrome and chronic renal failure .[16]

Additionally, recent studies underlined that the relation between inhibition of COXs and pharmacological effect (both therapeutic and adverse) could be more composite than it has been considered so far. This complex relation would generate from the finding that COX enzymes are homodimeric enzymes that act as conformational heterodimers.[17]

Molecular hybridisation and nitric oxide. The complexity of many pathologies often requires a multi-target strategy in order to get different and complementary pharmacodynamic mechanisms. The use of pharmacological cocktails is usually used to accomplish this task, in order to get different effects within the same administration, although one of the main drawbacks is related to poor compliance. In the last few years a novel strategy has been adopted to overcome the administration of cocktails. The aim of this strategy is to generate molecular entities that can activate different targets, reduce the toxicity or change the bioavailability of the parent drug in order to increase the therapeutic efficacy of the parent drug itself. The concept of molecular hybridisation is related to the concept of multiple ligands where conjugation or overlapping of two or more molecular portions, gives rise to drugs able to recognise more than one receptor.[18]

In recent years, many research groups have focused their efforts on the discovery and development of molecular hybrids characterised by a nitric oxide (*NO*) releasing moiety.[18] While at high concentrations *NO* is cytotoxic and is part of the immune system, at low concentrations it has important functions in the overall homeostasis of the organism. In the GI tract, *NO*

modulates the blood flow contributing to mucosal defence against luminal irritants and it also modulates mucus and bicarbonate secretion. It has a striking role in mucosal healing processes and modulates vascular permeability. Furthermore, *NO* is known to be a cardioprotective agent due to its vascular activity. Increasing evidence suggests a considerable modulating effect of nitric oxide as a pleiotropic agent.[19]

The role of *NO* in osteoarthritis (OA) has been recently reviewed.[20] Although *NO* is one of the pro-inflammatory factors associated with OA, its role in the joint is more complicated than previously understood and recent evidences point for protective roles of *NO* generated by the constitutive isoenzyme form of nitric oxide synthase (cNOS) in the joint. This suggests that *NO*-donors could be an asset in the OA treatment. Thus, while incubation of chondrocytes with a fast liberating *NO* donor such as glyceryl trinitrate (GTN) induces apoptosis, incubation with small amounts of *NO* or with diazenium diolates (a slow *NO*-donor) does not induce apoptotic cell death and has beneficial effects in chondrocytes, increasing alkaline phosphatase activity and collagen type X synthesis.[21] There is a body of evidence that the degenerative activity due to an increase of *NO* concentration could be a result of an increase in peroxy-nitrite concentration. In chondrocytes *NO* and peroxynitrite have opposite effects in the nuclear factor-kappa B (NF-$_k$B) activation. When cells are treated with a soft *NO*-donor, the number of cells with activated NF-$_k$B decreased, while addition of peroxy-nitrite gives rise to a dramatic increase in NF-kB activation. Also other cells in the joint are differently influenced by different *NO* concentrations and/or peroxy-nitrite presence. Exposure of osteoblast cells to low *NO* levels have protective effects while exposure to high concentrations induced apoptotic cell death.

Increasing evidence suggests that there is considerable cross talk between *NO* and prostaglandin (PG) biosynthesis.[22] Indeed, the amount of *NO* released by cNOS or inducible NOS (iNOS) and the redox state of the cell system play a relevant role in the modulation of COX enzymes leading to opposite responses. A mass of experimental evidences has recently shown that low concentrations of *NO* down-regulate PG biosynthesis, mainly via inhibition of COX-2

activation. *NO* donors added exogenously to lyphopolysaccarides-treated microglia, endothelial and J774 cells inhibit PG release and reduced COX-2 expression *in vitro* and *in vivo*.[23]

Accordingly, a beneficial role played by *NO* can be recognised for arthritis and other COX-mediated diseases. However, as in the cases of the treatment of CV and GI side-effects, the *NO* concentration and then the kinetic for its release is fundamental to make the difference between a protective/therapeutic effect and a deleterious effect. The release should be slow and the *NO* concentration should be maintained within the nanomolar levels, such as those liberated by cNOS, while agents which liberate in the short time micromolar levels of *NO* are expected to give rise to iNOS-like effects, in addition to possible hypotensive effects.

Generation of exogenous nitric oxide. Inorganic nitrates and nitrites are metabolised *in vivo* to form *NO*.[24] However the kinetic, the site, and the concentration of *NO* liberation cannot be controlled when these inorganic nitrates are administered. Organic nitrates are characterised with a more complex fate in the organism. For instance glyceryl trinitrate (GNT) has been therapeutically used for the treatment of angina since 1878. GNT and its congeners (i.e., isosorbide nitrate, ISDN) are absorbed almost unchanged and rapidly converted into the organism by enzyme-dependent mechanisms into their metabolites and *NO* (**Figure 6**).

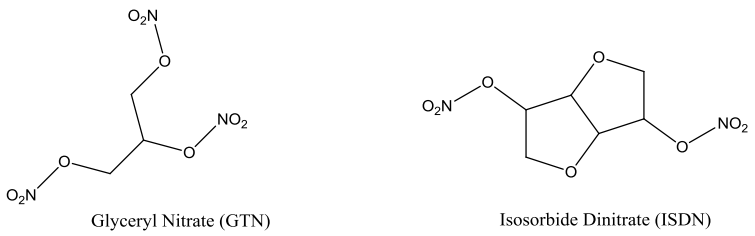


Figure 6. Glyceryl nitrate and isosorbide dinitrate structures

Two main biological pathways are responsible for the generation of *NO* from organic nitrates, the first involving an interaction with endogenous thiol-containing compounds (cysteine or glutathione, to form intermediate nitrosothiols), the other being a completely enzymatic redox reaction.[25]

CINODs and Naproxcinod. An interesting class of pharmacodynamics hybrids is represented by COX Inhibiting Nitric Oxide Donors, namely CINODs. This class of molecules was firstly developed with the aim to cut down the GI side effects related to the administration of NSAIDs, through the conjugation of existing tNSAIDs with a nitric oxide releasing moiety. This kind of strategy was first explored by NicOx through the development of two representative drugs of the CINODs family: NO-flurbiprofen (HTC-1026) and Naproxcinod (AZD3582) (**Figure 7**).[26]

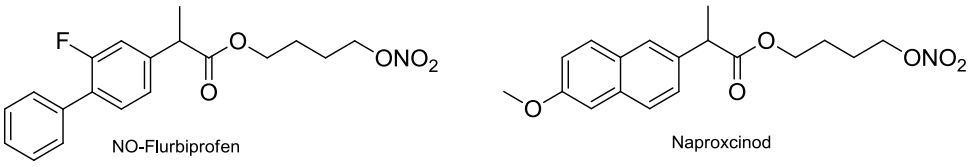


Figure 7. Structure of CINODs, NO-Flurbiprofen and Naproxcinod

Naproxcinod is the first drug of the CINODs class at the latest clinical trial. The phase III studies confirmed that Naproxcinod dosed at 375mg was comparable to 500mg dose of Naproxen in relieving signs and symptoms of OA and it was well-tolerated with most adverse effect being mild-to-moderate.[27] A large clinical program of 37 studies was conducted for Naproxcinod with about 6,500 patients and three large pivotal studies, where safety was completely assessed in 4,000 patients. Interestingly, even though GI side-effect related to Naproxcinod appeared only slightly better than those of Naproxen, an improved CV safety appeared during these studies.[28] Naproxcinod (750mg twice a day) reduced systolic blood pressure compared to Naproxen. In addition, the difference in mean change from baseline in systolic blood pressure between Naproxen and Naproxcinod was 6.5 mmHg in favour of Naproxcinod in patients with hypertension. The findings that Naproxcinod does not induce any increase of blood pressure, as with Naproxen and other NSAIDs, prompted NicOx to adjust the strategy focusing their drug for treating pain and inflammation in patients with congestive heart failure, liver disease, cirrhosis, pre-existing renal disease, elderly with renal impairment, chronic renal failure or essential hypertension.[29]

Naproxcinod is the ester of Naproxen with 4-(nitroxy)butanol (NOBA). In order to exert its analgesic and anti-inflammatory activity Naproxen must be liberated from Naproxcinod, which in this case acts as prodrug (**Figure 8**).

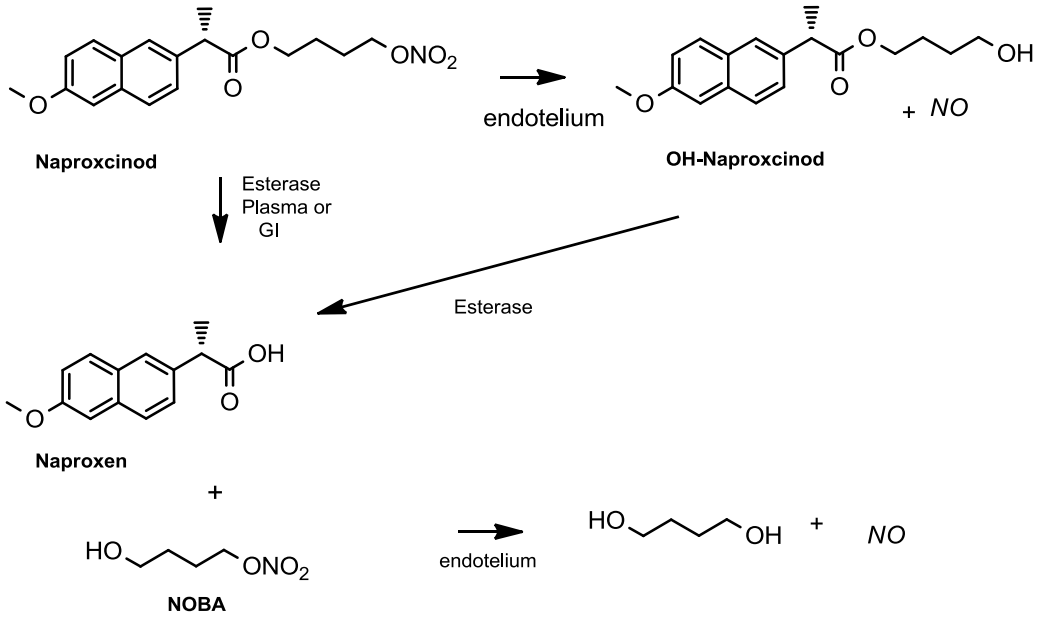


Figure 8. Conversion of Naproxcinod through two main metabolic pathways

In the blood and intestine Naproxcinod is converted into naproxen by esterases and only a modest amount of unchanged drug is found in plasma after oral administration. In humans about 9-20% of the dose appears to be taken up as intact substance. The circulating Naproxcinod is further metabolised with a half-life of about 3 hrs.[30] Accordingly, Naproxcinod is converted mainly in the GI and for the remaining part in the organism into Naproxen which acting as COX inhibitor gives rise to the analgesic and anti-inflammatory actions. Though Naproxcinod in theory can release NO according to two independent mechanisms (**Figure 8**), one involving *NO* liberation from the intact drug to give rise to the alcohol metabolite, while the other implies Naproxcinod hydrolysis into Naproxen and the *NO* releasing moiety NOBA, the extensive GI hydrolysis excludes the first mechanism as the main *NO* releasing pathway. Since plasma nitrate levels were found to be significantly elevated after single and repeated doses of

Naproxcinod, it should be deduced that the main source of *NO* is the organic nitrate NOBA, which is mainly liberated and adsorbed in the GI. A common pathway for *NO* generation between Naproxcinod and GNT was defined and later *NO*-flurbiprofen was shown to be characterised by the same kinetic of *NO*-release, highlighting the NOBA moiety as the active metabolite of Naproxcinod responsible for *NO* activity. Accordingly, Naproxcinod is mainly converted in the GI into two active metabolites: Naproxen responsible for analgesic and anti-inflammatory activity and NOBA responsible for *NO* related effects including the CV effects. The high in vivo metabolism of Naproxcinod into these metabolites can be at least in part responsible for some discrepancies found between in vitro and in vivo studies and explain some deviations from expectations seen with Naproxcinod. Moreover, NOBA, like GNT, acts as a relatively fast *NO* releaser (CV effects are seen up to 3hrs from drug administration) while the duration of action of naproxen is 7-10 hrs.[31] This does not completely match the pharmacokinetics of the *NO*-release with the COX inhibition and could give rise in the long term use of the drug to a more limited CV protection than that expected on the basis of the present data.

Rationale and aims. It appears at the present that only COX inhibition by NSAIDs represents an efficacious and cost effective therapy for the treatment of mild-to-moderate pain and inflammation. On the other hand GI side-effects seriously impact the long term use of traditional NSAIDs (tNSAIDs), while CV adverse effects hamper a widespread use of coxibs. Naproxcinod clinical data highlighted how the combination of a NO-donor moiety with a tNSAIDs, even though not giving rise to substantial improvement in GI toxicity of Naproxen, improved the CV safety.

Accordingly, the combination of a COX-2 inhibitor with an NO-donor should provide a drug endowed with strong analgesic and anti-inflammatory properties, sparing the GI and endowed with the appropriate CV safety. The novelty in developing a class of molecules that would exert both COX inhibition and NO donor actions, without the need to be hydrolysed is based on the

idea of providing a drug endowed with chimeric pharmacokinetic properties, assuring COX-2 inhibition and *NO* suitable concentration at the same time and in the same compartment. In addition, the NO-releasing properties could improve the CV-protective properties and gain an advantage of those favourable effects exerted by low NO concentrations on inflammatory and cartilage degradation processes, as discussed above. The scaffold present in the original COX-2 inhibitors of Rottapharm S.p.A., patented and granted, was used as scaffold for the design of dual COX-2-inhibitors/NO-donors (**Figure 9**).[32]

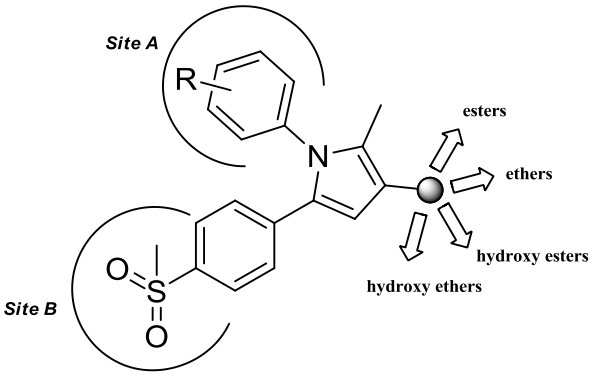


Figure 9. Core structure of the selective COX-2 scaffold by Rottapharm S.p.A.; **Site A** corresponds to the site which is likely to interact with Tyr-385 of the catalytic pocket, whereas **Site B** is responsible for interaction with the side pocket of the enzyme; it was suggested that the side chain of the molecule could be involved in selective interactions as well

Though several examples describe the "hybridisation" of NSAIDs, few of those involve the modification of a coxib-like structure, namely a selective COX-2 inhibitor.[33]

Selective COX-Inhibiting Nitric Oxide Donors. We followed the pioneering strategy adopted by NicOx. Nonetheless our idea was to conjugate the COX-2 structure identified previously (Rottapharm S.p.A.) with a nitric oxide releasing moiety to give access to new molecular entities endowed with reduced toxicity (CV and GI) and enhanced pharmacodynamic properties (**Figure 2**), where the molecule itself would present chimeric pharmacodynamic properties. The chemical manipulation was focused mainly on position 3 of the pyrrole core in order to retain

COX-2 inhibitory properties both in terms of potency and selectivity and to introduce an *NO*-releasing moiety with appropriate characteristics. It has been speculated that the addition of a polar substituent on the heterocyclic core could improve the selectivity and the affinity for the alternative binding site in COX-2 (through engagement of H-bond interactions with the close Arg513 side chain which is present only in this isoform), due to dynamic interactions between the enzyme pocket and the ligand. The development of this novel panel of compounds was based on the dynamical features of this ligand-binding mechanism, where the polarity of the side chain is considered extremely important for selectivity. Three main classes of compounds were explored for COX-inhibitory activity and *NO*-releasing properties (**Figure 10**).

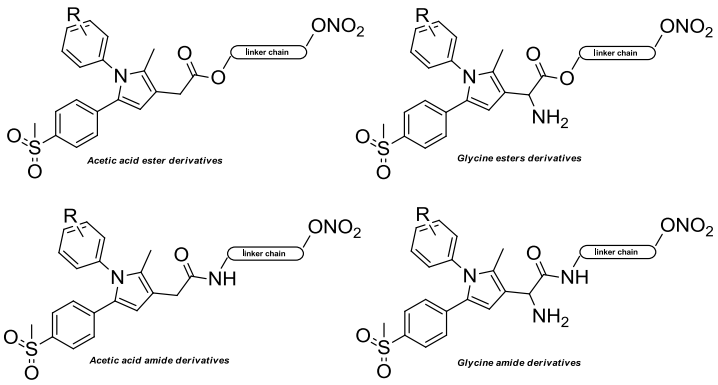


Figure 10. Structures of compounds belonging to the four generations of Selective COX-2 Inhibiting/Nitric Oxide Donors

Aiming at achieving a full understanding of all the dynamic interaction of these molecules with the human body and the role of the NO-release at any level, nitro-free derivatives were also synthesised and tested.

Stepwise approach to solubility and metabolic stability. When dealing with drug discovery and development, the concept of "bioavailability" is always to be considered of great importance. The bioavailability of any xenobiotic introduced in the human body is mainly determined by two parameters: solubility and metabolic stability. The former is determined by the amphiphilic character of biological membranes: in other words, balanced ratios between lipophilicity/hydrophilicity represent ideal conditions to allow the drug to reach the blood

stream and then the target. The latter is important in the sense that any alteration of the molecular structure could lead to inactivation of the biological properties or to an improvement of its toxicity. Moreover, as explained before, in the case of "pharmacodynamic hybrids" (as Selective COX-2 Inhibitors/Nitric Oxide Donors) the metabolic stability would provide a drug endowed with chimeric pharmacokinetic properties. It was reasoned that a stepwise approach would be more useful for a broader achievement ensuring COX-2 inhibition and *NO*-releasing properties. The starting point was a generation of molecules (acetic acid esters) that are characterised by low stability and solubility; the molecular structure was then adjusted stepwise in order to increase the solubility at first (glycine esters) and then stability (acetic acid and glycine amides).

Solubility in phosphate buffered saline (PBS) and simulated gastric fluid (SGF). Since this kind of molecules (if marketed) would be administered "*per os*", it is reasonable to check out the eventual solubility in the gastric and in the body fluids environment. To fulfil these goals, SGF (pH 1.5) without pepsin and PBS buffer were used.[34]

Metabolic stability in phosphate buffered saline, simulated gastric fluid and rat plasma. After administration of any drug "*per os*", this should be soluble enough to interact with the human body and then be absorbed. Metabolism is a crucial parameter that has to be considered when developing novel molecular entities since any metabolic action on a xenobiotic can dramatically change its therapeutic profile. To assess the sensitivity of these four classes of "pharmacodynamic hybrids", SGF, PBS and rat plasma were used: the formers to assess the stability before absorption, the latter after absorption in the blood stream.[35]

Biological and pharmacological evaluation. The evaluation of stability and solubility were always accompanied by biological and pharmacological issues related to every generation of molecules. Each compound was screened for activity and selectivity towards both cyclooxygenases (1 and 2). To correlate the physical chemical (solubility and metabolic stability) data and the *in vitro* data, *ex vivo* and *in vivo* tests were carried out. It is extremely interesting to notice how *in vitro*

and *in vivo* testing could show discrepancies that could be explained by means of pharmacokinetics. Cell-based assay. Both nitro-oxy and nitro-free compounds were subjected to biological evaluation through *in vitro* testing. The *in vitro* estimation of the title compounds was performed to assess their inhibitory activity on both COX-1 and COX-2. Abdominal constriction test. In the attempt to screen the analgesic activity, the writhing test was performed on compounds that were selected on the basis of the activity displayed in the cell-based assay. This test is extremely indicative when trying to predict the antinociceptive activity of any molecule. Paw pressure and oedema test. The carrageenan-induced inflammation test was used to assess the analgesic and anti-inflammatory activities of these compounds. This model of inflammation is based on determination of the animal threshold response to pain induced in the paw by the application of a uniformly increasing pressure. The measurement of the edema (carrageenan-induced) volume after administration of the compound is used to predict the actual antiedemigenic activity. Human whole blood assay. The selectivity towards the COX-2 isoform displayed through the cell-based assay cannot be considered completely reliable, because the use of exogenous AA in the assay for COX-1 activity *in vitro* (in the murine monocyte/macrophage J774 cell line) can interfere with the COX-1 isoform leading to a conformation change within the enzyme binding site. The human whole blood assay (HWB) is an important tool to assess selectivity towards COX-isoenzymes in humans. This information is very important to predict the clinical consequences of the administration of any drug as the *in vivo* degree of inhibition of COX-1 and COX-2 depends on the dose.[36] Nitric Oxide release. The evaluation of nitric oxide release is of utmost importance, for the development of the hybrids. The nitric oxide dependency, when vasorelaxation was observed, was evaluated *via* administration of a guanilate cyclase (GC) inhibitor, namely 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). Incubation of selected compounds with liver homogeneate allowed to study the kinetic of release and the "species" which is responsible for the actual vasorelaxing effect.

Acetic esters derivatives: the first generation

(Biava, M.; Porretta, G.C.; Poce, G.; Battilocchio, C.; Alfonso, S.; Rovini, M.; Valenti, S.; Giorgi, G.; Calderone, V.; Testai, L.; Martelli, A.; Sautebin, L.; Rossi, A.; Papa, G.; Ghelardini, C.; Di Cesare Mannelli, L.; Giordani, A.; Anzellotti, P.; Bruno, A.; Patrignani, P.; Anzini, M. *J. Med. Chem.* **2011**, 54, 77659-7771. Ref. 38).

A first class of molecules with significant analgesic and anti-inflammatory properties was identified (**Figure 11**). These compounds were obtained by simple "conjugation" of selective COX-2 structures[32] with nitrooxy alkyl chains of different lengths (two, three and four carbons) in order to investigate the influence of the *NO*-donor moiety size on nitric oxide release and COX-2 inhibition.

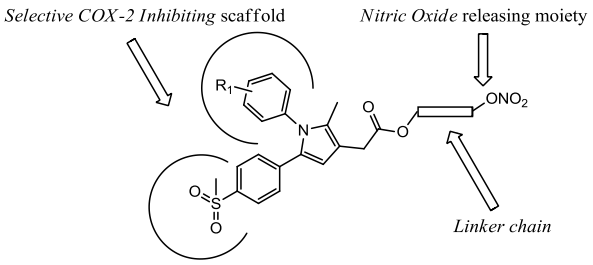
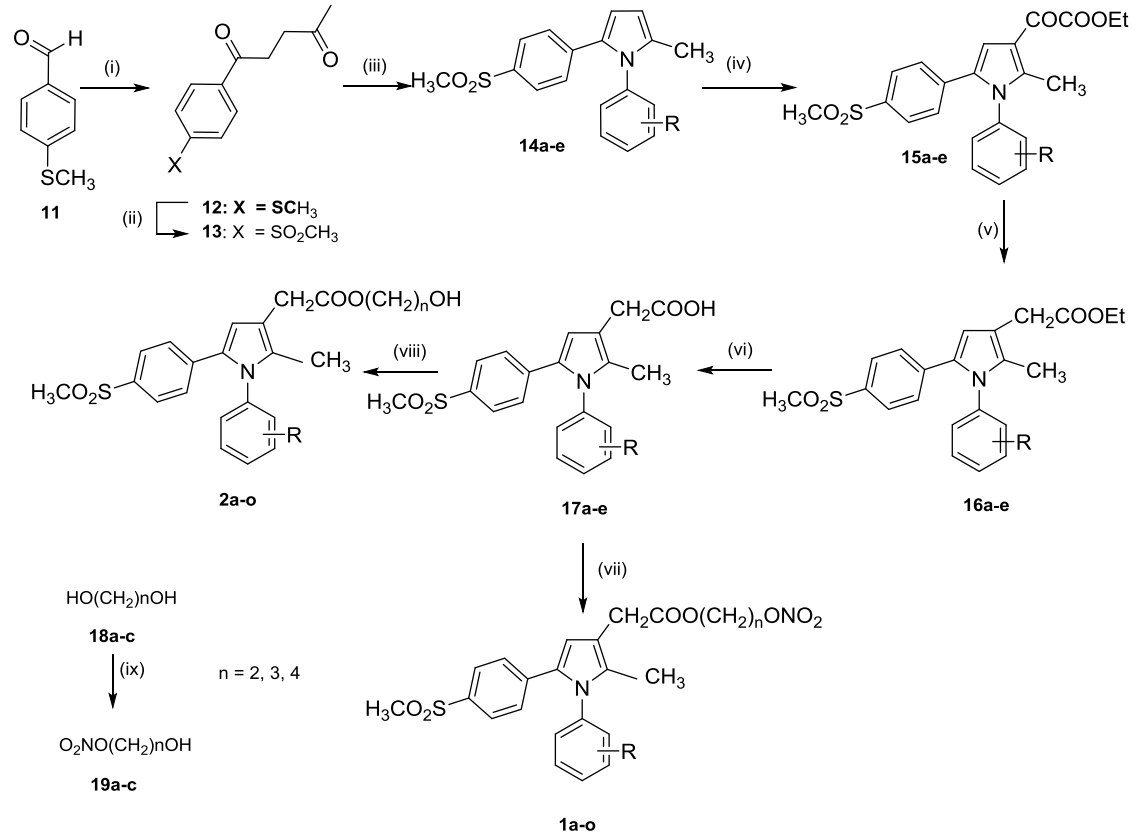


Figure 11. Chemical structure of the first generation of compounds

Considering that the nitro-oxy alkyl chains would be metabolised *in vivo*, the corresponding nitro-free derivatives were also synthesised and tested accordingly.

Chemistry. Access to the pyrrole core (**14a-e**) is given *via* a Stetter reaction between methyl-vinyl ketone and *p*-methylthiobenzaldehyde (**11**), an oxidation of compound (**12**) by means of Oxone and a final Paal-Knorr condensation with a selection of anilines. Then a regioselective acylation with titanium tetrachloride/ethoxalylchloride, followed by reduction with triethylsilane/trifluoracetic acid and hydrolysis in basic conditions afforded the acid derivatives (**17a-e**).[32] A final coupling of the acids with diols (**18a-c**) gave access to esters (**2a-o**), whereas the use of nitroxyalcohols (**19a-c**) as coupling partners afforded esters (**1a-o**). Nitro-oxy alcohols (**19a-c**) were obtained from diols (**18a-c**) using a simple nitrating overnight procedure.[37]

Scheme 1. Synthesis of compounds (**1a-o**) and (**2a-o**)



Reagents and conditions. (i) CH_2=CHCOMe, TEA, 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, MW, 15 min; (ii) Oxone, MeOH/H_2O, rt, 2 h; (iii) $RPhNH_2$, *p*-toluensulfonic acid, EtOH, MW, 45 min; (iv) EtOCOCOCl, $TiCl_4$, CH_2Cl_2, rt, 4 h; (v) Et_3SiH, TFA, rt, 2 h; (vi) 1N NaOH, MeOH, 1 h; (vii) **19a-c,** EDCI, DMAP, TEA, CH_2Cl_2, rt, 3h;(viii) **18a-c**, EDCI, DMAP, TEA, CH_2Cl_2, rt, 3h**;** (ix) HNO_3, EtOAc, $(CH_3CO)_2O$, 12h, rt.

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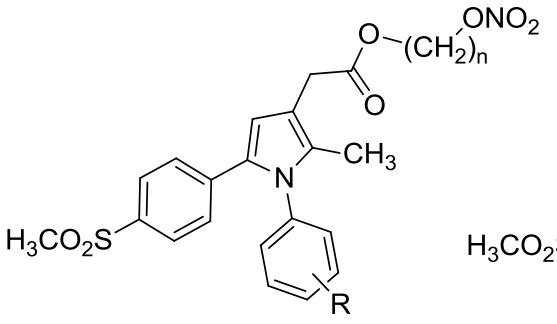
In vitro assay. This first class of esters (similar to Naproxcinod and *NO*-flurbiprofen) displayed selective low micro and nanomolar COX-2 inhibition (**Table 1**). Interestingly, these compounds were potent and selective COX-2 inhibitors as nitroesters (before releasing NO and/or to be hydrolyzed to the corresponding acid) and this represents the first striking difference in comparison with Naproxcinod.[30, 31] The corresponding alcohols (potential metabolites formed after *NO* release) are also effective in inhibiting COX-2, even although the presence of the nitro-oxy group is associated with higher extents of inhibition and selectivity. The data showed that substitution on the phenyl ring at N1 had an influence on selectivity and potency. Indeed,

activity could be modulated from low nanomolar (19 nM, **1d**) to low micromolar (0.12 μM, **1g**) simply by moving a fluorine substituent from position 3 to position 4. Side-chain length also had an impact on COX-2 activity as well (see: **1e**, IC_{50} is 7.3 nM, vs. **1d** and **1f**, respectively 19 nM and 69 nM).

Table 1. Cycloxygenases inhibition for compounds (**1**) and (**2**)



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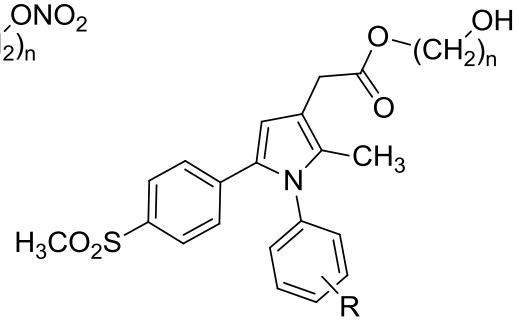


2

Compd	R	n	COX-1 inhibition (%)	COX-1 IC_{50}	COX-2 inhibition (%)	COX-2 IC_{50}	S.I.[a]
1a	H	2	19	>10	100	0.0430	>232
1b	H	3	27	>10	90	0.0420	>238
1c	H	4	10	>10	87	0.0900	>100
1d	3-F	2	36	>10	96	0.0190	>526
1e	3-F	3	75	1.1	97	0.0073	>150
1f	3-F	4	10	>10	98	0.0690	>100
1g	4-F	2	0	>10	100	0.1200	>344
1h	4-F	3	28	>10	91	0.0372	>268
1i	4-F	4	19	>10	92	0.0760	>100
1j	4-SCH_3	2	0	>10	100	0.0170	>588
1k	4-SCH_3	3	0	>10	99	0.0270	>370
1l	4-SCH_3	4	10	>10	82	0.1200	>100
1m	4-OCH_3	2	53	6.4	97	0.1700	>37.6
1n	4-OCH_3	3	0	>10	90	0.0024	>41667
1o	4-OCH_3	4	5	>10	87	0.1400	>100
2a	H	2	22	>10	100	0.096	>100
2b	H	3	0	>10	100	>10	n.c.
2c	H	4	2	>10	93	0.1100	>100
2d	3-F	2	33	>10	80	0.0850	>100
2e	3-F	3	0	>10	85	0.0230	>250
2f	3-F	4	17	>10	89	0.0920	>100
2g	4-F	2	21	>10	100	0.0390	>100
\2h	4-F	3	0	>10	100	0.0560	>100
2i	4-F	4	7	>10	96	0.1920	>100
2j	4-SCH_3	2	62	3.18	87	0.0850	37
2k	4-SCH_3	3	36	>10	85	0.2600	>30
2l	4-SCH_3	4	24	>10	80	0.9800	>10

2m	4-OCH_3	2	5	>10	84	1.1000	>9
2n	4-OCH_3	3	27	>10	93	0.7000	>10
2o	4-OCH_3	4	33	>10	92	0.9000	>10
Celecoxib	-	-	64	3.84	100	0.0610	>63

[a] Selectivity index: IC_{50} COX-1 vs. IC_{50} COX-2 ratio.

Stability and solubility assessment. Investigations on the chemical and enzymatic (esterases) sensitivity of these compounds highlighted the same problem described for Naproxcinod.[31] The series of compounds were unstable at both neutral and acid pH values (PBS and SGF) (**Table 2**). Due to this chemical sensitivity, compounds belonging to this generation are likely to be cleaved into the acid and the *NO*-donor (NOBA or a NOBA analogue) under biological conditions, exactly as happens with naproxcinod, the only difference being the selectivity.

Table 2. Stability assessment for compounds (**1d**) and (**1e**)

Compound	**Time**	**Parent molecule remained (%)**		
		PBS (pH 7.4)	**SGF (pH 1.5)**	**Rat Plasma**
1d	*30 min*	74.1	80.2	0.00
	60 min	53.6	65.7	0.00
	120 min	0.15	42.8	0.00
1e	*30 min*	88.10	87.40	0.10
	60 min	73.20	75.20	0.00
	120 min	62.90	61.00	0.00

Moreover, these compounds were found to be poorly soluble both in PBS and SGF (**Table 3**).

Table 3. Solubility assessment for compounds (**1d**) and (**1e**)

Compound	**PBS (pH 7.4) µM**	**SGF (pH 1.5) µM**
1d	1.6	<1
1e	1.1	<1

Vasorelaxing activity. Compounds (**1a**-**o**) were found to possess good vasorelaxing properties that were dependent on the nitric oxide pathway (nitric oxide release) (**Table 4**).

Table 4. NO-releasing efficacy and potency for compound (**1a-o**) and **GTN**

Compound	Emax	pIC50	ODQ
1a	65 ± 2	5.76 ± 0.08	+
1b	44 ± 8	< 5	+
1c	33 ± 5	< 5	+
1d	69 ± 4	6.48 ± 0.06	+
1e	39 ± 1	< 5	+
1f	29 ± 1	< 5	+
1g	58 ± 5	5.47 ± 0.07	+
1h	41 ± 2	< 5	+
1i	30 ± 3	< 5	+
1j	65 ± 1	6.34 ± 0.06	+
1k	41 ± 8	< 5	+
1l	28 ± 5	< 5	+
1m	77 ± 2	6.75 ± 0.05	+
1n	41 ± 11	< 5	+
1o	33 ± 3	< 5	+
GTN	73 ± 2	6.90 ± 0.07	+

The vasorelaxing activity of these molecules can be appreciated more when plotting the data in a graph (**Figure 12**). In the presence of ODQ no vasorelaxing activity was detected. This suggestes the mechanism is dependent on nitric oxide release.

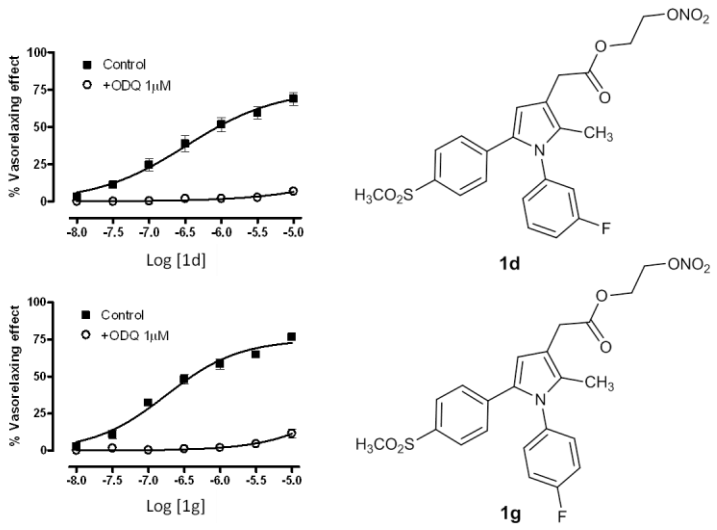


Figure 12. Representative concentration-response curves relative to the vasorelaxing effects evoked by compounds (**1d**) and **1g**), in the absence (control, black squares) or in the presence (white circles) of 1 μM ODQ

While the *NO*-releasing properties showed no dependence on the substitution pattern of the N1 aromatic ring, varying the side chain length did alter the activity. This is probably due to differing solubilities of the compounds. Indeed, the vasorelaxing activity seems to increase with the reduction of the side chain length. The experimental evidence suggests that the *NO*-release occurs through a modulated "slow donating process" which is preferred for hybrid drugs (**Figure 13**).

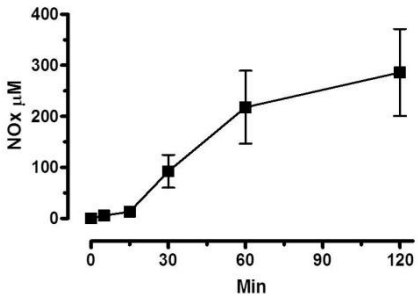


Figure 13. Time-dependent increase of the concentrations of NOx (nitrites + nitrates) following the incubation of 1 mM of (**1d**) in rat hepatic homogenate, containing the opportune cofactors

Whole human blood assay. In the *ex vivo* human whole blood (HWB) assay, the compounds showed a drop in inhibitory potency and selectivity toward COX-2 (**Table 5** and **Figure 14**) when compared to the cell-based results.

Table 5. Ex-vivo HWB assay for compounds (**1d**) and (**2d**) and reference compound Celecoxib

Compound	COX-1 IC_{50} (µM)[a]	COX-2 IC_{50} (µM)[a]	S. I.[b]
1d	9.6 (6.3 – 14.6)	3.2 (2 – 5.5)	3
2d	12.2 (7 – 21)	1.7 (0.94 – 3)	7.3
Celecoxib	12.47 (8.6 -17.9)	0.54 (0.29 – 0.52)	23

[a] IC_{50} values of COX-1 and COX-2 are reported as mean of three different experiments performed in duplicate and in brackets 95% confidence intervals (CI) are shown.
[b] Selectivity Index.

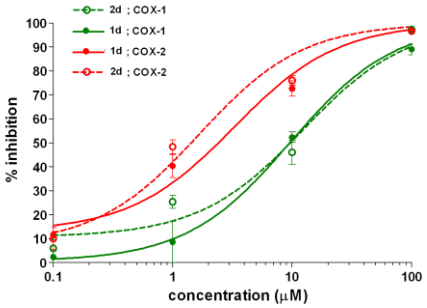


Figure 14. Inhibition of (**1d**) and (**2d**) on COX-1 and COX-2 activity in vitro in human whole blood; results are expressed as average percent of inhibition (N=3, MEAN+SEM) from three separate experiments

In vivo testing. The results of the writhing test for (**1d-f**) and (**2d-f**) underlined a striking dependence on the side-chain length. Indeed, activity of the compounds decreased with the increase of the side chain size. Compounds (**1d**) and (**2d**) display a 70% of activity at their max dosage, compounds (**1e**) and (**2e**) displayed only by a lower activity (nearly 40% of writhes reduction), while (**1f**) and (**2f**) present a mere analgesic activity (around 20% of writhes reduction) (**Table 6** and **Figure 15**).

Table 6. Writhes reduction evoked in the mice by administration of compound (**1d-f**) and (**2d-f**) after treatment of intraperitoneal acetic acid 0.1% solution

Compound[a]	n° mice	Dose po μmol/kg^{-1}	n° writhes	writhes reduction
CMC	43	-	32.6±2.1	-
1d	12	42	17.1±2.3*	48%
	12	84	9.4±2.5*	71%
1e	18	41	25.7±2.8^	30%
	10	82	24.9±2.2*	32%
1f	12	39	28.3±2.8^	18%
	15	78	26.4±2.1*	27%
2d	8	7	26.9±3.0*	26%
	8	23	17.4±2.9*	48%
	8	46	16.2±2.8*	51%
	8	70	8.9±2.1*	73%
2e	10	45	25.3±2.8	30%
	10	90	20.6±2.2*	40%
2f	13	42	26.8±2.2*	26%
	12	84	26.0±2.9*	24%
Celecoxib	10	26	13.4±2.6*	60%

^P<0.05; *P < 0.01 versus vehicle treated mice.

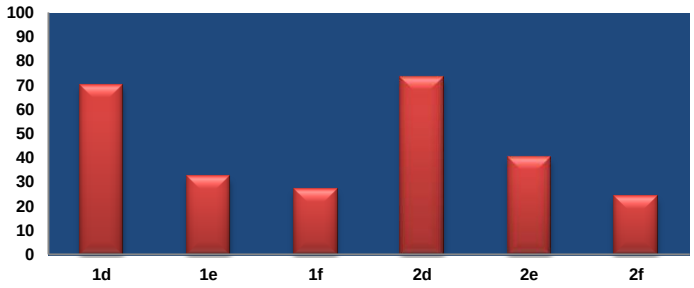


Figure 15. Percentage of writhes reduction of compounds belonging to the first generation

The results of the carrageenan-induced hyperalgesia and edema show good pharmacological profiles (**Table 7**). Compound (**2d**) is worthy of particular note since it displayed an 80% reduction of hyperalgesia 30 minutes after the treatment, when administered at a dose of 30 $mgkg^{-1}$. Comparison of (**2d**) with celecoxib showed similar features, with the main difference being a faster onset of the effect for (**2d**). The related nitroxy derivative (**1d**) displayed the same fast effect but its efficacy was abated after 60 minutes. This could be due to different metabolic pathways for the two compounds.

Table 7. Paw pressure test and edema for compounds (**1d-f**) and (**2d-f**) with reference compound Celecoxib

Pre-treatment	Treatment	Dose µmol/Kg	Paw Pressure				Edema Volume (mL)
			Before treatment	After Treatment			
				30 min	60 min	120 min	
Saline	CMC	32	62.6±2.1	62.5±1.8	63.4±2.2	62.7±2.5	1.33±0.07
Carrageenan	CMC	28	61.9±2.0	33.7±2.6	35.1±2.3	34.8±2.3	2.44±0.08
Carrageenan	**1d**	42	32.6±2.7	40.1±3.4	35.3±2.8	N.D.	2.52±0.06
Carrageenan	**1d**	84	29.8±3.1	54.1±3.7	38.7±3.1	N.D.	2.17±0.08
Carrageenan	**1d**	210	31.9±3.0	57.2±3.5	36.5±3.8	N.D.	1.88±0.08
Carrageenan	**1e**	41	62.5±3.1	43.2±3.2	45.1±3.2	40.7±2.6	2.05±0.08
Carrageenan	**1e**	82	63.2±3.3	45.5±3.7	44.9±4.1	36.8±3.8	1.96±0.09
Carrageenan	**1f**	39	62.4±3.1	39.2±4.3	40.1±2.7	33.7±2.6	2.15±0.08
Carrageenan	**1f**	78	61.4±3.3	41.5±3.6	43.7±4.8	37.9±4.0	2.00±0.08
Carrageenan	**2d**	70	31.8±2.5	55.7±3.1	52.3±2.8	39.3±3.5	2.51±0.05
Carrageenan	**2d**	45	62.6±3.1	41.2±3.0	45.8±3.1	36.4±3.1	N.D.
Carrageenan	**2e**	90	61.3±3.0	43.1±3.6	48.8±3.3	44.4±3.3	N.D.
Carrageenan	**2f**	84	61.1±3.2	40.1±3.0	43.3±3.2	39.4±2.3	N.D.
Carrageenan	Celecoxib	26	61.5±3.4	54.3±3.9	57.1±4.0	54.9±3.6	1.36±0.07

Conclusions. The slow donating properties, good selective inhibition towards COX-2 and the acceptable *in vivo* activity are an excellent starting point for this novel class of compounds. However, the extremely low solubility and the high sensitivity to enzymatic and non-enzymatic metabolic routes were considered major problems which needed to be overcome. The high *in vivo* metabolism of these molecules could be responsible of the discrepancies between the biological (*in vitro*) and pharmacological data (*in vivo*), indicating that the pharmacokinetic behaviour plays a crucial role in the fate of this class of molecules.

Glycine esters derivatives: improving solubility

(Biava, M.; Battilocchio, C.; Poce, G.; Alfonso, S.; Consalvi, S.; Porretta, G.C.; Schenone, S.; Calderone, V.; Martelli, A.; Testai, L.; Ghelardini, C.; Di Cesare Mannelli, L.; Sautebin, L.; Rossi, A.; Giordani, A.; Patrignani, P.; Anzini, M. *Eur. J. Med. Chem.* **2012**, *58*, 287–298. Ref. 40)

Poor solubility can be caused by either high crystallinity of the molecule (due to zwitterion formation, insoluble salts, strong H-bonding networks, etc) or hydrophobicity. Hence high values of logP (absence of an ionisable moiety, high molecular weight) are undesirable.[34,35] In the case of molecules belonging to the first generation of esters, the problem was the latter (**Figure 15**). Indeed, a rapid calculation of the cLogP for all compounds highlighted values between 3.25 to 4.50.

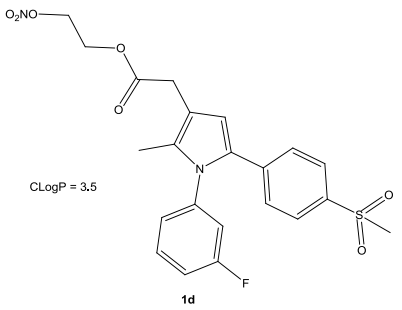


Figure 15. CLogP calculated for compound **1d** using ChemDraw software (version 12.0)

Formulation strategies represent a fashionable and successful way to improve the solubility and bioavailability of active compounds; nevertheless, the study of these systems adds further parameters which results in added complexity and expense in the early stages of development.[34] It was therefore decided to improve the solubility through chemical modification of the identified core (**Figure 16**).

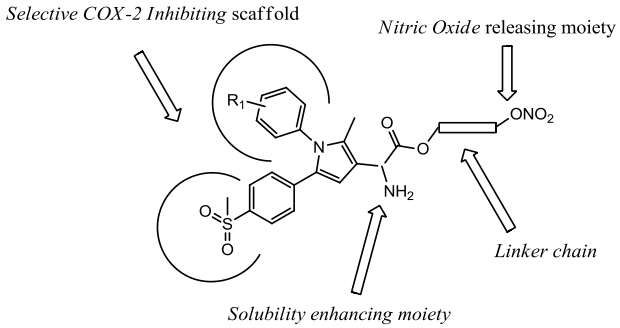
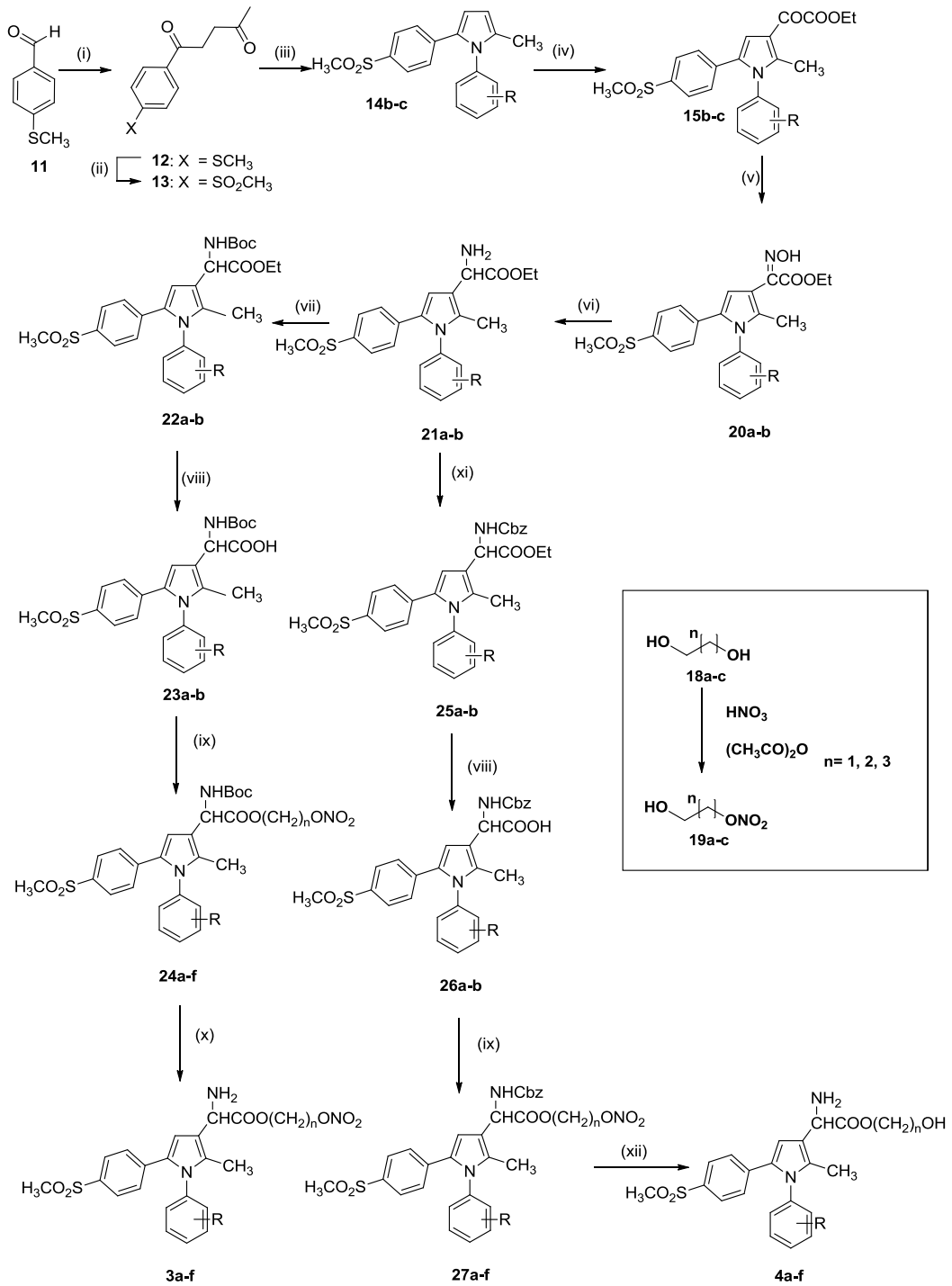


Figure 16. Chemical structure of compounds belonging to the second generation

The introduction of an amino group would represent an effective improvement in order to give access to molecules endowed with higher solubility and higher diffusion within the hydrophilic environment of the cartilage. It was decided that the second generation of esters would be characterised by a *meta*-fluoro or *para*-fluoro substituent at the N-phenyl ring, for a higher degree of interaction shown in the first generation.[32,38] Since we expected that the *NO* release could give rise to the nitro-free intermediates as metabolites, we decided to further synthesise and analyse the activity of these molecules to better understand the biological profile of this class of derivatives.

Scheme 2. Synthesis of the second generation of compounds



Reagents and conditions. (i) CH_2=CHCOMe, TEA, 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, MW, 15 min; (ii) Oxone, MeOH/H_2O, rt; (iii) $RPhNH_2$, *p*-toluensulfonic acid, EtOH, MW; (iv) EtOCOCOCl, $TiCl_4$, CH_2Cl_2, rt; (v) NH_2OH, NaAcO, dioxane; (vi) Zn, HCOOH; (vii) $(Boc)O_2$;(viii) NaOH, MeOH; (ix) **19a-c**, EDCI, DMAP, TEA, DCM; (x) TFA, DCM; (xi) CbzCl, Na_2CO_3, MeOH; (xii) Pd/c, NH_4COO, isopropanol.

Chemistry. These molecules were prepared using a route to a common core, namely (**21a-b**) and then following two different approaches for the generation of nitro-oxy and nitro-free compounds (**Scheme 2**). As for compounds belonging to the first generation, the synthesis commences with a Stetter reaction between the *para*-methythiobenzaldehyde (**11**) and methyl vinyl ketone in the presence of a thiazolium salt catalyst. The compound obtained (**12**) is then oxidised by means of Oxone and subsequently a Paal-Knorr reaction affords the 1,5-diarylpyrroles (**14b-c**). Regioselective acylation of the pyrroles is achieved by a titanium-catalysed Friedel-Crafts reaction to obtain compounds (**15b-c**). The installation of the amino functionality was first attempted using a direct reductive-amination with ammonium acetate and screening different boron hydrides: unfortunately, we did not obtain very good results with yields ranging between 30 to 50%.[39] Moreover, the long reaction time and the need to column the final mixture discouraged us from pursuing this approach further. However, we found that formation of the oxime, its subsequent reduction to the amine and protection were easily achieved without the use of chromatography, giving the products (**22a-b**) and (**25a-b**) in very good yields. Hydrolysis of these ethyl esters under mild conditions and subsequent coupling gave access to compounds (**26a-f**) and (**27a-f**). A final deprotection step yielded nitro-free compounds (**4a-f**) and nitro-oxy compounds (**3a-f**). As shown in the scheme above, the installation of the α-functionality is obtained using the same approach (oxime formation, reduction and protection) but employing two different protection strategies for the nitroxy and nitro-free derivatives. The *tert*-butyl-oxy carbonyl (Boc) protecting group was selected for the synthesis of the nitro-oxy compounds (**3a-f**), whose deprotection in mild acid conditions did not affect the stability of the nitro-oxy side chain. On the other side, the benzyl-oxy carbonyl (Cbz) group was used for the route to the nitro-free derivatives since a late stage double-deprotection was envisaged to yield compounds (**4a-f**). Indeed, compounds (**27a-f**) represent a double protected scaffold where catalytic hydrogenation can eliminate both the nitroxy and Cbz functionalities.

Solubility assessment. As shown in **Table 8**, the introduction of an ionisable moiety within the scaffold hugely increased the solubility as expected. However, it was clear that even though changes on that position were tolerated, alteration of activity could occur.

Table 8. Solubility assessment for compounds (**3d-f**) (second generation)

Compound	PBS (pH 7.4) µM	SGF (pH 1.5) µM
3d	89.0	>200
3e	80.0	>200
3f	70.8	>200

Analysis of the solubility profiles underlines a 200-fold improvement of solubility in SGF and around 100-fold in PBS(Figure 17).

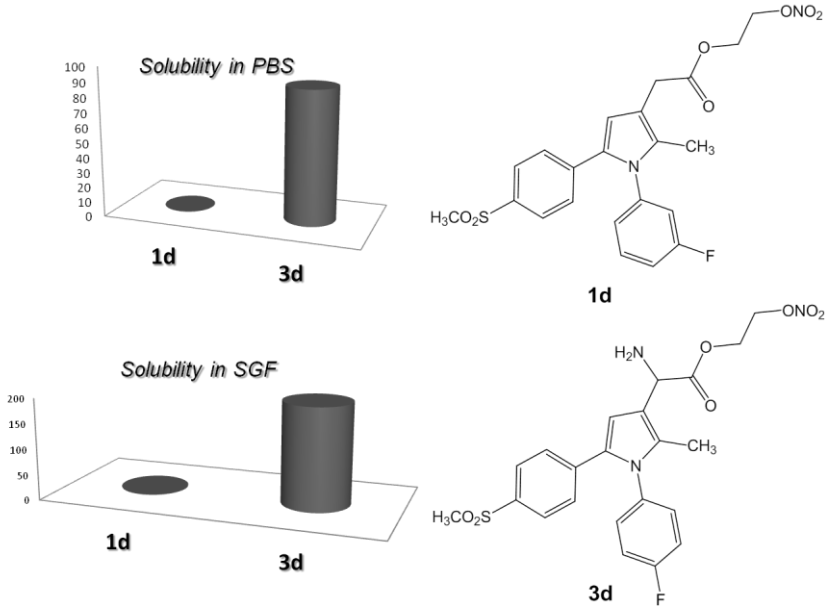
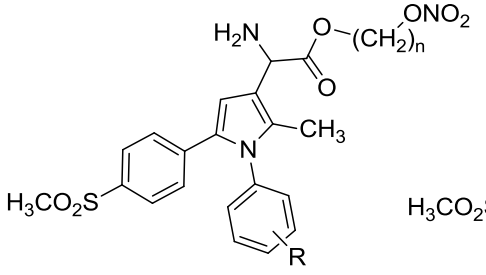


Figure 17. Solubility of (**3d**) and (**1d**) in both SGF and PBS

In vitro testing. As anticipated, the introduction of an ionisable moiety resulted in reduction of the activity in the cell based-assay. Indeed, this second generation of esters is endowed with lower extents of inhibition towards both COX-1 and -2. This can be clearly seen in **Table 9**, where activities of compounds range in the millimolar values, while first generation compounds exhibited low millimolar/nanomolar inhibition.

Table 9. In vitro activity for compounds (**3a-f**) and (**4a-f**)



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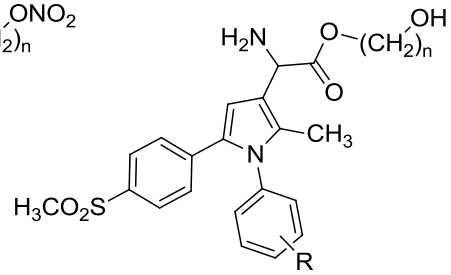

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Compound	R	N	% inhib COX-1 (10 µM)	% inhib COX-2 (10 µM)	IC_{50} (COX-1) (µM)	IC_{50} (COX-2) (µM)	COX-1/ COX-2 (S.I.)[a]
3a	3-F	2	0	89	>10	1.10	>10.00
3b	3-F	3	0	95	>10	0.82	>12.20
3c	3-F	4	1	82	>10	1.00	>10.00
3d	4-F	2	0	80	>10	1.89	>10.00
3e	4-F	3	5	100	>10	1.51	>6.62
3f	4-F	4	15	59	>10	1.41	>1.35
4a	3-F	2	14	92	>10	1.09	>10.00
4b	3-F	3	23	100	>10	0.76	>13.16
4c	3-F	4	10	100	>10	0.22	>45.45
4d	4-F	2	23	100	>10	1.45	>10.00
4e	4-F	3	30	79	>10	1.19	>8.40
4f	4-F	4	23	100	>10	1.50	>6.67

[a] Selectivity index: IC_{50} COX-1 vs. IC_{50} COX-2 ratio.

However, an average micromolar activity is still present in all compounds and selectivity towards the COX-2 isoenzyme is maintained. Amongst the nitro-oxy derivatives, (**3b**) proved to exert the best inhibition towards COX-2 (IC_{50} 0.82 uM) while the nitro-free compound (**4c**) inhibited COX-2 isoform at the lowest IC_{50} value (0.22 uM).

In vivo testing. The results of the writhing test highlight that even though the introduction of the amino group in the original scaffold reduced the in activity in the cell-based assay, an appreciable analgesic activity is still detected *in vivo* (**Table 10**).

Table 10. Writhing reduction for compounds (**3d-e**) in comparison with their first generation homologues **(1e-f**)

Compound	n° mice	Dose *po* µmol/kg^{-1}	n° writhes	writhes reduction
CMC	43	-	32.6±2.1	-
3e	13	5.5	26.9±2.6	19%
	10	18.4	21.3±2.5	36%
	12	36.8	18.1±3.0	46%
	14	73.6	13.5±2.0	59%
3f	13	35.9	24.5±3.2	27%
	15	71.8	19.8±2.4	41%
1e	18	41	25.7±2.8^	30%
	10	82	24.9±2.2*	32%
1f	12	39	28.3±2.8^	18%
	15	78	26.4±2.1*	27%
Celecoxib	10	26	13.4±2.6	60%

^P<0.05; *P < 0.01 versus vehicle treated mice.

Comparing the activities of compounds (**3e**) and (**3f**) it can be speculated that the side chain length inversely relates to the analgesic/anti-inflammatory potency of these molecules as observed in the first generation of compounds. Besides the outcast (**3d**) (not active), compounds (**3e**) and (**3f**) present higher activities than the parent compounds of the first generation. We hypothesise that although the molecular interactions of the amino derivatives are less favourable (cell-based assay), the solubility enhancement gained with the introduction of an ionisable group causes an overall increase in *in vivo* activity (**Figure 18**).

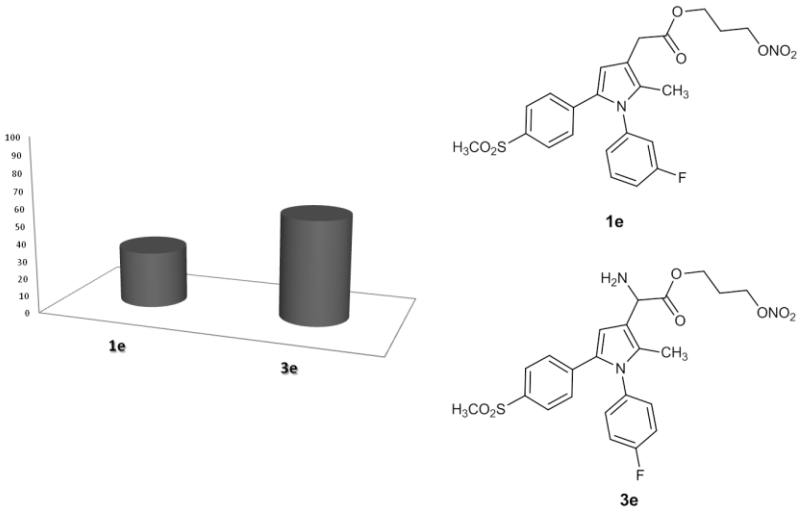


Figure 18. Comparison of writhes reduction between (**3e**) and (**1e**) (40 $mgkg^{-1}$ dosage)

As for the carrageenan-induced inflammation test, the data were collected every 15 min until activity of compounds was not detected anymore (**Table 11**).

Table 11. Activity of compounds **3e** in comparison with (**1d-e**) in the carrageenan induced inflammation test

Pre-treatment i.pl.	**Treatment p.o.**	**Dose (mg/kg)**	**Before treat.**	**Paw-pressure**				**Edema volume (mL)**
				After treatment				
				15 min	30 min	45 min	60 min	
Saline	CMC	-	61.2±2.5	65.6±3.4	58.9±3.8	61.4±4.1	63.6±4.0	1.44±0.05
Carrageenan	CMC	-	36.1±3.3	32.8±2.9	30.6±3.2	31.8±2.2	34.8±3.1	2.71±0.08
Carrageenan	**3e**	20	33.8± 2.8	40.2± 3.6 (25%)	43.7± 3.7 (44%)	38.3±3.1 (27%)	37.1±3.0 (15%)	2.58±0.05 (15%)
Carrageenan	**3e**	40	32.9±3.0	45.4±3.2 (40%)	50.2±4.4 (70%)	45.6±4.3 (53%)	37.5±2.8 (16%)	2.11±0.09 (45%)
Carrageenan	**1d**	20	35.7±3.1	38.7±3.3 (18%)	40.1±3.4 (35%)	38.7±3.4 (29%)	35.2±2.8 (10%)	2.52±0.06 (17%)
Carrageenan	**1d**	40	29.8±3.1	48.3±3.3 (50%)	54.1±3.7 (80%)	49.7±3.0 (60%)	38.7±3.1 (23%)	2.17±0.08 (40%)
Carrageenan	**1e**	40	33.5±2.6	41.2±2.2 (40%)	42.3±3.8 (41%)	40.7±2.6 (40%)	37.9±3.0 (17%)	2.35±0.07 (35%)
Carrageenan	Celecoxib	10	31.7±2.7	45.7±4.2 (45%)	52.9±3.1 (75%)	48.3±3.4 (56%)	42.5±2.9 (39%)	2.53±0.05 (17%)

A good extent of activity was already present after just 15 min. The results concerning (**3e**) show the maximum activity after 30 min (70% pressure reduction at 40 $mgkg^{-1}$) and then after 45 min (53% reduction) a decrease in activity. The same trend is observed for (**1d**) and (**1e**) (first generation), indicating that the pharmacokinetics plays a similar role whether or not an amino group is present on the side chain.[37] As observed in the writhing test, the solubility enhancement of compound (**3e**) seems to be favourable as this compound is more active than the corresponding parent molecules (**1e**) (**Figure 19**).[38]

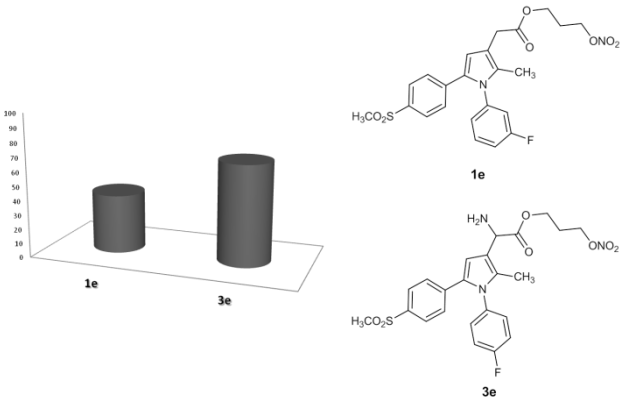


Figure 19. Comparison of paw pressure reduction between (**9e**) and (**13e**) (40$mgkg^{-1}$ dosage)

The same cannot be said for (**3d**) which showed complete lack of activity (data not reported). Moreover, (**3e**) is endowed with an activity which is very close to that displayed by (**1d**).[38]

Vasorelaxing effect. The vasorelaxing efficacy of compounds belonging to the second generation is definitely improved and can be accounted for by the enhanced solubility due to the introduction of the amino moiety (**Table 12**). Nevertheless, compound (**1d**) (first generation) is endowed with the best potency profile.[38]

Table 12. Vasorelaxing activity of compounds (**3d-e**) in comparison with their first generation homologues **(1d-f**)

Compound	*E max*	*pIC_{50}*	*+ODQ 1μM*
1d	69.0±4.0	6.48±0.06	+
1e	39.0±1.0	n.c.	+
1f	30±1.0	n.c.	+
3d	80.0±1.9	5.95±0.05.	~
3e	84.0±2.0	5.66±0.03	~
3f	93.0±1.3	5.81±0.01	~

These compounds show a unique behaviour because their vasorelaxing properties are not completely *NO*-dependent. This is shown in **Figure 19**, where the vasorelaxing activity is partially detected even when ODQ is present (especially for **3f**), suggeting a different mechanism. Investigation of this alternative mechanism is outside the scope of this research dissertation.

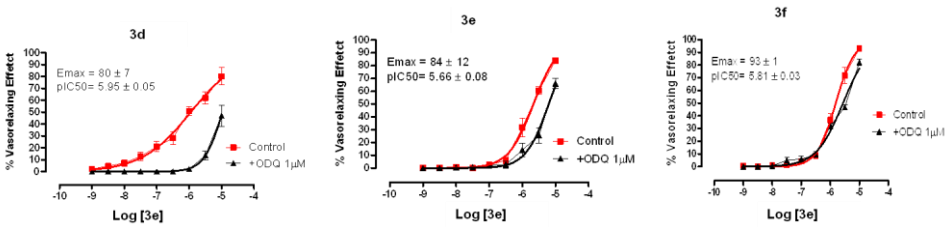


Figure 20. Response vs. concentration curves for the vasorelaxing activity of (**3d-f**)

Stability assessment. Even though the introduction of the amino group doesn't reduce the activity *in vivo*, the drop of activity in the cell-based assay (which may exclude the activity on COXs), the potential interaction with other vasorelaxing routes (other than the *NO*-dependent one) and the "high" metabolic sensitivity to esterases (**Table 13**) could be considered negative features of this compound series.

Table 13. Stability assessment for compound **3e**

Compound	***Time***	***Parent molecule remained (%)***		
		PBS (pH 7.4)	***SGF (pH 1.5)***	***Rat Plasma***
3e	*30 min*	75.0	100	0.00
	60 min	75.0	100	0.00
	120 min	75.0	100	0.00

In a stability assessment, (**13e**) exhibited a moderate stability in PBS and high stability in SGF, meaning that the introduction of the α-amino moiety reduces the sensitivity to non-enzymatic hydrolysis.

Conclusions. This class of molecules proved to be more soluble and the vasorelaxing effect associated with their administration was far better than for the previous class of compounds. However, the reduced interaction with the enzyme *in vitro* (IC_{50} ranging in the millimolar concentration) and the absence of substantial increase of the *in vivo* activity led us to undertake further structural modifications of these molecules.

Three generations of amides: improving stability whilst keeping solubility

(Biava, M.; Battilocchio, C.; Poce, G.; Alfonso, S.; Consalvi, S.; Porretta, G.C.; Schenone, S.; Calderone, V.; Martelli, A.; Testai, L.; Ghelardini, C.; Di Cesare Mannelli, L.; Sautebin, L.; Rossi, A.; Giordani, A.; Patrignani, P.; Anzini, M. *J. Med. Chem. Submitted for publication* Ref. 41)

The chemical and enzymatic sensitivities of the first two generations of esters were speculated to be responsible for the gap between the biological and pharmacological issues.[38, 40, 41]

Another class of compounds was generated through bioisosteric replacement of the ester moiety with the amide one. We had previously identified a need to generate more stable and soluble derivatives in order to enhance the pharmacokinetics. In particular, we designed and developed a subclass of "linear" acetic amides (first generation amides), "serine/homoserine" acetic amides (second generation amides) and glycine amides (third generation amides) (**Figure 21**).

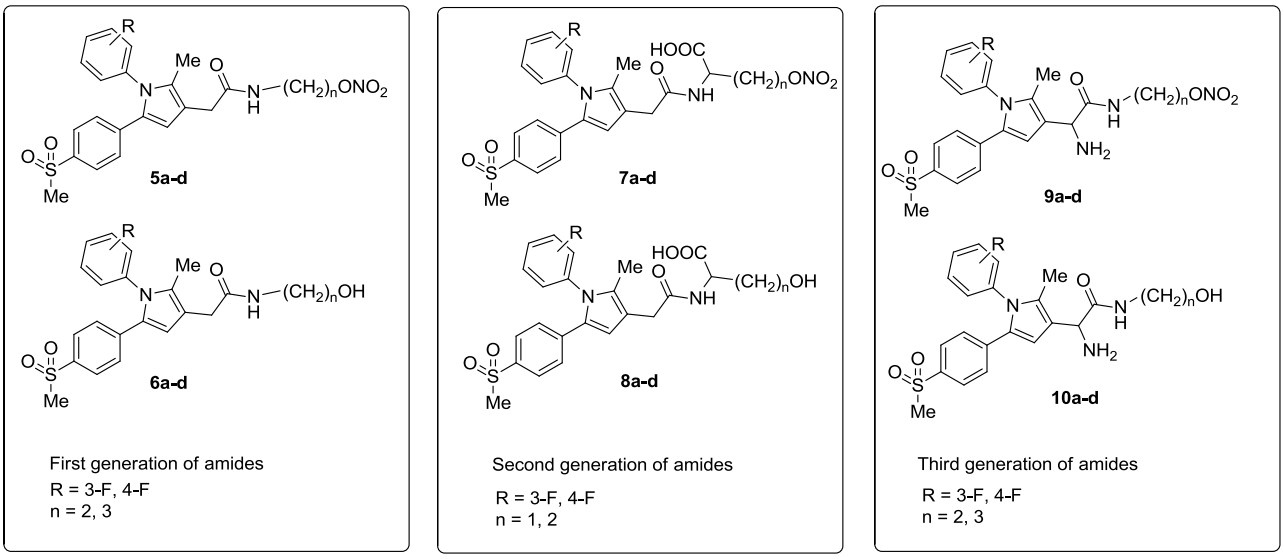


Figure 21. Structures of amides

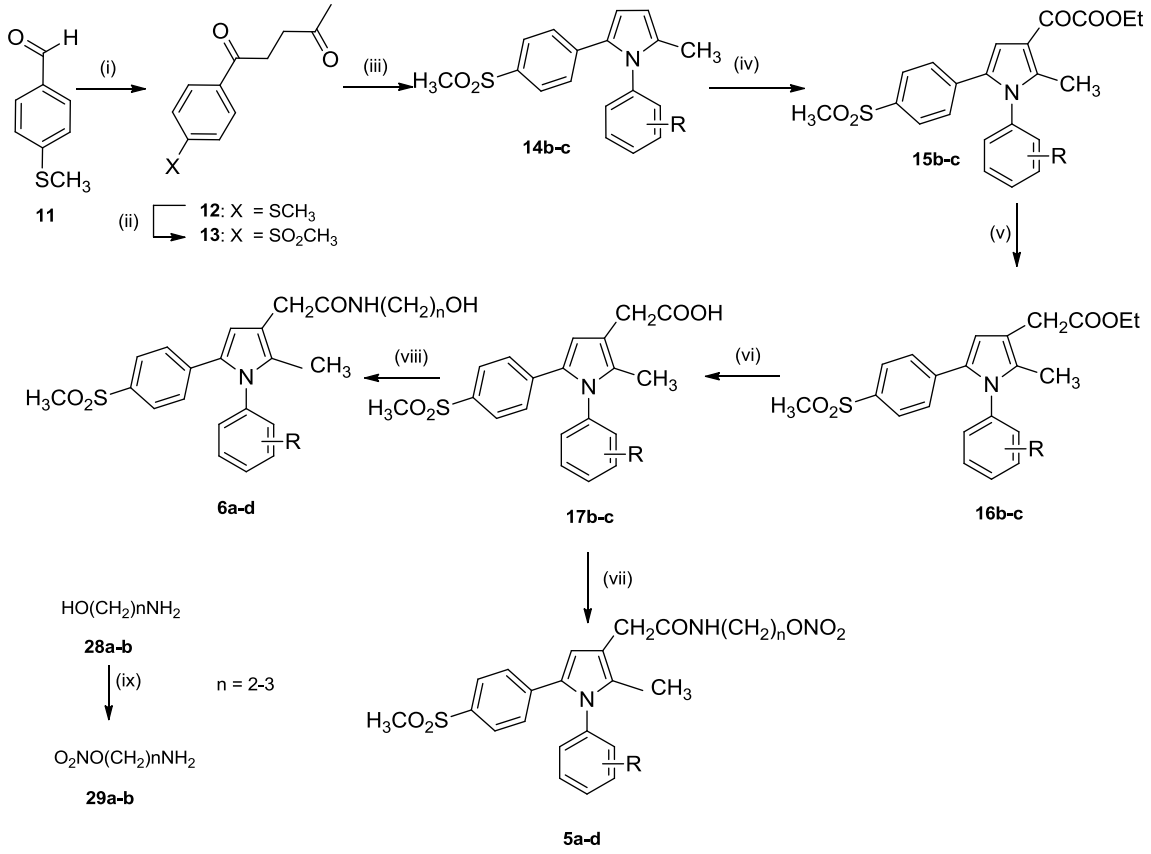
Due to the presence of an amino group which is protonated at physiological pH, the glycine derivatives were expected to better penetrate into the cartilage. Indeed, positively charged compounds are reported to better diffuse into the cartilage matrix due to electrostatic interaction with the negatively charged GAG-sulphates.[42]

The substitution pattern of compounds was based on the results obtained during the previous studies, where it was clearly underlined that a fluorine substituent on the N1 phenyl ring (*meta* or *para*) was associated to the best bio-pharmacological profiles.[32,38,40] In the glycine amides series, only the *meta* fluoro substituent was selected.

The phenyl ring in C5 of the central core presented a methansulfonyl group which has been proven to be responsible for selectivity towards COX-2. In order to understand the importance of the *NO* release (nitric oxide dependent effects), nitro-free derivatives were synthesised and tested accordingly.

Chemistry. The first generation of amides was obtained as shown in the scheme below (**scheme 3**). Access to the acetic acid derivative (**17b-c**) was achieved through a first Stetter "umpolung" reaction, followed by an oxidation using oxone, a Paal-Knorr condensation to get to the pyrrole ring (**14b-c**), a titanium-catalysed regioselective acylation, a reduction with triethylsilane and a final saponification.[32,38,40] The nitro-oxy compounds (**5a-d**) were obtained using EDCI as activating agent, DMAP as covalent nucleophilic catalyst and then the nitro-oxyalkyl amines (**29a-b)**. The nitro-oxyalkyl amines (**29a-b**) were obtained *via* nitration of amino alcohols (**28a-b**) employing fuming nitric acid in dichloromethane and then precipitated as nitrated salts after addition of acetic anhydride.[43] The nitro-free derivatives (**6a-d**) are obtained *via* a EDCI/HOBt coupling system in order to discriminate the nucleophilicity of the oxygen and the nitrogen.

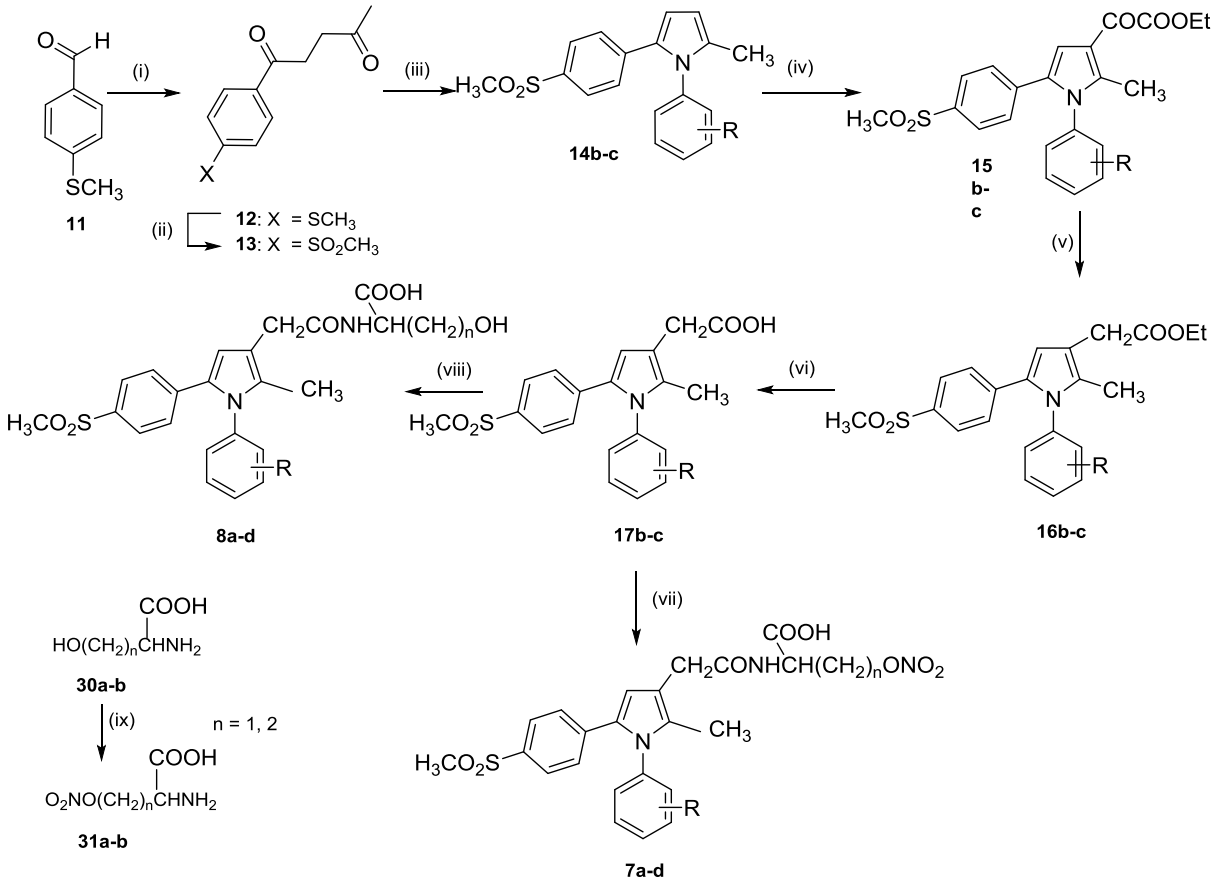
Scheme 3. Synthesis of acetic acid derivatives bearing a non-ionisable side chain



Reagents and conditions. (i) CH_2=CHCOMe, TEA, 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, MW, 15 min; (ii) Oxone, MeOH/H_2O, rt, 2 h; (iii) $RPhNH_2$, *p*-toluensulfonic acid, EtOH, MW, 45 min; (iv) EtOCOCOCl, $TiCl_4$, CH_2Cl_2, rt, 4 h; (v) Et_3SiH, TFA, rt, 2 h; (vi) 1N NaOH, MeOH, 1 h; (vii) **29a-b,** EDCI, DMAP, TEA, CH_2Cl_2, rt, 3h;(viii) **28a-b**, HOBt, EDCI, TEA, acetonitrile, rt, 3h; (ix) HNO_3 fuming , CH_2Cl_2, 1h, then $(CH_3CO)_2O$, 45 min.

A second generation of amides was obtained following the same route to the acid derivatives (**17b-c**) as shown in the previous scheme. Activation of the acid derivative with benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and subsequent reaction with the amino acid (**30a-b** and **31a-b**) gave access to the final molecules (nitro-oxy **7a-d** and nitro-free **8a-d**). Compounds (**31a-b**) were obtained following the same nitrating procedure described for compounds (**29a-b**) (**scheme 3**).[43] The amino acids (**30a-b**) used were racemic serine and homoserine (**scheme 4**).

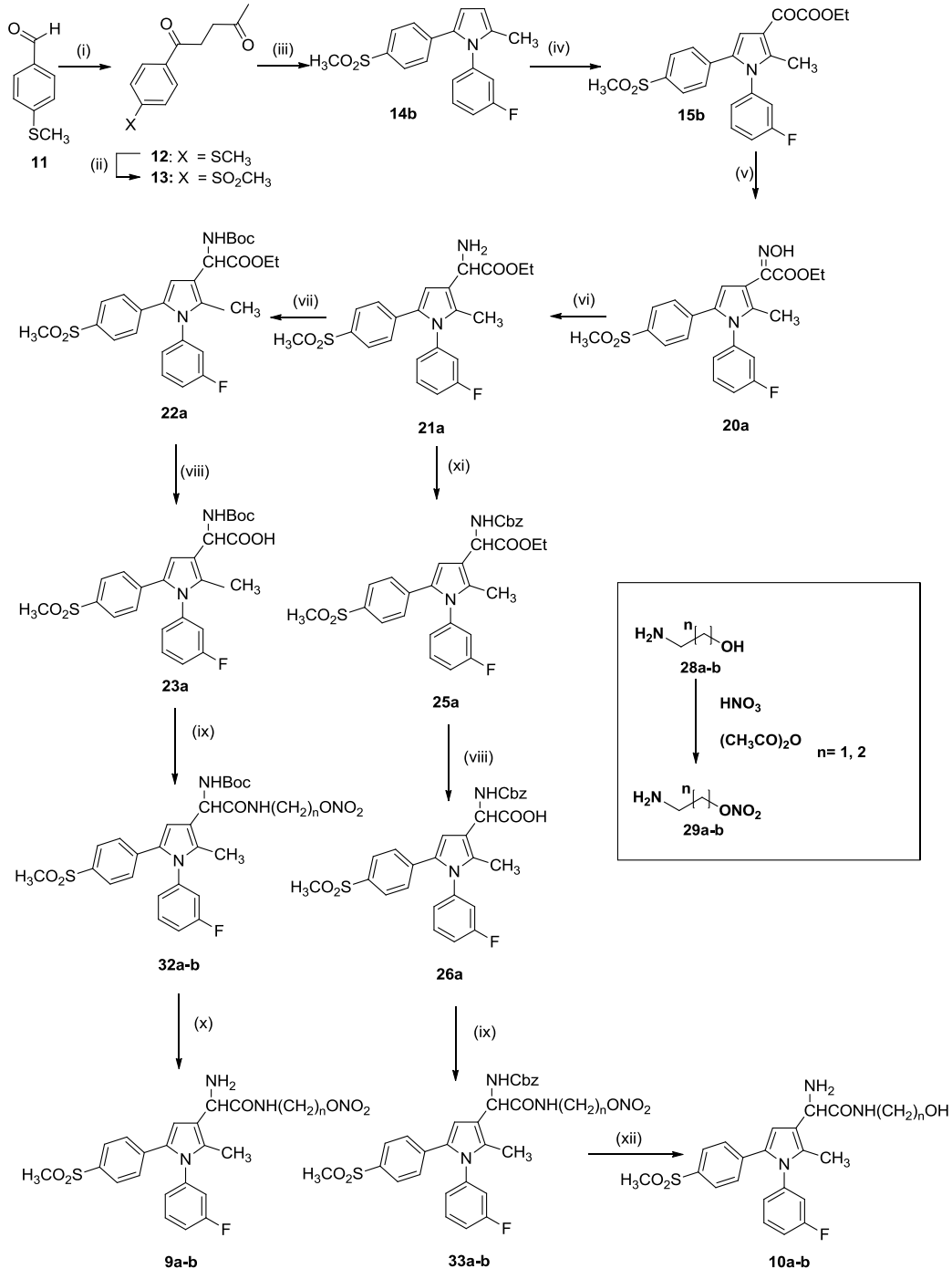
Scheme 4. Synthesis of acetic acid derivatives bearing an amino acid side chain



Reagents and conditions. (i) CH_2=CHCOMe, TEA, 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, MW, 15 min; (ii) Oxone, MeOH/H_2O, rt, 2 h; (iii) $RPhNH_2$, *p*-toluensulfonic acid, EtOH, MW, 45 min; (iv) EtOCOCOCl, $TiCl_4$, CH_2Cl_2, rt, 4 h; (v) Et_3SiH, TFA, rt, 2 h; (vi) 1N NaOH, MeOH, 1 h; (vii) **31a-b,** PyBop, TEA, CH_2Cl_2, rt, 3h;(viii) **30a-b**, PyBop, TEA, acetonitrile, rt, 3h; (ix) HNO_3 fuming , CH_2Cl_2, 1h, then $(CH_3CO)_2O$, 45 min.

Glycine derivatives were prepared using a common route to derivative (**21a**) and then following two different strategies for the generation of nitro-oxy and nitro-free compounds (**scheme 5**).[40] Starting from the intermediate (**15b**) already described in the previous schemes, the installation of the amino functionality was achieved *via* oxime formation and then reduction to the glycine ethyl ester to afford (**21a**). A subsequent protection using two different protecting groups (Boc for **22**, Cbz for **25**), hydrolysis of the ethyl ester and coupling gave access to compounds (**32a-b**) and (**33a-b**). A final deprotection step yielded nitro-free (**10a-b**) and nitro-oxy compounds (**9a-b**).

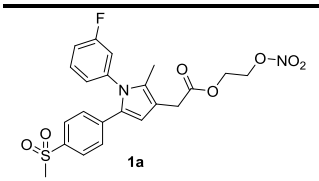
Scheme 5. Synthesis of acetic acid derivatives bearing an amino acid side chain

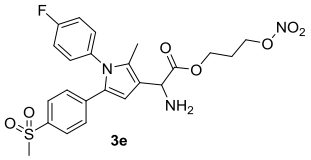


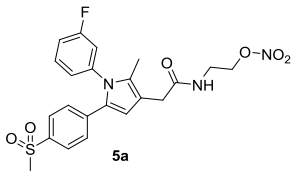
Reagents and conditions. (i) CH_2=CHCOMe, TEA, 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, MW, 15 min; (ii) Oxone, MeOH/H_2O, rt; (iii) $RPhNH_2$, *p*-toluensulfonic acid, EtOH, MW; (iv) EtOCOCOCl, $TiCl_4$, CH_2Cl_2, rt; (v) NH_2OH, NaAcO, dioxane; (vi) Zn, HCOOH; (vii) $(Boc)O_2$;(viii) NaOH, MeOH; (ix) **29a-b**, EDCI, DMAP, TEA, DCM; (x) TFA, DCM; (xi) CbzCl, Na_2CO_3, MeOH; (xii) Pd/c, NH_4COO, isopropanol.

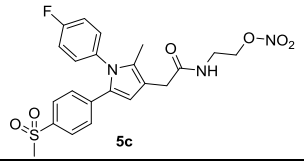
Solubility assessment. As expected the replacement of the ester functionality with the amide one gave rise to more soluble molecules.

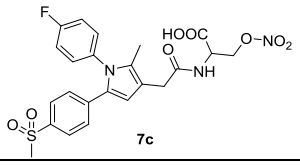
Table 14. Solubility assessment in SGF and PBS for compounds (**1d**), (**3e**), (**5a**), (**5c**), (**7c**) and **(9a**)

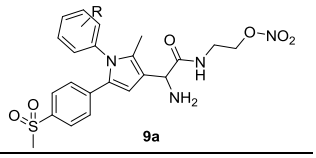












Compound	SGF (pH 1.5) (μM)	PBS (pH 7.4) (μM)
1d	<1	1.6
3e	>200	80.0
5a	150.0	>200
5c	147.0	>200
7c	112.0	90.5
9a	>200	>200

Surprisingly, the introduction of a carboxylic acid moiety (compound **3c**) was endowed with a lower extent of solubility (**Table 14**). This could be due to a participation of the carboxylic acid moiety to intramolecular H-bonding networks that stabilise the folding of the molecule leading to reduced solubility. On the other side "linear" amides showed good solubility in both SGF and PBS. However, the best solubility profile was displayed by the glycine amide (**9a**) whose solubility was higher than 200μM.

Stability in simulated gastric fluid (SGF-without pepsin), phosphate buffered solution (PBS) and rat plasma. Stability studies on (**1d**) and (**3e**) revealed the expected sensitivity of these structures to any metabolic pathway. However, it is interesting pointing out that the presence of the amino moiety, probably due to its steric hindrance or electronics conferred more stability, especially in SGF (100% of parent compound detected after 120 minutes). The effect of the bioisosteric replacement reduced dramatically the metabolic liability. The presence of an amide bond was indeed responsible of better stability especially for compounds belonging to the first and third subclass of amides (**5a** and **9a**) (**Table 15**). In our conditions, compound (**5a**) was insensitive to any form of metabolic stress, whereas compound (**9a**) is relatively more sensitive in rat plasma (even though after 120 min of incubation 77% of the compound can still be detected).

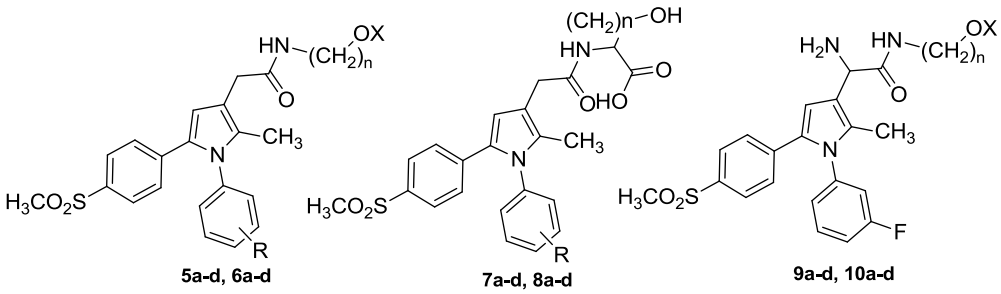
Table 15. Stability assessment in SGF and PBS for compounds (**1d**), (**3e**), (**5a**), (**7a**) and (**9a**)

Compound	Time	Parent molecule remained (%)		
		PBS (pH 7.4)	SGF (pH 1.5)	Rat Plasma
1d	30 min	74.1	80.2	0.00
	60 min	53.6	65.7	0.00
	120 min	0.15	42.8	0.00
3e	30 min	75.0	100	0.00
	60 min	75.0	100	0.00
	120 min	75.0	100	0.00
5a	30 min	100	100	100
	60 min	100	100	100
	120 min	100	100	100
7a	30 min	75.0	100	71.4
	60 min	50.0	93.8	57.1
	120 min	37.5	87.5	28.6
9a	30 min	100	100	94
	60 min	100	100	93
	120 min	100	100	77

The higher profile of sensitivity of the serine derivative (**7a**) could be explained by intra-molecular interactions due to the presence of the carboxylic acid moiety, that could ease any chemical transformation on the side chain (enzyme mediated or not).

In vitro COX-2 inhibition. Linear derivatives (**5a-d** and **6a-d**) displayed an average inhibition towards COX-2 of 0.2-0.3 μM. Surprisingly, derivatives 6b and 6d (which are nitro-free compounds p-fluoro substituted) exhibited inhibitions in the nanomolar range that could be due to a non-specific cooperation between the N1 substitution and the hydroxyl chain, allowing very strong and effective interactions with the enzyme. As for the nitro-oxy amino acid derivatives, the data clearly show that serine derivatives are more active *in vitro* than the homoserine ones. The nitro-free compounds (**8a**) and (**8d**) displayed outstanding inhibitions against the COX-2 isoform. Low COX-2 inhibitory potencies and high selectivity values are obtained within the third generation of amides (**9a-b** and **10a-b**), demonstrating that the negative effect obtained *in vitro* with the previous class of glycine esters was due probably to a mismatch-interaction rather than to the presence of the amino group itself (**Table 16**).

Table 16. In vitro COXs inhibition and selectivity for compounds (**5a-d**), (**6a-d**), (**7a-d**), (**8a-d**), (**9a-b**) and (**10a-b**)



5a-d, 6a-d 7a-d, 8a-d 9a-d, 10a-d

Compound	R	X	n	% inhib COX-1 (10 μM)	% inhib COX-2 (10 μM)	IC_{50} (COX-1) (μM)	IC_{50} (COX-2) (μM)	COX-1/ COX-2 (S.I.)[a]
5a	3-F	NO_2	2	0	95	>10	0.300	>33
5b	3-F	NO_2	3	0	100	>10	0.250	>40
5c	4-F	NO_2	2	0	100	>10	0.240	>42

5d	4-F	NO_2	3	0	100	>10	0.310	>32
6a	3-F	H	2	0	95	>10	0.240	>34
6b	3-F	H	3	21	100	>10	0.045	>220
6c	4-F	H	2	0	95	>10	0.300	>33
6d	4-F	H	3	17	100	>10	0.068	>147
7a	3-F	NO_2	1	0	100	>10	0.140	>70
7b	3-F	NO_2	2	27	43	>10	n.d.	n.d.
7c	4-F	NO_2	1	0	100	>10	0.310	>32
7d	4-F	NO_2	2	6	89	>10	1.600	>6.2
8a	3-F	H	1	23	100	>10	0.057	>167
8b	3-F	H	2	16	79	>10	n.d.	n.d.
8c	4-F	H	1	23	100	>10	0.160	>62.5
8d	4-F	H	2	16	79	>10	0.086	>116
9a	-	NO_2	2	10	90	>10	0.054	>185
9b	-	NO_2	3	10	86	>10	0.140	>71
10a	-	H	2	10	25	>10	n.d.	n.d.
10b	-	H	3	10	83	>10	0.047	>200

[a] Selectivity index: IC_{50} COX-1 vs. IC_{50} COX-2 ratio.

Writhes reduction in the acetic acid-induced abdominal constrictions in mice. All compounds showed very good activity in reducing the abdominal writhes induced by acetic acid (**Table 17**). Compounds belonging to the first generation of amides were endowed with a higher extent of reduction compared to the serine/homoserine derivatives. In particular, compound (**5c**) displayed a 78% of reduction when dosed at 20 mg/kg. On the other side, the introduction of the amino moiety (which is associated to great stability and solubility, as shown before) gave access to a compound with outstanding properties. Derivative (**9a**) was active at 3 mg/kg with a reduction of writhes around 18%; the reduction extent increases with the dose and reached 56% when the compound was administered at 10 mg/kg and reached a plateau of 80% when the dose was above 20 mg/kg, meaning that the maximum efficacy of this molecule had been reached already at 20 mg/kg. It is worth mentioning that nitro-free derivatives (**6c**) and (**8a**) displayed a lower extent of inhibition in this test when compared to the related nitro-oxy compounds (**5c**) and (**9a**) and this could account for a direct *NO* dependent effect. The only

exception was the couple (**7c**)/(**8c**) where the nitro-free compounds were more active than the nitro-oxy ones and this could be explained by different metabolic pathways of activation/disactivation.

Table 17. Writhes reduction for compounds **5a-d**, **6c**, **7a-d**, **8c**, **9a** and **9b**

Compound	n° mice	Dose *po* mg/kg^{-1}	n° writhes after 30 min	writhes reduction
CMC	43	-	32.6±2.1	-
5a	10	10	26.8±2.1	18%
	9	20	20.6±3.0	37%
	10	40	14.7±2.1	56%
5b	10	10	33.2±3.5	0%
	10	20	29.8±3.6	12%
	10	40	22.9±2.2	31%
5c	10	10	12.2± 2.3	62%
	10	20	7.4±1.3	78%
	10	40	8.1±1.2	75%
5d	10	3	33.3±3.2	0%
	9	10	20.4±2.2	37%
	10	20	21.6±2.8	34%
6c	10	10	20.7±3.5	37%
	11	20	12.6±2.7	62%
	9	40	12.5±2.2	62%
7a	10	10	28.3± 2.5	12%
	10	20	17.1±3.1	46%
	10	40	16.4±2.9	48%
7b	10	10	33.4±3.6	0%
	10	20	29.2±2.4	9%
	10	40	25.2±3.3	22%
7c	10	10	33.1± 3.5	0%
	10	20	23.2±2.3	28%
	11	40	16.3±3.2	50%
7d	9	10	22.8±3.0	31%
	11	20	14.6±2.7	56%
	10	40	14.7±2.8	56%
8c	9	10	23.7±3.0	28%
	10	20	13.4±2.8	59%
	10	40	12.9±2.1	62%
9a	10	3	25.1±2.3	18%
	10	10	14.2±2.9	56%
	10	20	6.7±2.0	80%
	11	40	6.5±2.1	81%
9b	11	3	31.6±2.1	3%
	10	10	30.5±2.6	6%
	10	20	21.4±3.7	34%
	9	40	18.8±3.1	43%
Celecoxib	13	10	13.5±3.0	61%

Reduction of hyperalgesia in the carrageenan induced inflammation and edema reduction.

Compound (**5c**) and (**9a**) were selected for a further assessment of their *in vivo* profile through the carrageenan-induced edema and hyperalgesia. Surprisingly, the data on the carrageenan induced inflammation showed that (**5c**) was associated with a good but not outstanding activity. Indeed, at 40 mg/kg the maximum activity was reached after 60 minutes with a reduction of hyperalgesia equating to 60%. A mere activity was found when testing the compound for its anti-edemigenic properties (10%). On the other hand, compound (**9a**) proved to be highly active with a reduction of hyperalgesia of 80% after just 30 minutes. The activity is maintained after 60 minutes (70%) and after 90 minutes a good reduction was still displayed (50%). After 2 hours, a residual analgesic activity could still be detected (26% reduction). An anti-edemigenic effect was observed with the administration of this compound at 20mg/kg. In these conditions the edema volume was reduced by about 76% (**Table 18**).

Table 18. Hyperalgesia and edema reduction in the carrageenan induced inflammation for compounds (**5c**) and (**9a**) in comparison with celecoxib

Pretreat. (i.pl.)	Comp.	Dose (mg/kg)	Before treatment	Paw-pressure				Edema volume (mL)
				After treatment				
				30min	60 min	90 min	120 min	
Saline	**CMC**	-	61.2±2.5	61.6±3.4	58.9±3.8	61.4±4.1	61.4±4.1	1.44±0.05
Carrageenan	**CMC**	-	36.1±3.3	32.8±2.9	30.6±3.2	31.8±2.2	31.8±2.2	2.71±0.08
Carrageenan	**5c**	20	33.8± 2.9	40.7±3.5 (28%)	42.8 ±3.4 (30%)	37.8 ± 2.6 (20%)	32.7± 2.6 (0%)	2.63 ± 0.05 (4%)
Carrageenan	**5c**	40	32.9±3.3	44.2±4.0 (32%)	48.3±4.6 (60%)	41.5 ± 3.7 (29%)	31.5± 2.6 (0%)	2.50 ± 0.09 (10%)
Carrageenan	**9a**	20	33.8± 2.9	55.2±3. (80%)	52.1±4.2 (70%)	47.3±3.7 (50%)	39.5±3.0 (26%)	1.79±0.09 (76%)
Carrageenan	**Celecoxib**	10	33.8± 2.9	55.2±3. (80%)	54.9±4.2 (78%)	49.3±3.4 (60%)	45.3±3.0 (40%)	1.83±0.07 (70%)

Ex vivo nitric oxide releasing properties. All nitro-oxy compounds showed *NO*-dependent vasorelaxing properties (**Table 19**). Derivatives belonging to the second subclass of amides (serine and homoserine derivatives, **7a-d**) were associated to a mere vasorelaxing effect. Compounds (**5a-c**) were endowed with very good efficacy and potency but the vasorelaxing activity of these compounds was lower than for the ester (**3e**). However, the introduction of the amide functionality within the glycine skeleton gave rise to a class of compounds with outstanding vasorelaxing properties (**9a** and **9b**).

Table 19. Vasorelaxing properties for compounds (**5a-c**), (**7a-c**) and (**9a-b**) in comparison with **Naproxcinod**, (**1d**) and (**3e**)

Compound	E max	pIC_{50}	+ODQ 1µM
Naproxcinod	68.0±3.0	6.33±0.06	+
1d	69.0±4.0	6.48±0.06	+
3e	80.0±1.9	5.95±0.05.	+
5a	49.0±2.0	n.c.	+
5b	69.0±8.0	5.78±0.07	+
5c	68.0±0.5	5.31±0.05	+
7a	18.0±9.0	n.c.	+
7b	25.0±2.0	n.c.	+
7c	25.0±1.9	n.c.	+
7d	42.0±9.0	n.c.	+
9a	74.1±1.0	5.38±0.20	+
9b	86.0±2.0	5.38±0.03	+

The kinetic of *NO* release was also investigated for compounds (**5c**) and (**9a**). The concentration over time curve of both compounds described a class of molecules which were endowed with a slow kinetic of *NO* release, which is absolutely important for the development of this kind of hybrids (**Figure 22**).

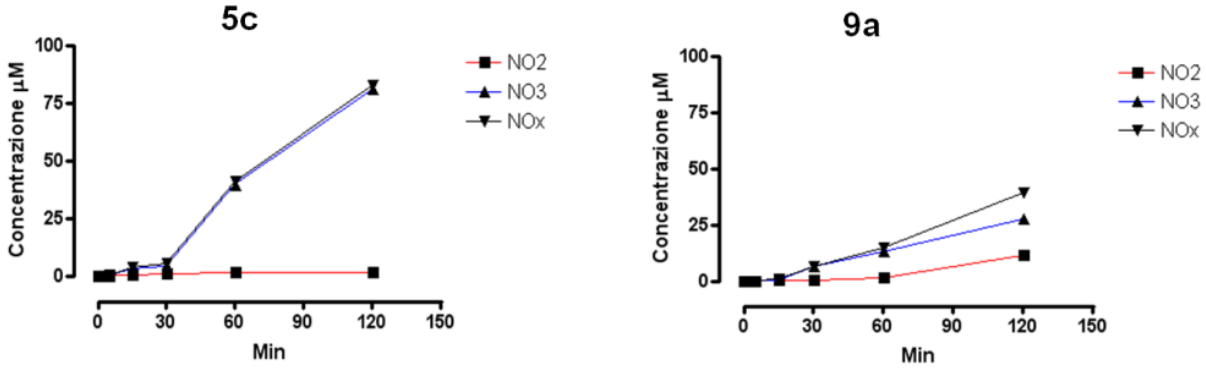


Figure 22. Time-dependent increase of the concentrations of NOx (nitrites + nitrates) following the incubation of 1 mM (**5c**) and (**9a**) in rat hepatic homogenate, containing the opportune cofactors

The first difference between the two compounds is that nitrated species were released faster with compound (**5c**). More interestingly, the other difference between the two compounds is that the overall amount of nitrated species for (**5c**) was generated by a direct release of nitrates, whilst for (**9a**) both nitrates and nitrites could be detected.

Human whole blood assay. *C*ompounds (**5c**) and (**9a**) were tested in the human whole blood assay.[36] Along with the nitro-oxy amides (**5c**) and (**9a**) the respective nitro-free derivatives (**6c** and **10a**) were studied in the human whole blood assay. Compound (**9a**) inhibited COX-2 activity in a concentration-dependent fashion with an IC_{50} value of 21.23 μM (**Table 20**).

Table 20. COX-1 and COX-2 IC_{50} values for (**9a**) and (**10a**) in the HWB assay

Compd	n	R	COX-1 IC_{50} μM (95% CI)	COX-2 IC_{50} μM (95% CI)	S.I.
9a	2	NO_2	>100	21.23 (15.17- 29.71)	>5
10a	2	H	> 100	144 (66-311)	<1
Celecoxib			12.47 (8.6 -17.9)	0.54 (0.29 – 0.52)	23

At 100μM, this compound inhibited COX-2 activity by 80.2%, while it inhibited only marginally COX-1 activity (**Figure 23**).

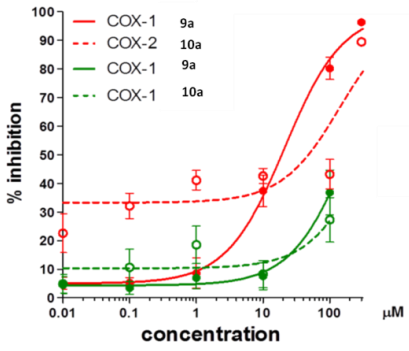


Figure 23. Inhibition/concentration curves for (**9a**) and (**10a**) in the HWB assay

COX-2 activity of the nitro-free derivative (**10a**) was characterised by an unusual concentration-response curve. In fact, up to 100 µM, COX-2 activity was inhibited in a concentration-dependent fashion reaching a maximal inhibitory effect of 43%. At 100 µM, COX-1 activity was only marginally inhibited.

Compound (**5c**) caused a concentration dependent inhibition of COX-1 and COX-2 activities with a respective IC_{50} of 0.8 and 3.2 µM, respectively. Surprisingly, this compound was 4-fold more potent towards COX-1 than COX-2. Its nitro-free derivative (**6c**) inhibited COX-1 and COX-2 activities with similar IC_{50} values of 1.9 and 1.4 µM, respectively (**Figure 24** and **Table 21**).

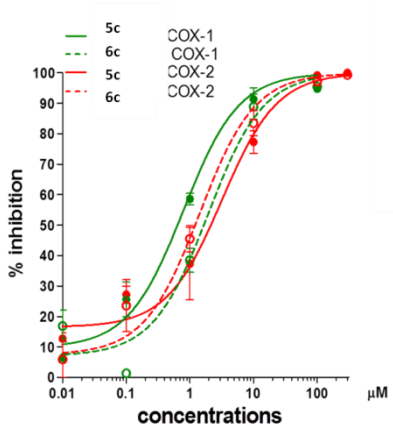


Figure 24. Inhibition/concentration curves for (**5c**) and (**6c**) in the HWB assay

Table 21. COX-1 and COX-2 IC_{50} values for (**1c**) and (**2c**) in the HWB assay

Compd	n	R	COX-1 IC_{50} µM (95% CI)	COX-2 IC_{50} µM (95% CI)	S.I.
5c	2	NO_2	0.8 (0.5 - 1.3)	3.2 (1.8 - 5.6)	0.23
6c	2	H	1.9 (1.1 - 3.3)	1.4 (0.9 - 2.3)	1.33
Celecoxib			12.47 (8.6 -17.9)	0.54 (0.29 – 0.52)	23

As shown in **Table 21**, (**5c**) and (**6c**) inhibited COX-1 activity with similar IC_{50} values. The IC_{50} values for the inhibition of COX-2 activity by (**5c**) and its derivative were not significantly different.

Conclusions. The replacement of the ester moiety with an amide one gave access to a stable and soluble class of hybrid compounds. Nitro-oxy derivatives highlighted a very good COX-2 inhibiting feature both in rats and in humans. The *NO*-dependent vasorelaxing properties and the slow kinetic release of *NO* make these compounds an ideal class of pharmacodynamic hybrids. Among all derivatives, (**5a**) is endowed with an outstanding *in vivo* profile (comparable and in some extent superior to celecoxib). It is quite interesting to observe in the HWB assay that (**5a**) is a selective inhibitor of COX-2 and that its potential metabolite (**6a**) is endowed with very low affinity towards COX-2. This finding could mean that (**5a**) can efficiently inhibit COX-2 and release *NO* whereas, when transformed to its metabolite, it loses its activity towards COX-2.

Conclusions

A novel class of molecules that inhibits selectively COX-2 has been developed. These molecules proved to be effective nitric oxide donors and the biopharmacological tests have shown them to be potential analgesic and anti-inflammatory agents.

Further work will focus on developing methabolically stable analogues (**Figure 25**). The future plan is to conjugate the identified molecular core with an adamantyl group. It has been proven that the adamantyl moiety, beyond increasing the partition coefficient of molecules when introduced, positively modulates the activity of therapeutics. One of the most outstanding features displayed by this group is the metabolic stabilization towards hydrolytic enzymes due to its steric hindrance.

The 2-aminoadamantane 2-carboxylic acid core is a very challenging moiety to achieve. Although in the literature two synthetic pathways are described, both of them have been proven problematic in our hands. Though commercially available, this amino acid is extremely expensive. We developed a novel economic and scalable synthesis for this amino acid which is described in the following section.

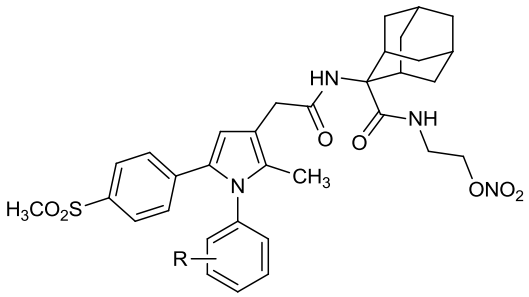
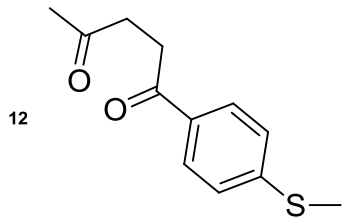


Figure 25. Representative structure of adamantyl-bearing molecules

Experimental procedures

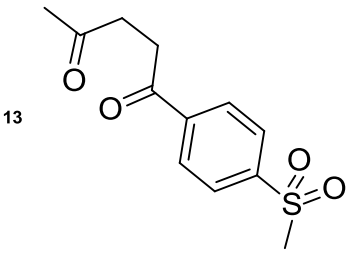
Chemistry. All chemicals used were of reagent grade. Yields refer to purified products and are not optimized. A CEM Discovery microwave system apparatus was used for the Stetter and Paal–Knorr reactions. Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Microanalyses were performed on a Perkin-Elmer 240C or a Perkin-Elmer series II CHNS/O analyzer 2400 instrument. Sigma-Aldrich silica gel 60 (230–400 mesh) was used for column chromatography. Merck TLC plates (silica gel 60 F 254) were used for thin-layer chromatography (TLC). Sigma-Aldrich aluminum oxide (activity II–III, according to Brockmann) was used for chromatographic purifications. Sigma-Aldrich Stratocrom aluminum oxide plates with a fluorescent indicator were used for TLC to check the purity of the compounds. ^{13}C-NMR and ^{1}H-NMR spectra were recorded with a Bruker AC 400 spectrometer in the indicated solvent (TMS as the internal standard). The values of the chemical shifts are expressed in ppm and the coupling constants (J) in hertz. The purity of the title compounds was assessed by means of a Waters Alliance 2695 instrument equipped with an UV–vis Waters PDA 996 as the detector and working at 333 nm. Millennium Empower with Windows XP was used. A Phenomenex LUNA C8, 5µm (150 mm×4.6 mm) column (code 00F-4249-E0) at 40°C was used as the chromatographic column at a flow rate of 1.0 mL/min. The eluent was (A) a 65/15/20 (v/v/v) 10 mM (pH 2.5) KH_2PO_4/ MeOH/acetonitrile mixture or (B) a 15/75/10 (v/v/v) 10 mM (pH 2.5) KH_2PO_4/MeOH/acetonitrile mixture. A gradient from 100% A to 100% B was run in 25 min, and then isocratic conditions at 100% B were maintained for an additional 10 min. Using the analytical conditions reported above, the retention times of the compounds range between 7.44 and 17.25 min. All the synthesized compounds were generally more than 95% pure. Elemental analyses were determined at the ISS (Istituto Superiore della Sanita') and are in accordance with the theoretical values within a tolerance of ±0.3%.

1-[4-(Methylthio)phenyl]pentane-1,4-dione (12).



4-Methyl-thiobenzaldehyde (**11**) (11.97 mL, 90 mmol), triethylamine (19.5 mL, 600 mmol), methyl vinyl ketone (5.8 mL, 90 mmol), and 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (14 mmol) were microwave irradiated in a 80 mL vessel for 15 min (150 W, 70°C, 170 psi). The mixture obtained was treated with 2 N HCl (10 mL) until pH 2 was reached. After extraction with ethyl acetate, the organic layer was washed with aqueous sodium bicarbonate and water. The organic fractions were dried over sodium sulfate, filtered, and concentrated to give a crude orange liquid. After crystallization from cyclohexane, intermediate (**12**) was isolated. White needles (78% yield), m.p. 75 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.87 (d, 2H, *J* = 8.0 Hz), 7.26 (d, 2H, *J* = 8.0 Hz), 3.23 (t, 2H, *J* = 7 Hz), 2.87 (t, 2H, *J* = 7.0 Hz), 2.52 (s, 3H), 2.25 (s, 3H); Anal. ($C_{12}H_{14}O_2S$) C, H.

1-[4-(Methylsulfonyl)phenyl]pentane-1,4-dione (13).

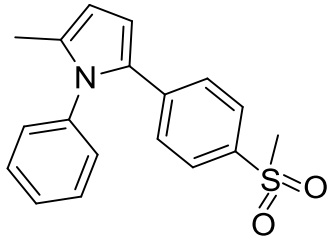


A solution of Oxone (61.4 mmol) in water (150 mL) was added dropwise over 15 min to a solution of (**12**) (35 mmol) in methanol (250 mL). The reaction mixture was stirred for 2 h at room temperature, then diluted with water (400 mL) and extracted with dichloromethane. The organic layer was washed with brine (200 mL), water (200 mL) and dried over sodium sulfate. Filtration and concentration under reduced pressure, give access to compound (**13**). White

crystalline solid (>92% yield), m.p. 138-140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.16 (d, 2H, *J* = 8.0 Hz), 8.06 (d, 2H, *J* = 8.0 Hz), 3.28 (t, 2H, *J* = 7.0 Hz), 3.08 (s, 3H), 2.96 (t, 2H, *J* = 7.0 Hz), 2.26 (s, 3H); Anal.($C_{12}H_{14}O_4S$) C, H.

1-Phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrole (14a).

14a



Following the Paal–Knorr condensation conditions, p-(methylthio)aniline (2.28 mmol) and p-toluenesulfonic acid (0.17 mmol) were added to a solution of compound (**13**) (2.28 mmol) in ethanol (2 mL). were added. The flask was inserted into the cavity of the microwave system apparatus and heated (150 W for 45 min, temperature 160°C, pressure 150 psi). The reaction mixture was cooled to room temperature and subsequently concentrated. The crude material was purified by chromatography on aluminum oxide using cyclohexane/ethyl acetate, 3:1 (v/v), as the eluent to give the expected 1,5-diarylpyrroles solids in satisfactory yields. Yellowish needles (79% yield), mp 105–110°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.78 (d, 2H, *J* =8.6 Hz), 7.26 (d, 2H, *J* = 8.5 Hz), 7.13-7.06 (m, 5H), 6.39 (d, 1H, *J* = 5.0 Hz), 6.11 (d, 1H, *J* = 5.0 Hz), 3.01 (s, 3H), 2.08 (s, 3H); Anal.($C_{18}H_{17}NO_2S$) C, H, N.

1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrole (14b). Yellowish needles (76% yield), m.p. 106–110 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.80 (d, 2H, *J* = 8.6 Hz), 7.48 (d, 2H, *J* = 8.5 Hz), 7.20 (m, 1H), 7.04 (m, 2H), 6.41 (d, 1H, *J* = 5.0 Hz), 6.12 (d, 1H, *J* = 5.0 Hz), 3.06 (s, 3H), 2.09 (s, 3H); Anal.($C_{18}H_{16}NO_2SF$) C, H, N.

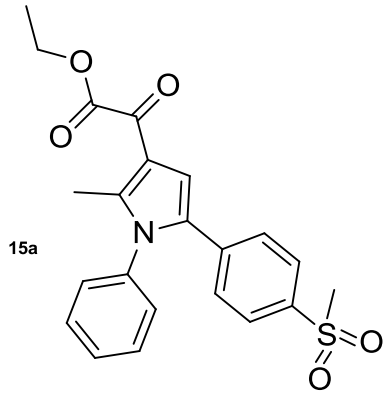
1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrole (14c). Yellowish needles (78% yield), m.p. 110–112 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.71 (d, 2H, *J* =8.6

Hz), 7.46 (d, 2H, *J* = 8.5 Hz), 7.23 (d, 2H, *J* = 8.6 Hz), 7.02 (d, 2H, *J* = 8.5 Hz), 6.38 (d, 1H, *J* = 3.0 Hz), 6.11 (d, 1H, *J* = 3.0 Hz), 3.01 (s, 3H), 2.06 (s, 3H); Anal.($C_{18}H_{16}NO_2SF$) C, H, N.

1-[(4-Methylthio)phenyl]-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrole (14d). Yellowish needles (79% yield), m.p. 100–103 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.77 (d, 2H, *J* =8.6 Hz), 7.46 (d, 2H, *J* = 8.5 Hz), 7.17 (d, 2H, *J* = 8.6 Hz), 6.98 (d, 2H, *J* = 8.5 Hz), 6.39 (d, 1H, *J* = 3.0 Hz), 6.11 (d, 1H, *J* = 3.0 Hz), 3.01 (s, 3H), 2.51 (s, 3H), 2.06 (s, 3H); Anal.($C_{19}H_{19}NO_2S_2$) C, H, N.

1-[(4-Methoxy)phenyl]-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrole (**14e**). Yellowish needles (78% yield), m.p. 105–108 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.78 (d, 2H, *J* =8.6 Hz), 7.47 (d, 2H, *J* = 8.5 Hz), 7.20 (d, 2H, *J* = 8.6 Hz), 7.01 (d, 2H, *J* = 8.5 Hz), 6.40 (d, 1H, *J* = 3.0 Hz), 6.13 (d, 1H, *J* = 3.0 Hz), 3.88 (s, 3H), 3.06 (s, 3H), 2.09 (s, 3H); Anal.($C_{19}H_{19}NO_3S$) C, H, N.

Ethyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15a).



15a

Ethoxalyl chloride (4.82 mmol) and $TiCl_4$ (4.82 mmol) were added in sequence to a solution of pyrrole (**14a**) (4.82 mmol) in dry dichloromethane (5 mL), at 0°C under inert atmosphere. The solution was stirred for 4 h at room temperature and then quenched with ice water. The mixture was extracted with chloroform, and the organic layer washed with brine, dried under sodium sulfate and evaporated *in vacuo* to afford an oily mixture. Purification of this mixture by chromatography on silica gel employing petroleum ether/chloroform/ ethyl acetate, 4:3:1

(v/v/v), as the eluent gave a solid which, after recrystallization from hexane, afforded the expected product. Yellowish needles (77% yield), m.p. 105–110°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75 (d, 2H, *J* = 8.6 Hz), 7.49 (d, 2H, *J* = 8.5 Hz), 7.18-7-10 (m, 5H), 6.49 (s, 1H), 4.12 (q, 2H, *J* = 7.0 Hz), 3.09 (s, 3H), 2.11 (s, 3H), 1.43 (t, 3H, *J* = 7.0 Hz); Anal.($C_{22}H_{21}NO_5S$) C, H, N.

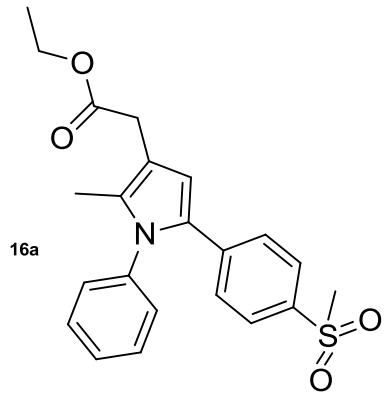
Ethyl 2-[1-[(3-fluoro)phenyl]-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15b). Yellowish needles (79% yield), m.p. 115-113 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.86 (d, 2H, *J* = 8.4 Hz), 7.43 (d, 2H, *J* = 8.4 Hz), 7.20 (m, 1H), 7.12 (m, 3H), 6.48 (s, 1H), 4.12 (q, 2H, *J* = 7.1 Hz), 3.08 (s, 3H), 2.11 (s, 3H), 1.39 (t, 3H, *J* = 7.1 Hz); Anal.($C_{22}H_{20}NO_5SF$) C, H, N.

Ethyl 2-[1-[(4-fluoro)phenyl]-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15c). Yellowish needles (77% yield), m.p. 113-115 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.79 (d, 2H, J = 8.5 Hz), 7.56 (d, 2H, *J* = 8.5 Hz), 7.20 (m, 2H), 7.10 (m, 2H), 6.98 (s, 1H), 4.12 (q, 2H, *J* = 7.1 Hz), 3.04 (s, 3H), 2.10 (s, 3H), 1.40 (t, 3H, *J* = 7.1 Hz); Anal.($C_{22}H_{20}NO_5SF$) C, H, N.

Ethyl 2-[1-[(4-methylthio)phenyl]-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15d). Yellowish needles, mp 105–110°C (yield 73%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.60 (d, 2H, *J* = 8.6 Hz), 7.25 (d, 2H, *J* = 8.5 Hz), 7.17 (d, 2H,J= 8.6 Hz), 7.03–6.99 (m, 3H,), 4.10 (q, 2H, *J* = 7.0 Hz), 3.00 (s, 3H), 2.54 (s, 3H), 2.09 (s, 3H), 1.55 (t, 3H, *J* = 7.0 Hz); Anal.($C_{23}H_{23}NO_5S_2$) C, H, N.

Ethyl 2-[1-[(4-methoxy)phenyl]-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]glyoxylate (15e). Yellowish needles (yield 71%), mp 100–103°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.84 (d, 2H, *J* = 8.6 Hz), 7.50 (d, 2H,J = 8.5 Hz), 7.23 (d, 2H, *J* = 8.6 Hz), 7.11 (d, 2H, *J* = 8.6 Hz), 6.99 (s, 1H), 4.11 (q, 2H, *J* = 7.0 Hz), 3.88 (s, 3H), 3.00 (s, 3H), 2.09 (s, 3H), 1.45 (t, 3H, *J* = 7.0 Hz); Anal.($C_{23}H_{23}NO_6S$) C, H, N.

Ethyl 2-[1-pheny-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (16a).



16a

Triethylsilane (0.75 mL, 4.7 mmol) was added slowly to a solution of 1,5-diarylpyrrol-3-glyoxylic ester (**15a**) (2.3 mmol) in trifluoroacetic acid (9 mL, 0.12 mol), stirred at 0°C under nitrogen atmosphere. The mixture was reacted for 2 h at room temperature and then the pH adjusted to 12m using a 40% aqueous ammonia solution. The mixture obtained was extracted with chloroform. The organic solution was dried over sodium sulfate, filtered, and evaporated in vacuo. The resulting residue was purified by chromatography on silica gel using petroleum ether/chloroform/ethyl acetate, 4:3:1 (v/v/v), as the eluent to give a solid which, after recrystallization from ethanol, afforded compound (**16a**) Yellowish needles (77% yield), m.p. 123−127°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.88 (d, 2H, *J* = 8.6 Hz), 7.47 (d, 2H, *J* = 8.5 Hz), 7.20-7.13 (m, 5H), 6.46 (s, 1H), 4.10 (q, 2H, *J* = 7.0 Hz), 3.51 (s, 2H), 3.00 (s, 3H), 2.54 (s, 3H), 2.09 (s, 3H), 1.55 (t, 3H, J = 7.0 Hz); Anal.($C_{22}H_{23}NO_4S$) C, H, N.

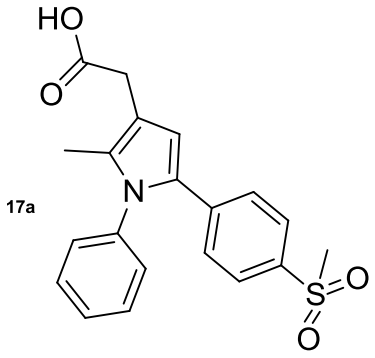
Ethyl-2-methyl-1-[3-(fluoro)-phenyl]-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-acetate (16b). Yellowish needles (73% yield), m.p. 133 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.90 (d, 2H, *J* = 8.4 Hz), 7.50 (d, 2H, *J* = 8.4 Hz), 7.23 (m, 1H), 7.10 (m, 3H), 6.32 (s, 1H), 4.12 (q, 2H, *J* = 7.1 Hz), 3.38 (s, 2H), 3.04 (s, 3H), 2.10 (s, 3H), 1.34 (t, 3H, *J* = 7.1 Hz). Anal.($C_{22}H_{22}NO_4SF$) C, H, N.

Ethyl-2-methyl-1-[4-(fluoro)-phenyl]-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-acetate (16c). Yellowish needles (71% yield), m.p. 133 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.99 (m, 2H), 7.76 (m, 2H), 7.20 (m, 2H), 7.00 (m, 2H), 6.30 (s, 1H), 4.12 (q, 2H, *J* = 7.1 Hz), 3.39 (s, 2H), 2.84 (s, 3H), 2.16 (s, 3H), 1.30 (t, 3H, *J* = 7.1 Hz); Anal.($C_{22}H_{22}NO_4SF$) C, H, N.

Ethyl-2-methyl-1-[4-(methylthio)-phenyl]-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-acetate (16d). Yellowish needles (76% yield), m.p. 123-127 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.83 (d, 2H, *J* = 8.6 Hz), 7.49 (d, 2H, *J* = 8.5 Hz), 7.21 (d, 2H, *J* = 8.6 Hz), 7.05 (d, 2H,J= 8.5 Hz), 6.43 (s, 1H), 4.10 (q, 2H, *J* = 7.0 Hz), 3.51 (s, 2H), 3.00 (s, 3H), 2.54 (s, 3H), 2.09 (s, 3H), 1.40 (t, 3H, *J* = 7.0 Hz); Anal.($C_{23}H_{25}NO_4S_2$) C, H, N.

Ethyl-2-methyl-1-[4-(methoxy)-phenyl]-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-acetate (16e). Yellowish needles (70% yield), m.p. 118-122 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.85 (d, 2H, *J* = 8.6 Hz), 7.48 (d, 2H, *J* = 8.5 Hz), 7.22 (d, 2H, *J* = 8.6 Hz), 7.03 (d, 2H, *J* = 8.5 Hz), 6.42 (s, 1H), 4.11 (q, 2H, *J* = 7.0 Hz), 3.49 (s, 2H), 3.87 (s, 3H), 3.04 (s, 3H), 2.10 (s, 3H), 1.39 (t, 3H, *J* = 7.0 Hz); Anal.($C_{23}H_{25}NO_5S$) C, H, N.

2-[1-Phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17a).



17a

Sodium hydroxide solution (1N, 4.83 mL) was added to a solution of ethyl 1,5-diarylpyrrole-3-acetic ester (**16a**) (0.67 mmol) in methanol (4.83 mL). The resulting mixture was refluxed for 1.5 h, cooled, and concentrated in vacuo. The residue was solubilized in water (5 mL) and then acidified with concentrated HCl. The precipitate was filtered off to give the expected acid as a white solid. White needles (>95% yield), m.p. 147–152°C; ^{1}H NMR (400 MHz, CDCl3) δ (ppm): 11.00 (br s, 1H), 7.60 (d, 2H, *J* = 8.6 Hz), 7.25 (d, 2H, *J* = 8.5 Hz),7.17 (d, 2H, *J* = 8.6 Hz), 7.00 (d, 2H, *J* = 8.5 Hz), 6.49 (s, 1H), 3.02 (s, 3H), 2.55 (s, 3H), 2.10 (s, 3H). Anal.($C_{20}H_{19}NO_4S$) C, H, N.

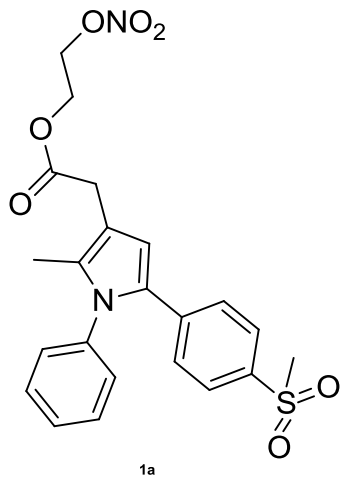
2-[1-(3-fluoro)phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17b). White needles (>95% yield), m.p. 166–170°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 11.20 (br s, 1H), 7.72 (d, 2H, *J* = 8.6 Hz), 7.25 (d, 2H, *J* = 8.5 Hz), 7.20 (m, 1H), 7.07-7.00 (m, 2H), 6.45 (s, 1H), 3.02 (s, 3H), 2.10 (s, 3H); Anal.($C_{20}H_{18}NO_4SF$) C, H, N.

2-[1-(4-fluoro)phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17c). White needles (>95% yield), m.p. 170–175°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 11.17 (br s, 1H), 7.71 (d, 2H, *J* = 8.6 Hz), 7.26 (d, 2H, *J* = 8.5 Hz), 7.22 (m, 2H), 7.09 (m, 2H), 6.44 (s, 1H), 3.07 (s, 3H), 2.11 (s, 3H); Anal.($C_{20}H_{18}NO_4SF$) C, H, N.

2-[1-(4-methylthio)phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17d). White needles (>95% yield), m.p. 147–152°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 11.00 (br s, 1H), 7.70 (d, 2H, *J* = 8.6 Hz), 7.25 (d, 2H, *J* = 8.5 Hz),7.17 (d, 2H, *J* = 8.6 Hz), 7.00 (d, 2H, *J* = 8.5 Hz), 6.49 (s, 1H), 3.02 (s, 3H), 2.55 (s, 3H), 2.10 (s, 3H); Anal.($C_{21}H_{21}NO_4S_2$) C, H, N.

2-[1-(4-methoxy)phenyl-2-methyl-5-[4-(methylsulfonyl)-phenyl]-1H-pyrrol-3yl]acetic acid (17e). White needles (>95% yield), m.p. 139–143°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 11.09 (br s, 1H), 7.69 (d, 2H, *J* = 8.6 Hz), 7.28 (d, 2H, *J* = 8.5 Hz),7.19 (d, 2H, *J* = 8.6 Hz), 7.02 (d, 2H, *J* = 8.5 Hz), 6.47 (s, 1H), 3.86 (s, 3H), 3.10 (s, 3H), 2.10 (s, 3H); Anal.($C_{21}H_{21}NO_5S$) C, H, N.

2-(Nitroxy)ethyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1a).



1a

Nitro-oxy alcohol (**19a**) (0.3 mmol), DMAP (0.1 mmol), and EDCI (0.2 mmol) were added in sequence to a solution of 1,5-diarylpyrrole-3-acetic acid (**17a**) (0.1 mmol) in dichloromethane (5 mL), under nitrogen atmosphere. After 3h stirring, the reaction was quenched with water and extracted with chloroform. The organic layer was washed with 1 N HCl, sodium bicarbonate saturated solution, brine and dried over sodium sulfate. After filtration and concentration of the organic phase a crude material was obtained. The material was then purified by chromatography on silica gel using petroleum ether/chloroform/ethyl acetate, 4:4:1 (v/v/v), as the eluent to give the desired product in good yield. White needles (yield 80%), mp 124-126 °C; FT-IR (neat, cm^{-1}): 1740, 1622, 1277, 1153, 881; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.63 (d, 2H, *J* = 8.6 Hz), 7.36-7.40 (m, 3H), 7.11-7.15 (m, 4H), 6.48(s, 1H), 4.67-4.70 (m, 2H), 4.37-4.42 (m, 2H), 3.53 (s, 2H), 2.96 (s, 3H), 2.04 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 10.98, 32.26, 44.50, 60.77, 69.90, 112.78, 113.79, 115.50, 120.20, 124.35, 130.60, 130.59, 131.39, 134.67, 137.22, 138.01, 139.45, 176.88; MS-ESI = 459.12 m/z [M + H^+]; HPLC: >98% (Rt=13.87 min.)

3-(Nitroxy)propyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1b). Obtained with the same procedure as (**1a**) using (**19b**) as alcohol. White needles (yield 77%), mp 116-118 °C; FT-IR (neat, cm^{-1}): 1737, 1285, 1152, 1633, 877; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.62 (d, 2H, *J* = 8.5 Hz), 7.38-7.40 (m, 3H), 7.12-7.16 (m, 4H), 6.49(s, 1H), 4.52 (t, 2H, *J* = 6.4), 4.23 (t, 2H, *J* = 6.0 Hz), 3.51 (s, 2H), 2.97 (s, 3H), 2.06-2.15 (m, 5H).^{13}C-NMR (400 MHz, CDCl3) δ (ppm): 11.03, 27.45, 32.26, 44.50, 60.77, 69.90, 112.78, 113.79, 115.50, 120.20, 124.35, 130.60, 130.59, 131.39, 134.67, 137.22, 138.01, 139.45, 176.88; Anal.($C_{23}H_{24}N_2O_7S$) C, H, N; MS-ESI: m/z 473.15 [M + H^+]; HPLC: >98% (Rt=14.31 min.).

3-(Nitroxy)butyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1c). Obtained with the same procedure as (**1a**) using (**19c**) as alcohol. White needles (yield 59%), mp 110-112 °C; FT-IR (neat, cm^{-1}): 1734, 1630, 1282, 1150, 866; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.69 (d, 2H, *J* = 8.5 Hz), 7.42-7.39 (m, 3H), 7.12-7.16 (m, 4H), 6.49(s, 1H), 4.69 (t, 2H, *J* = 6.4), 4.26 (t, 2H, *J* = 6.0 Hz), 3.51 (s, 2H), 2.97 (s, 3H), 2.10 (s, 5H), 1.99-1.85

(m, 4H).^{13}C-NMR (100 MHz, CDCl3) δ (ppm): 11.09, 27.45, 31.03, 32.29, 44.50, 60.77, 69.92, 112.81, 113.79, 115.50, 120.24, 124.35, 131.60, 130.59, 131.39, 134.67, 137.22, 138.01, 139.45, 176.79. Anal. ($C_{24}H_{26}N_2O_7S$) C, H, N; MS-ESI: m/z 487.14 [M + H^+]; HPLC: >98% (Rt=15.01 min.).

2-(Nitroxy)ethyl 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1d). Obtained with the same procedure as (**1a**). Yellowish needles (89% yield), mp 130-133 °C; FT-IR (neat, cm^{-1}): 1738, 1625, 1280, 1144, 843; 1H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, *J* = 8.6 Hz), 7.38-7.36 (m, 1H), 7.14-7.10 (m, 3H), 6.91-6.87 (m, 2H), 6.49 (s, 1H), 4.68 (t, 2H, *J* = 7.0 Hz), 4.20 (t, 2H, *J* = 6.8 Hz), 3.50 (s, 2H), 3.00 (s, 3H), 2.09 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 10.93, 32.22, 44.46, 60.69, 69.88, 112.78, 113.87, 115.55, 120.13, 124.33, 127.07, 130.59, 130.66, 131.44, 131.90, 137.20, 138.02, 139.40, 166.69, 177.00; Anal.($C_{22}H_{21}FN_2O_7S$) C, H, N; MS-ESI: m/z 477.14 [M + H^+]; HPLC: >97% (Rt=13.95 min.)

3-(Nitroxy)propyl 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1e). Obtained with the same procedure as (**1a**) using (**19b**) as alcohol. Yellowish needles (92% yield), mp 120-125 °C; FT-IR (neat, cm^{-1}): 1742, 1630, 1281, 1150, 870; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.69 (d, 2H, *J* = 8.6 Hz), 7.40-7.38 (m, 1H), 7.18 (d, 2H, *J* = 8.6 Hz), 7.12-7.10 (m, 1H), 6.95-6.93 (m, 2H), 6.48 (s, 1H), 4.69 (t, 2H, *J* = 7.0 Hz), 4.25 (t, 2H, *J* = 6.8 Hz), 3.50 (s, 2H), 3.00 (s, 3H), 2.09-2.04 (m, 5H). ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 10.94, 26.42, 32.19, 44.45, 60.70, 69.82, 112.77, 113.85, 115.53, 120.12, 124.30, 127.07, 130.58, 130.64, 131.41, 131.88, 137.15, 138.00, 139.55, 166.71, 177.06; Anal.($C_{23}H_{23}FN_2O_7S$) C, H, N; MS-ESI: m/z 513 [M + Na^+]; HPLC: >97% (Rt=14.50 min.)

4-(Nitroxy)butyl 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1f). Obtained with the same procedure as (**1a**) using (**19c**) as alcohol. Yellowish needles (yield 89%), mp 113-115 °C. FT-IR (neat, cm^{-1}): 1739, 1633, 1280, 1144, 865; ^{1}H-NMR (400 MHz, CDCl3) δ (ppm): 7.70 (d, 2H, *J* = 8.6 Hz), 7.40-7.37 (m, 1H), 7.14-7.10 (m, 3H), 6.91-6.87 (m, 2H), 6.49 (s, 1H), 4.70 (t, 2H, *J* = 7.0 Hz), 4.17 (t, 2H, *J* = 6.8 Hz), 3.50 (s, 2H), 3.00 (s, 3H), 2.09 (s, 3H), 2.01-1.92 (m, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 10.94, 26.42,

30.11, 32.20, 44.50, 60.71, 69.88, 112.79, 113.80, 115.51, 120.12, 124.30, 127.07, 130.58, 130.64, 131.41, 131.90, 137.65, 138.11, 139.51, 166.67, 177.10; Anal.($C_{24}H_{25}FN_2O_7S$) C, H, N; MS-ESI: m/z 505.20 [M + H^+]. HPLC: >98% (Rt=15.05 min.)

2-(Nitroxy)ethyl 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1g). Obtained with the same procedure as (**1a**). White needles, mp 110-112 °C, (yield 78%); FT-IR (neat, cm^{-1}): 1745, 1634, 1283, 1155, 890; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.64 (d, 2H, *J* = 8.6 Hz), 7.25-7.15 (m, 6H), 6.47 (s, 1H), 4.68 (t, 2H, *J* = 6.2 Hz), 4.32 (t, 2H, *J* = 6.2 Hz), 3.52 (s, 2H), 3.00 (s, 3H), 2.03 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 10.99, 32.17, 44.50, 60.30, 69.91, 112.96, 113.50, 116.00, 120.13, 124.30, 127.08, 130.59, 131.31, 131.92, 137.16, 138.01, 166.67, 177.08; Anal.($C_{22}H_{21}FN_2O_7S$) C, H, N; MS-ESI: m/z 477.14 [M + H^+]; HPLC: >98% (Rt =14.01 min.)

3-(Nitroxy)propyl 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1h). Obtained with the same procedure as (**1a**) using (**19b**) as alcohol. White needles, mp 126-128 °C, (yield 81%). FT-IR (neat, cm^{-1}): 1739, 1626, 1277, 1144, 871; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.64 (d, 2H, *J* = 8.6 Hz), 7.06-7.15 (m, 6H), 6.47 (s, 1H), 4.69 (t, 2H, *J* = 7.1 Hz), 4.21 (t, 2H, *J* = 7.0 Hz), 3.49 (s, 2H), 2.96 (s, 3H), 2.00-2.13 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 11.00, 26.44, 32.20, 44.49, 60.33, 69.90, 112.97, 113.49, 116.01, 120.12, 124.30, 127.07, 130.58, 131.30, 131.90, 137.15, 138.00, 166.60, 176.99; Anal. ($C_{23}H_{23}FN_2O_7S$) C, H, N; MS-ESI: m/z 491.11 [M + H^+]. HPLC: >96% (Rt = 14.89 min.)

4-(Nitroxy)butyl 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1i). Obtained with the same procedure as (**1a**) using (**19c**) as alcohol. White needles (yield 81%), mp 116-118 °C; FT-IR (neat, cm^{-1}): 1739, 1626, 1277, 1144, 871; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68 (d, 2H, *J* = 8.6 Hz), 7.06-7.15 (m, 6H), 6.47 (s, 1H), 4.71 (t, 2H, *J* = 7.1 Hz), 4.16 (t, 2H, *J* = 7.0 Hz), 3.49 (s, 2H), 2.96 (s, 3H), 2.09 (s, 3H), 2.01-1.92 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 11.02, 26.50, 31.00, 32.23, 44.55, 60.39, 69.90, 113.00,

113.49, 116.00, 120.12, 124.30, 127.07, 130.58, 131.30, 131.90, 137.15, 138.00, 166.55, 177.01; Anal. ($C_{24}H_{25}FN_2O_7S$) C, H, N; MS-ESI: m/z 505.13 [M + H^+]. HPLC: >98% (Rt = 15.09 min.)

2-(Nitroxy)ethyl 2-[1-(4-methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1j). Obtained with the same procedure as (**1a**). Yellow powder (yield 70%), mp 118–123°C; FT-IR (neat, cm^{-1}): 1738, 1628, 1280, 1144, 862; ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.80 (d, 2H, *J* = 8.6 Hz), 7.24 (d, 2H, *J* = 8.5 Hz), 7.17 (d, 2H, *J* = 8.6 Hz), 6.99 (d, 2H, *J* = 8.5 Hz), 6.51 (s, 1H), 4.73 (t, 2H, *J* = 6.3 Hz), 4.27 (m, 2H, *J* = 6.3 Hz), 3.50 (s, 2H), 3.00 (s, 3H), 2.55 (s, 2H), 2.10 (s, 3H). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.89, 29.79, 32.21, 44.60,60.41, 69.90, 112.96, 113.50, 115.89, 120.67, 125.39, 127.00, 130.59, 130.70, 131.30, 131.80, 136.10, 138.00, 177.03; Anal.($C_{23}H_{24}N_2O_7S_2$) C, H, N; MS-ESI: m/z 505 [M + H^+]. HPLC: >98% pure (tR= 14.19 min).

3-(Nitroxy)propyl 2-[1-(4-methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1k). Obtained with the same procedure as (**1a**) using (**19b**) as alcohol. Yellowish needles (yield 68%), mp 118-122 °C; FT-IR (neat, cm^{-1}): 1737, 1632, 1281, 1142, 865; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.69 (d, 2H, *J* = 8.6 Hz), 7.30 (d, 2H, *J* = 8.6 Hz), 7.12 (d, 2H, J = 8.6 Hz), 6.96 (d, 2H, *J* = 8.6 Hz), 6.56 (s, 1H), 4.66 (t, 2H, *J* = 7.0 Hz), 4.18 (t, 2H, *J* = 6.9 Hz), 3.59 s, 2H), 3.09 (s, 3H), 2.53 (s, 3H), 2.50 (s, 3H), 2.10 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 10.89, 26.44, 29.79, 32.20, 44.63, 60.40, 69.87, 112.99, 113.54, 115.91, 120.67, 125.39, 127.02, 130.55, 130.70, 131.30, 131.77, 136.11, 138.07, 177.07; Anal.($C_{24}H_{26}N_2O_7S_2$) C, H, N; MS-ESI: m/z 519 [M + H^+]. HPLC: >97% (Rt = 17.25 min.)

4-(Nitroxy)butyl 2-[1-(4-methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (1l). Obtained with the same procedure as (**1a**) using (**19c**) as alcohol. Yellowish needles, mp 112-115 °C (yield 68%). FT-IR (neat, cm^{-1}): 1741, 1626, 1282, 1149, 866; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68 (d, 2H, *J* = 8.6 Hz), 7.30 (d, 2H, *J* = 8.6 Hz), 7.12 (d, 2H, J = 8.6 Hz), 6.96 (d, 2H, *J* = 8.6 Hz), 6.46 (s, 1H), 4.66 (t, 2H, *J* = 7.0 Hz), 4.18 (t, 2H, *J* = 6.9 Hz), 3.56 (s, 2H), 3.09 (s, 3H), 2.53 (s, 3H), 2.10 (s, 3H), 2.00-1.93 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$)

δ(ppm): 10.98, 26.44, 29.82, 30.98, 32.20, 44.63, 60.40, 69.91, 113.01, 113.54, 115.91, 120.67, 125.39, 127.02, 130.55, 130.68, 131.29, 131.75, 136.13, 138.09, 177.07; Anal.($C_{25}H_{28}N_2O_7S_2$) C, H, N; MS-ESI: m/z 519 [M + H^+]. HPLC: >97% (Rt = 17.25 min.)

2-(Nitroxy)ethyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (1m). Obtained with the same procedure as (**1a**). Yellowish needles (yield 78%), mp 120-125 °C; FT-IR (neat, cm^{-1}): 1742, 1629, 1280, 1144, 867; ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.70 (d, 2H, *J* = 8.6 Hz), 7.20 (d, 2H, *J* = 8.5 Hz), 7.10 (d, 2H, *J* = 8.6 Hz), 6.89 (d, 2H, *J* = 8.5 Hz), 6.50 (s, 1H), 4.68 (t, 2H, *J* = 6.3 Hz), 4.24 (t, 2H, *J* = 6.0 Hz), 3.60 (s, 3H), 3.50 (s, 2H), 3.01 (s, 3H), 2.10 (s, 3H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.04, 32.35, 44.70, 54.09, 60.42, 69.83, 112.87, 113.40, 115.67, 120.60, 125.44, 127.10, 130.59, 130.70, 131.20, 131.70, 138.10, 138.17, 177.06; Anal.($C_{23}H_{24}N_2O_8S$) C, H, N; MS-ESI: m/z 489 [M + H^+]. HPLC: >96% (Rt = 14.12 min.)

3-(Nitroxy)propyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (1n). Obtained with the same procedure as (**1a**) using (**19b**) as alcohol. Yellowish powder (yield 69%), mp 115-119 °C; FT-IR (neat, cm^{-1}): 1742, 1630, 1281, 1150, 870; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.67 (d, 2H, *J* = 8.6 Hz), 7.18 (d, 2H, *J* = 8.5 Hz), 7.07 (d, 2H, *J* = 8.6 Hz), 6.92 (d, 2H, *J* = 8.5 Hz), 6.49 (s, 1H), 4.67 (t, 2H, *J* = 7.0 Hz), 4.24 (t, 2H, *J* = 6.8 Hz), 3.85 (s, 3H), 3.52 (s, 2H), 3.00 (s, 3H) 2.12-2.03 (m, 5H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.00, 26.09, 32.36, 44.70, 54.10, 60.44, 69.90, 112.90, 113.40, 115.66, 120.61, 125.42, 127.10, 130.61, 130.68, 131.20, 131.72, 138.11, 138.15, 177.06; Anal.($C_{24}H_{26}N_2O_8S$) C, H, N; MS-ESI: m/z 503 [M + H^+]. HPLC: >98% (Rt = 15.08 min.).

4-(Nitroxy)butyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (1o). Obtained with the same procedure as (**1a**) using (**19c**) as alcohol. Yellowish powder (yield 69%), mp 105-108 °C. FT-IR (neat, cm^{-1}): 1741, 1632, 1281, 1152, 870; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.69 (d, 2H, *J* = 8.6 Hz), 7.18 (d, 2H, *J* = 8.5 Hz), 7.07 (d, 2H, *J* = 8.6 Hz), 6.92 (d, 2H, *J* = 8.5 Hz), 6.47 (s, 1H), 4.68 (t, 2H, *J* = 7.0 Hz), 4.23 (t, 2H, *J* = 6.8 Hz), 3.84

(s, 3H), 3.50 (s, 2H), 3.00 (s, 3H) 2.10 (s, 3H), 2.01-1.92 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.02, 26.09, 30.18, 32.38, 44.76, 54.16, 60.49, 69.92, 112.90, 113.40, 115.66, 120.60, 125.42, 127.10, 130.61, 130.56, 131.18, 131.72, 138.11, 138.15, 177.02; Anal.($C_{25}H_{28}N_2O_8S$) C, H, N; MS-ESI: m/z 503 [M + H^+]. HPLC: >98% (Rt = 15.08 min.).

2-Hydroxyethyl 2-[1-phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2a). Obtained with the same procedure as (**1a**) using ethylene glycol as alcohol. White needles (66% yield), mp 121-123 °C; 1H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.62 (d, 2H, *J* = 8.6 Hz), 7.35-7.40 (m, 3H), 7.09-7.14 (m, 4H), 6.50 (s,1H), 4.24 (t, 2H, *J* = 6.9 Hz), 3.85 (t, 2H, *J* = 6.9 Hz), 3.77 (br s, 1H), 3.54 (s, 2H), 2.96 (s, 3H), 2.05 (s, 3H). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.98, 32.26, 44.50, 60.77, 66.30, 112.79,113.78, 115.50, 120.20, 124.35, 130.60, 130.59, 131.39, 134.67, 137.20, 138.00, 139.45, 176.85.

3-Hydroxypropyl 2-[1-Phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2b). Obtained with the same procedure as (**1a**) using propylene glycol as alcohol. White needles (62% yield), mp 108-110 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.62 (d, 2H, *J* = 8.6 Hz), 7.37-7.40 (m, 3H), 7.12-7.16 (m, 4H), 6.50 (s,1H), 4.28 (t, 2H, *J* = 6.8 Hz), 3.71 (t, 2H, *J* = 6.7 Hz), 3.52 (s, 2H), 2.97 (s, 3H), 2.06 (s, 3H), 1.86-1.95 (m, 2H), 1.83 (br s, 1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 11.00, 27.45, 32.27, 44.51, 60.78, 65.99, 112.78, 113.80, 115.50, 120.20, 124.35, 130.60, 130.59, 131.39, 134.67, 137.22, 138.01, 139.46, 176.80.

4-Hydroxybutyl 2-[1-Phenyl-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2c). Obtained with the same procedure as (**1a**) using propylene glycol as alcohol. White needles (53% yield), mp 108-110 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.65 (d, 2H, *J* = 8.6 Hz), 7.37-7.41 (m, 3H), 7.12-7.18 (m, 4H), 6.48 (s,1H), 4.34 (t, 2H, *J* = 6.8 Hz), 4.12 (t, 2H, *J* = 6.7 Hz), 3.52 (s, 2H), 3.07 (s, 3H), 2.08 (s, 3H), 1.90-1.77 (m, 5H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 11.01, 27.43, 30.22, 32.29, 44.50, 60.78, 65.85, 112.78, 113.86, 115.50, 120.20, 124.35, 130.60, 130.59, 131.39, 134.55, 137.20, 138.01, 139.40, 176.78.

2-Hydroxyethyl 2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3 yl]acetate (2d). Obtained with the same procedure as (**1a**) using ethylene glycol as alcohol. Yellowish powder (77% yield), mp 115-120 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.71 (d, 2H, *J* = 8.6 Hz), 7.19-7.10 (m, 6H), 6.46 (s, 1H), 4.33 (t, 2H, *J* = 6.8 Hz), 4.12 (t, 2H, *J* = 6.8 Hz), 3.47 (s, 2H), 3.04 (s, 3H), 2.09 (s, 3H), 2.08(s broad, 1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 10.89, 32.42, 44.60, 60.49, 66.03, 112.72, 113.67, 115.63, 120.03, 124.93, 127.15, 130.86, 130.57, 131.44, 131.90, 137.20, 138.02, 139.40, 166.69, 177.00.

3-Hydroxypropyl 2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2e). Obtained with the same procedure as (**1a**) using propylene glycol as alcohol. Yellowish powder (81% yield), mp 110-113 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68 (d, 2H, *J* = 8.6 Hz), 7.37-7.36 (m, 1H), 7.15-7.11 (m, 3H), 6.90-6.88 (m, 2H), 6.50 (s, 1H), 4.24 (t, 2H, *J* = 6.8 Hz), 3.83 (t, 2H, J = 6.5 Hz), 3.50 (s, 2H), 3.00 (s, 3H), 2.10-2.03 (m, 5H),1.50 (s broad, 1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 10.94, 27.13, 32.22, 44.46, 60.69, 66.45, 112.78, 113.87, 115.55, 120.13, 124.30, 127.07, 130.60, 130.70, 131.44, 131.88, 137.20, 138.03, 139.40, 166.70, 177.01.

3-Hydroxypropyl 2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2f). Obtained with the same procedure as (**1a**) using propylene glycol as alcohol. Yellowish powder (58% yield), mp 110-113 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, *J* = 8.6 Hz), 7.37-7.35 (m, 1H), 7.15-7.10 (m, 3H), 6.90-6.88 (m, 2H), 6.48 (s, 1H), 4.33 (t, 2H, *J* = 6.8 Hz), 4.13 (t, 2H, *J* = 6.5 Hz), 3.50 (s, 2H), 3.00 (s, 3H), 2.10 (s, 3H), 1.92-1.76 (m, 4H), 1.50 (s broad, 1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 10.99, 27.13, 31.08, 33.00, 44.61, 60.70, 67.05, 112.80, 113.88, 115.61, 120.10, 124.30, 127.17, 130.61, 130.70, 131.44, 131.88, 137.20, 138.03, 139.22, 166.73, 177.02.

2-Hydroxyethyl 2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2g). Obtained with the same procedure as (**1a**) using ethylene glycol as alcohol. White needles, mp 129-131 °C, (yield 76%). 1H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68

(d, 2H, J = 8.3 Hz), 7.08-7.17 (m, 6H), 6.52 (s, 1H), 4.25 (t, 4H, *J* = 6.8 Hz), 4.11 (t, 2H, *J* = 6.8 Hz), 3.80 (br s, 1H), 3.50 (s, 2H), 2.99 (s, 3H), 2.01 (s, 3H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 10.95, 32.21, 44.50, 60.30, 66.33, 112.89, 113.50, 116.01, 120.13, 124.31, 127.08, 130.59, 131.31, 131.92, 137.16, 138.01, 166.70, 177.02.

3-Hydroxypropyl 2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2h). Obtained with the same procedure as (**1a**) using propylene glycol as alcohol. White needles, mp 112-115 °C, (yield 75%). 1H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.62 (d, 2H, *J* = 8.3 Hz), 7.12-7.16 (m, 6H), 6.50 (s, 1H), 4.25 (t, 2H, *J* = 6.9 Hz), 3.75 (t, 2H, *J* = 6.5 Hz), 3.50 (s, 2H), 2.98 (s, 3H), 2.05 (s, 3H), 1.84-1.94 (m, 2H), 1.80 (br s, 1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.01, 26.40, 32.10, 44.4, 60.33, 66.23, 112.97, 113.49, 116.01, 120.12, 124.30, 127.07, 130.58, 131.30, 131.91, 137.15, 138.00, 166.57, 176.95.

3-Hydroxypropyl 2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2i). Obtained with the same procedure as (**1a**) using propylene glycol as alcohol. White needles, mp 106-108 °C, (yield 75%). 1H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68 (d, 2H, *J* = 8.3 Hz), 7.17-7.14 (m, 6H), 6.44 (s, 1H), 4.28 (t, 2H, *J* = 6.9 Hz), 4.12 (t, 2H, *J* = 6.5 Hz), 3.50 (s, 2H), 2.98 (s, 3H), 2.05 (s, 3H), 1.92-1.81 (m, 4H), 1.79 (br s, 1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.01, 26.42, 31.00, 32.11, 44.51, 60.33, 66.23, 112.91, 113.45, 116.09, 120.10, 124.30, 127.11, 130.43, 131.30, 131.90, 137.15, 138.00, 166.52, 176.09.

2-Hydroxyethyl 2-[1-(4-Methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (2j). Obtained with the same procedure as (**1a**) using ethylene glycol as alcohol. Yellow powder, mp 110−112 °C (yield 55%). ^{1}H-NMR (400 MHz,$CDCl_3$) δ(ppm): 7.80 (d, 2H, J = 8.6 Hz), 7.24 (d, 2H, *J* = 8.5 Hz), 7.17 (d, 2H, *J*= 8.6 Hz), 7.00 (d, 2H, *J*= 8.5 Hz), 6.48 (s, 1H), 4.25 (t, 2H,J= 6.8 Hz), 3.89 (t, 2H,J = 6.8 Hz), 3.52 (s, 2H), 2.55 (s, 3H), 2.07 (s, 3H), 2.01 (br s, 1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.89, 29.79, 32.20, 44.54, 60.40, 66.29, 112.96, 113.50, 115.90, 120.67, 125.39, 127.01, 130.63, 130.71, 131.27, 131.80, 136.10, 138.00, 177.00.

3-Hydroxypropyl 2-[1-(4-Methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2k). Obtained with the same procedure as (**1a**) using propylene glycol as alcohol. Yellow solid, mp104-107 °C (yield 53 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.79 (d, 2H, J = 8.6 Hz), 7.34 (d, 2H, J = 8.6 Hz), 7.12 (d, 2H, J = 8.6 Hz), 6.96 (d, 2H, J = 8.6 Hz), 6.46 (s, 1H), 4.26 (t, 2H, *J* = 6.8 Hz), 3.85 (t, 2H, *J* = 6.6 Hz), 3.50 (s, 2H), 3.09 (s, 3H), 2.50 (s, 3H), 2.19 (s, 3H), 2.09 (m, 2H), 2.02 (br s, ,1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 10.89, 26.44, 29.79, 32.20, 44.63, 60.40, 67.00, 112.90, 113.53, 115.93, 120.67, 125.39, 127.02, 130.56, 130.70, 131.30, 131.77, 136.10, 138.00, 177.01.

3-Hydroxybutyl 2-[1-(4-methylthiophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetate (2l). Obtained with the same procedure as (**1a**) using propylene glycol as alcohol. Yellow solid, mp100-103 °C (yield 50 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.69 (d, 2H, *J* = 8.6 Hz), 7.34 (d, 2H, *J* = 8.6 Hz), 7.12 (d, 2H, *J* = 8.6 Hz), 6.96 (d, 2H, *J* = 8.6 Hz), 6.45 (s, 1H), 4.26 (t, 2H, *J* = 6.8 Hz), 4.10 (t, 2H, *J* = 6.6 Hz), 3.50 (s, 2H), 3.09 (s, 3H), 2.50 (s, 3H), 2.19 (s, 3H), 2.01-1.85 (m, 5H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 10.97, 26.39, 29.79, 30.12, 32.30, 44.61, 60.40, 67.02, 112.91, 113.53, 115.90, 120.69, 125.40, 127.00, 130.56, 130.70, 131.30, 131.77, 136.10, 138.10, 176.98.

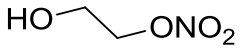
2-Hydroxyethyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (2m). Obtained with the same procedure as (**1a**) using ethylene glycol as alcohol. Yellowish powder (yield 59%), mp 105-110 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.60 (d, 2H, *J* = 8.6 Hz), 7.18 (d, 2H, *J* = 8.5 Hz), 7.00 (d, 2H, *J* = 8.6 Hz), 6.96 (d, 2H, *J* = 8.5 Hz), 6.43 (s, 1H), 4.30 (t, 2H, *J* = 6.8 Hz), 3.82 (t, 2H, *J* = 6.8 Hz), 3.58 (s, 3H), 3.47 (s, 2H), 2.40 (br,s, 1H), 2.37 (s, 3H), 2.01 (s, 3H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.04, 32.35, 44.70, 54.09, 60.42, 66.77, 112.85, 113.34, 115.67, 120.56, 125.44, 127.10, 130.59, 130.70, 131.20, 131.72, 138.11, 138.20, 177.01.

3-Hydroxypropyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (2n). Obtained with the same procedure as (**1a**) using propylene glycol as

alcohol. Yellow solid (yield 52%), mp 103-110 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.67 (d, 2H, *J* = 8.6 Hz), 7.18 (d, 2H, *J* = 8.5 Hz), 7.07 (d, 2H, *J* = 8.6 Hz), 6.92 (d, 2H, *J* = 8.5 Hz), 6.49 (s, 1H), 4.25 (t, 2H, *J* = 6.8 Hz), 3.83 (t, 2H, *J* = 6.6 Hz), 3.85 (s, 3H), 3.52 (s, 2H), 3.00 (s, 3H), 2.12-2.05 (m, 5H), 1.70 (s broad, 1H). ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 11.01, 26.08, 32.34, 44.71, 54.10, 60.45, 66.80, 112.91, 113.40, 115.66, 120.61, 125.42, 127.10, 130.66, 130.70, 131.20, 131.72, 138.11, 138.15, 177.04.

3-Hydroxypropyl 2-[1-(4-methoxyphenyl)-2-methyl-5-[4-(methylsulfonyl)pheny]-1H-pirrol-3yl]acetate (2o). Obtained with the same procedure as (**1a**) using propylene glycol as alcohol. Yellow solid (yield 52%), mp 97-100 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68 (d, 2H, *J* = 8.6 Hz), 7.18 (d, 2H, *J* = 8.5 Hz), 7.07 (d, 2H, *J* = 8.6 Hz), 6.92 (d, 2H, *J* = 8.5 Hz), 6.46 (s, 1H), 4.25 (t, 2H, *J* = 6.8 Hz), 4.09 (t, 2H, *J* = 6.6 Hz), 3.85 (s, 3H), 3.52 (s, 2H), 3.00 (s, 3H), 2.10 (s, 3H), 2.00-1.90 (m, 4H), 1.70 (s broad, 1H). ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 11.00, 26.11, 30.12, 32.30, 44.70, 54.11, 60.45, 66.85, 112.91, 113.40, 115.66, 120.61, 125.42, 127.10, 130.66, 130.70, 131.20, 131.72, 138.11, 138.15, 177.00.

2-Nitroxyethanol (19a).



19a

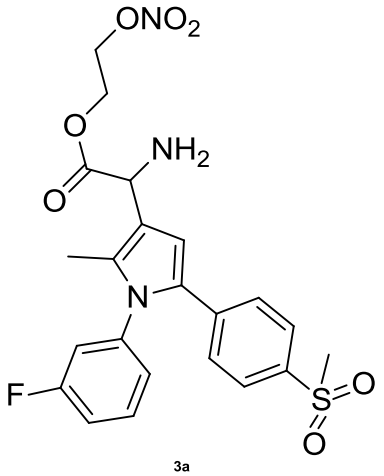
Nitric acid (65% w/v, 16 mmol), glacial acetic acid (97 mmol), and acetic anhydride (97% w/v, 59 mmol) were carefully added in sequence to a solution of ethylene glycol (**18a**, 20 mmol) in ethyl acetate (100 mL). The mixture was allowed to react overnight and then quenched with 20% KOH solution. The pH was adjusted to 12–13, and the aqueous phase was extracted with ethyl acetate (3×100 mL), washed with NaCl saturated solution, and dried over Na_2SO_4. After filtration, the organic layers were concentrated in vacuo, and the crude product was purified by chromatography on silica gel, using petroleum ether/chloroform/ethyl acetate, 3:1:1 (v/v/v), as

eluent, to obtain the expected nitroester in good yield. Yellowish oil (70% yield); FT-IR (neat, cm^{-1}): 1623, 850; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.67 (t, 2H, *J* = 6.9 Hz), 4.20 (t, 2H, *J* = 6.9 Hz), 2.10 (br s, 1H); MS-ESI: m/z 108.19 [M + H^+].

3-Nitroxypropanol (19b). Obtained with the same procedure as (**11a**) using 1,3-propandiol (**18b**). Colorless oil (68% yield); FT-IR (neat, cm^{-1}): 1638, 881 ; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.71 (t, 2H, *J* = 6.9 Hz), 4.18 (t, 2H, *J* = 6.9 Hz), 2.00 (br s, 1H), 1.91 (m, 2H); MS-ESI: m/z 122.39 [M + H^+].

4-Nitroxybutanol (19c). Obtained with the same procedure as (**11a**) using butylene glycol as alcohol. Colorless oil (55% yield); FT-IR (neat, cm^{-1}): 1640, 867; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.62 (t, 2H, *J* = 6.9 Hz), 4.19 (t, 2H, *J* = 6.9 Hz), 2.09-2.01 (m, 5H); MS-ESI: m/z 136.22 [M + H^+].

2-(Nitroxy)ethyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3a).



3a

An excess of trifluoroacetic acid (4mL) was added dropwise at 0 °C to a solution of (**24a**) (0.40 mmol) in dioxane (2 mL). The solution was microwave irradiated at 60 °C for 40 minutes (power 150 W, pressure 170 psi). The reaction mixture was poured into ice/water (20 mL) and the pH adjusted to 12-13 (using ammonia solution) and the mixture extracted with chloroform.

The organic layer was then dried over sodium sulfate. The filtration and concentration of the organic phase gave a crude material, which was purified through column chromatography (alumina) using MeOH/DCM 1:20 (v/v) to obtain the product as a yellowish powder. Yellowish powder, m.p. 137°C (83% yield); FT-IR (neat, cm^{-1}): 3340, 2889, 1738, 1681, 1590, 1300, 1145, 1091, 890; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, *J* = 8.5 Hz), 7.39 (m, 1H), 7.18 (d, 2H, *J* = 8.5 Hz), 7.10 (m, 1H), 7.03 (m, 2H), 6.60 (s, 1H), 4.66 (t, 2H, *J* = 7.2 Hz), 4.48 (s, 1H), 4.38 (t, 2H, *J* = 7.2 Hz), 3.05 (s, 3H), 2.20 (s, 3H), 1.90 (s br., 2H); ^{13}C-NMR (100 MHz, CDCl3): δ (ppm) 171.44, 158.57, 148.78, 144.00, 139.50, 138.56, 137.66, 135.54, 131.92, 128.90, 128.66, 126.20, 123.44, 122.59, 117.11, 74.58, 64.11, 61.03, 43.00, 11.11; Anal. ($C_{22}H_{22}FN_3O_7S$) C, H, N; ESI-Mass: m/z 528.122 $[M + Na]^+$.

3-(Nitroxy)propyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3b). Obtained following the procedure as (**3a**). Yellowish powder, m.p. 117°C (73% yield); FT-IR (neat, cm-1): 3340, 2887, 1738, 1681, 1592, 1300, 1149, 1091, 891; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, J = 8.5 Hz), 7.39 (m, 1H), 7.18 (d, 2H, J = 8.5 Hz), 7.10 (m, 1H), 7.03 (m, 2H), 6.60 (s, 1H), 4.67 (t, 2H, J = 7.2 Hz), 4.48 (s, 1H), 4.38 (t, 2H, J = 7.2 Hz), 3.07 (s, 3H), 2.23 (s, 3H), 1.98 (m, 2H), 1.90 (s br., 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 171.44, 158.57, 148.78, 144.00, 139.50, 138.56, 137.66, 135.54, 131.92, 128.90, 128.66, 126.20, 123.44, 122.59, 117.11, 74.58, 64.11, 61.03, 43.00, 27.69, 11.11; Anal. ($C_{23}H_{24}FN_3O_7S$) C, H, N; MS-ESI: m/z 528.122 $[M + Na]^+$.

4-(Nitroxy)butyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3c). Obtained following the procedure as (**3a**). Yellowish powder (57% yield), m.p. 113 °C; FT-IR (neat, cm^{-1}): 3350, 2892, 1740, 1693, 1580, 1292, 1150, 1088, 870; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68 (d, 2H, *J* = 8.4 Hz), 7.42 (m, 1H), 7.18 (d, 2H, J = 8.4 Hz), 7.10 (m, 1H), 7.05 (m, 2H), 6.61 (s, 1H), 4.70 (t, 2H, J = 7.2 Hz), 4.50 (s, 1H), 4.37 (t, 2H, J = 7.2 Hz), 3.12 (s, 3H), 2.20 (s, 3H), 2.03 (m, 4H), 1.90 (s br., 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 171.39, 158.60, 148.90, 144.05, 139.54, 138.56, 137.66, 135.50, 131.91, 128.88, 128.60,

126.20, 123.44, 122.59, 117.18, 74.70, 64.10, 61.20, 43.88, 25.69, 23.44, 11.09; Anal. ($C_{24}H_{26}FN_3O_7S$) C, H, N; MS-ESI: m/z 542.137 $[M + Na]^+$.

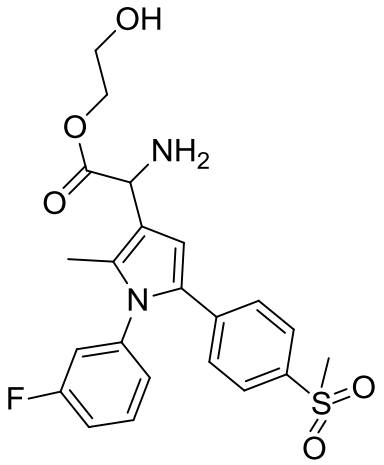
2-(Nitroxy)ethyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3d). Obtained following the procedure as (**3a**). Yellowish powder (85% yield), m.p. 133°C; FT-IR (neat, cm^{-1}): 3339, 2896, 1738, 1685, 1590, 1299, 1147, 1090, 880; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.73 (d, 2H, *J* = 8.4 Hz), 7.28 (d, 2H, *J* = 8.4 Hz), 7.12 (d, 2H, *J* = 8.4 Hz), 7.04 (m, 2H), 6.60 (s, 1H), 4.67 (t, 2H, *J* = 7.2 Hz), 4.50 (s, 1H), 4.37 (t, 2H, *J* = 7.2 Hz), 3.00 (s, 3H), 2.20 (s, 3H), 1.80 (s br., 2H); ^{13}C-NMR (100 MHz, CDCl3): δ (ppm) 171.33, 159.91, 147.33, 146.80, 141.53, 135.00, 130.32, 129.22, 128.76, 126.19, 123.45, 122.50, 117.09, 74.34, 64.08, 60.98, 43.07, 11.06; Anal. ($C_{22}H_{22}FN_3O_7S$) C, H, N; MS-ESI: m/z 528.122 $[M+Na]^+$.

3-(Nitroxy)propyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3e). Obtained following the procedure as (**3a**). Yellowish powder, m.p. 121°C (75% yield); FT-IR (neat, cm^{-1}): 3344, 2890, 1736, 1685, 1590, 1302, 1147, 1090, 880; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.70 (d, 2H, *J* = 8.4 Hz), 7.29 (d, 2H, *J* = 8.4 Hz), 7.11 (d, 2H, *J* = 8.4 Hz), 7.08 (m, 2H), 6.61 (s, 1H), 4.65 (t, 2H, *J* = 7.2 Hz), 4.50 (s, 1H), 4.39 (t, 2H, *J* = 7.2 Hz), 3.01 (s, 3H), 2.20 (s, 3H), 1.95 (m, 2H), 1.80 (s br., 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 171.33, 159.91, 147.33, 146.80, 141.53, 135.00, 130.32, 129.22, 128.76, 126.19, 123.45, 122.50, 117.09, 74.34, 64.08, 60.98, 53.07, 27.62, 11.06; Anal. ($C_{23}H_{24}FN_3O_7S$) C, H, N; MS-ESI: m/z 528.122 $[M+Na]^+$.

4-(Nitroxy)butyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (3f). Obtained following the procedure as (**3a**).Yellowish powder, m.p. 102 °C (58% yield); FT-IR (neat, cm^{-1}): 3347, 2894, 1742, 1689, 1587, 1298, 1157, 1099, 877; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.69 (d, 2H, *J* = 8.5 Hz), 7.25 (d, 2H, *J* = 8.5 Hz), 7.15 (d, 2H, *J* = 8.5 Hz), 7.07 (m, 2H), 6.60 (s, 1H), 4.68 (t, 2H, *J* = 7.2 Hz), 4.48 (s, 1H), 4.39 (t, 2H, *J* = 7.2 Hz), 3.07 (s, 3H), 2.23 (s, 3H), 2.01 (m, 4H), 1.90 (s br., 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 171.31, 159.90, 147.40, 146.71, 141.50, 135.02, 130.32, 129.67, 128.80, 126.21, 123.43, 122.51,

117.03, 74.34, 64.10, 60.90, 43.11, 24.62, 22.88, 11.16; Anal. ($C_{24}H_{26}FN_3O_7S$) C, H, N; MS-ESI: m/z 542.137 $[M+Na]^+$.

2-Hydroxyethyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4a).



4a

Ammonium formate (0.081g, 1.3 mmol) and Pd/C (0.07g) were added to a solution of (**27a**) (0.32 mmol) in isopropanol (2 mL). The solution was microwave irradiated at 80 °C for 5 minutes (power 150 W, pressure 170 psi). The reaction mixture was passed through Celite® and then poured into water (20 mL). The pH was adjusted to 10-12 (using NaOH 10% solution) and the mixture extracted with chloroform. The organic layer was then dried over Na_2SO_4. Filtration and concentration of the organic phase gave a material, which was identified to be the product without the need of any further purification. Yellowish powder, m.p. 118°C (>95% yield); FT-IR (neat, cm^{-1}): 3345, 3322, 2858, 1741, 1590, 1300, 1145, 1090; ^{1}H-NMR (400 MHz, DMSO-*d6*): δ (ppm) 7.71 (d, 2H, *J* = 8.4 Hz), 7.41 (m, 1H), 7.15 (d, 2H, *J* = 8.4 Hz), 7.05 (m, 1H), 6.97 (m, 2H), 6.59 (s, 1H), 4.79 (t app., 1H), 4.40 (s, 1H), 4.13 (m, 2H), 3.56 (m, 2H), 3.11 (s, 3H), 2.20 (s br., 2H), 2.19 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 172.44, 158.57, 148.78, 144.00, 139.50, 138.56, 137.66, 135.54, 131.92, 128.90, 128.66, 126.20, 123.44, 122.55, 117.11, 64.09, 60.88, 58.80, 43.10, 11.01; Anal. $C_{22}H_{23}FN_2O_5S$ (C, H, N); MS-ESI: m/z 468.107 $[M+Na]^+$.

3-Hydroxypropyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4b). Obtained following the procedure as (**4a**), using (**27b**) as starting material. Yellowish powder (>95% yield), m.p. 110°C; FT-IR (neat, cm^{-1}): 3345, 3322, 2858, 1741, 1590, 1300, 1145, 1090; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.71 (d, 2H, *J* = 8.4 Hz), 7.41 (m, 1H), 7.15 (d, 2H, *J* = 8.4 Hz), 7.05 (m, 1H), 6.97 (m, 2H), 6.59 (s, 1H), 4.79 (t app., 1H), 4.40 (s, 1H), 4.13 (m, 2H), 3.56 (m, 2H), 3.11 (s, 3H), 2.20 (s br., 2H), 2.19 (s, 3H), 1.92 (m, 2H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 171.44, 158.57, 148.78, 144.00, 139.50, 138.56, 137.66, 135.54, 131.92, 128.90, 128.66, 126.20, 123.44, 122.59, 117.11, 64.09, 60.88, 58.80, 43.10, 27.60, 11.01; Anal. ($C_{23}H_{25}FN_2O_5S$)C, H, N; MS-ESI: m/z 483.137 $[M + Na]^+$.

4-Hydroxybutyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4c). Obtained following the procedure as (**4a**), using (**27c**) as starting material. Yellowish powder (>95% yield), m.p. 98°C; FT-IR (neat, cm^{-1}): 3353, 3311, 2861, 1744, 1590, 1300, 1147, 1092; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.74 (d, 2H, *J* = 8.5 Hz), 7.43 (m, 1H), 7.15 (d, 2H, *J* = 8.5 Hz), 7.11 (m, 1H), 6.97 (m, 2H), 6.52 (s, 1H), 4.80 (t app., 1H), 4.39 (s, 1H), 4.18 (m, 2H), 3.53 (m, 2H), 3.13 (s, 3H), 2.21 (s br., 2H), 2.18 (s, 3H), 1.86 (m, 4H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 171.39, 158.60, 148.90, 144.05, 139.54, 138.56, 137.66, 135.50, 131.91, 128.88, 128.60, 126.20, 123.44, 122.59, 117.18, 74.70, 64.10, 61.20, 43.34, 25.69, 23.44, 11.09; Anal. ($C_{24}H_{27}FN_2O_5S$) C, H, N; MS-ESI: m/z 497.152 $[M+Na]^+$.

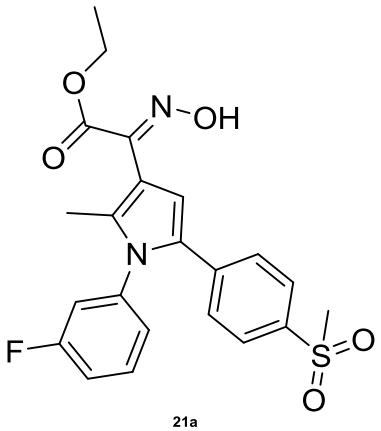
2-Hydroxyethyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4d). Obtained following the procedure as (**4a**), using (**27d**) as starting material.Yellowish powder (>95% yield), m.p. 119°C; FT-IR (neat, cm^{-1}): 3340, 3320, 2852, 1736, 1593, 1302, 1140, 1098; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.90 (d, 2H, *J* = 8.4 Hz), 7.70 (d, 2H, *J* = 8.4 Hz), 7.29 (d, 2H, *J* = 8.4 Hz), 7.08 (m, 2H), 6.56 (s, 1H), 4.78 (t app., 1H), 4.45 (s, 1H), 4.15 (m, 2H), 3.57 (m, 2H), 3.06 (s, 3H), 2.24 (s br., 2H), 2.18 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-*d6*): δ (ppm) 171.34, 159.87, 147.30, 146.78, 141.51, 135.00, 130.32, 129.25, 128.78,

126.20, 123.48, 122.53, 117.12, 64.10, 61.00, 58.88, 44.07, 11.06; Anal. ($C_{22}H_{23}FN_2O_5S$) C, H, N; MS-ESI: m/z 468.107 $[M+Na]^+$.

3-Hydroxypropyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4e). Obtained following the procedure as (**4a**), using (**27e**) as starting material. Yellowish powder (>95% yield), m.p. 107°C; FT-IR (neat, cm^{-1}): 3340, 3320, 2852, 1736, 1593, 1302, 1140, 1098; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.90 (d, 2H, *J* = 8.4 Hz), 7.70 (d, 2H, *J* = 8.4 Hz), 7.29 (d, 2H, *J* = 8.4 Hz), 7.08 (m, 2H), 6.56 (s, 1H), 4.78 (t app., 1H), 4.45 (s, 1H), 4.15 (m, 2H), 3.57 (m, 2H), 3.06 (s, 3H), 2.24 (s br., 2H), 2.18 (s, 3H), 1.90 (m, 2H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 171.30, 159.87, 147.30, 146.78, 141.51, 135.00, 130.32, 129.25, 128.78, 126.20, 123.48, 122.53, 117.12, 64.10, 61.00, 58.88, 43.07, 27.62, 11.06; Anal. ($C_{23}H_{25}FN_2O_5S$) C, H, N; MS-ESI: m/z 483.137 $[M+Na]^+$.

4-Hydroxybutyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (4f). Obtained following the procedure as (**4a**) using (**27f**) as starting material. Yellowish powder, m.p. 101°C (>95% yield);); FT-IR (neat, cm^{-1}): 3349, 3324, 2861, 1731, 1588, 1298, 1132, 1079; ^{1}H-NMR (400 MHz, DMSO-d6) δ (ppm): 7.72 (d, 2H, *J* = 8.5 Hz), 7.37 (d, 2H, *J* = 8.5 Hz), 7.15 (d, 2H, *J* = 8.5 Hz), 7.09 (m, 2H), 6.60 (s, 1H), 4.78 (t app., 1H), 4.41 (s, 1H), 4.17 (m, 2H), 3.55 (m, 2H), 3.10 (s, 3H), 2.20 (s br., 2H), 2.19 (s, 3H), 1.89 (m, 4H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 171.31, 159.90, 147.40, 146.71, 141.50, 135.02, 130.32, 129.67, 128.80, 126.21, 123.43, 122.51, 117.03, 74.34, 64.10, 60.90, 43.11, 24.62, 22.88, 11.16; Anal. ($C_{24}H_{27}FN_2O_5S$) C, H, N; MS-ESI: m/z 497.152 $[M+Na]^+$.

Ethyl 2-(hydroxyimino)-2-[2-methyl-5-[4-(methylsulfonyl)phenyl]-1-(3-fluorophenyl)-1H-pyrrol-3-yl]acetate (20a).



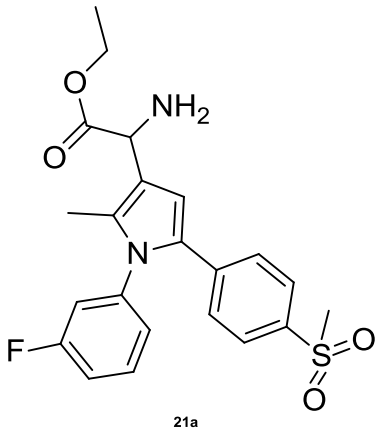
21a

Hydroxylamine hydrochloride (6.07 g, 0.0874 mol) and sodium acetate (11.9g, 0.1456 mol) were added to a suspension of (**15b**) (25g, 0.0582 mol) in ethanol/1,4-dioxane (100 mL, 1:1 *v*/*v*). The resulting solution was heated at 90 °C under stirring for 40 hours. The resulting mixture was concentrated *in vacuo*, extracted with ethyl acetate (500 mL) and then washed with water (100 mL). The organic phase was dried over sodium sulfate, filtered and concentrated to afford the oxime (**20a**) as yellowish solid. Yellowish solid (79% yield), m.p. 104 °C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.73 (d, 2H, *J* = 8.5 Hz), 7.33 (m, 4H), 7.18 (d, 2H, *J* = 8.5 Hz), 6.55 (s, 1H), 5.67 (s, 1H), 4.18 (q, 2H), 3.14 (s, 3H), 2.05 (s, 3H), 1.18 (t, 3H); ^{13}C-NMR (100 MHz, DMSO-d6) δ (ppm): 170.10, 159.66, 149.51, 144.67, 141.33, 138.78, 135.00, 130.75, 129.20, 128.22, 126.06, 123.40, 122.50, 120.66, 118.34, 115.37, 65.99, 43.10, 27.62, 11.06; Anal. ($C_{22}H_{21}FN_2O_5S$) C, H, N; MS-ESI: m/z 467.105 $[M+Na]^+$.

Ethyl 2-(hydroxyimino)-2-[2-methyl-5-[4-(methylsulfonyl)phenyl]-1-(4-fluorophenyl)-1H-pyrrol-3-yl]acetate (20b). Obtained following the procedure as (**20a**), using compound (**15c**) as starting material. Yellowish solid (80% yield), m.p. 103 °C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.73 (d, 2H, *J* = 8.5 Hz), 7.33 (m, 4H), 7.18 (d, 2H, *J* = 8.5 Hz), 6.55 (s, 1H), 5.67 (s, 1H), 4.18 (q, 2H, *J* = 7.4 Hz), 3.14 (s, 3H), 2.05 (s, 3H), 1.18 (t, 3H, *J* = 7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-*d6*): δ (ppm) 170.02, 159.70, 149.49, 144.50, 141.33, 135.20, 130.25, 129.20, 128.22,

126.11, 123.45, 122.50, 118.03, 115.34, 65.84, 43.15, 27.60, 10.96; Anal. ($C_{22}H_{21}FN_2O_5S$) C, H, N; MS-ESI: m/z 467.105 $[M + Na]^+$.

Ethyl 2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (21a).

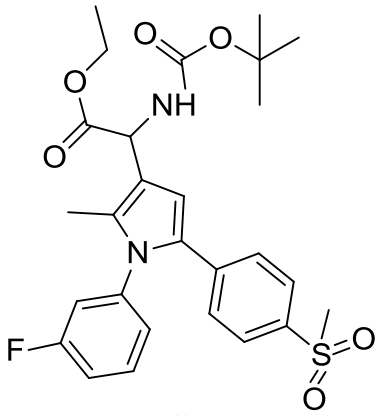


21a

Formic acid (100 mL) was added to a solution of the crude oxime (**20a**) in ethanol (100 mL) and the solution was cooled to °C. Then zinc dust (0.2522 mol) was added in portions over a period of 1h. The mixture was allowed to warm up to rt very slowly and then stirred for 19 h. Afterwards, zinc salts were filtered off and the solvents evaporated under reduced pressure. The residue obtained was dissolved in water and the pH adjusted to 10 with 10% NaOH solution and extracted with EtOAc. The combined organic fractions were dried over sodium sulfate, filtered and concentrated in vacuo to afford the crude product. The material was then purified by column chromatography (silica gel) eluting with MeOH/CHCl3 (1:20) (v/v) to afford the product. White powder (47% yield), m.p. 97 °C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.73 (d, 2H, *J* = 8.4 Hz), 7.32 (m, 4H), 7.19 (d, 2H, *J* = 8.4 Hz), 6.59 (s, 1H), 4.76 (app. t, 1H), 4.09 (q, 2H, *J* = 7.4 Hz), 3.16 (s, 3H), 2.05 (s, 3H), 1.74 (s, broad 2H), 1.16 (t, 3H, *J* = 7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 169.89, 159.27, 143.85, 140.59, 135.72, 130.93, 128.96, 128.13, 125.89, 124.91, 123.92, 122.48, 117.02, 114.84, 108.04, 65.95, 57.99, 42.97, 27.31, 11.02; Anal. ($C_{22}H_{23}FN_2O_4S$) C, H, N; MS-ESI: m/z 453.126 [M+Na]+;

Ethyl 2-amino-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetate (21b). Obtained following the same procedure as (**21a**), using (**20b**) as starting material. White powder (50% yield), m.p. 95°C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.73 (d, 2H, *J* = 8.5 Hz), 7.30 (m, 4H), 7.18 (d, 2H, *J* = 8.5 Hz), 6.55 (s, 1H), 4.60 (s, 1 H), 4.04 (q, 2H, *J* = 7.3 Hz), 3.14 (s, 3H), 2.05 (s, 3H), 1.74 (s broad, 2H), 1.18 (t, 3H, *J* = 7.3 Hz); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 169.77, 159.42, 144.50, 141.33, 135.20, 130.25, 129.20, 128.22, 126.11, 123.45, 122.50, 115.34, 108.59, 65.84, 58.23, 43.33, 27.60, 10.96; Anal. ($C_{22}H_{23}FN_2O_4S$) C, H, N; MS-ESI: m/z 453.126 $[M+Na]^+$.

***tert*-Butyl (ethoxycarbonyl)-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]methyl carbamate (22a).**



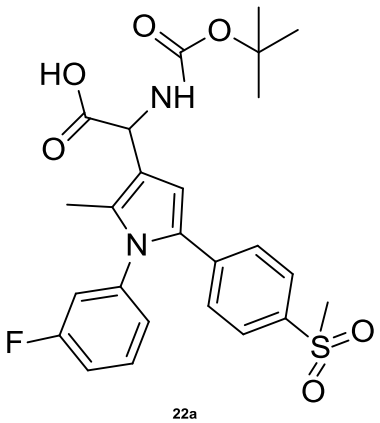
22a

Boc anhydride (1.01g, 0.004651 mol) was added to a solution of the amino ester (**21a**) (1g, 0.002326 mol) in MeOH (15 mL) and the reaction mixture stirred for 18h. The mixture was then diluted with water, adjusted the pH to 2-3 with 1N HCl and extracted with EtOAc. The combined organic fractions were washed with water. Dried over sodium sulfate and evaporated to give the crude product. The residue was purified by column chromatography (silica gel) eluting with EtOAc/hexane (1:20) (v/v) to afford the desired product as white solid. White solid (67% yield), m.p. 109°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.69 (d, 2H, *J* = 8.4 Hz), 7.35 (m, 1H), 7,13 (d, 2H, *J* = 8.4 Hz), 7.09 (m, 1H), 6.90 (m, 2H), 6.59 (s, 1H), 5.65 (d, 1H, *J* = 7.8 Hz), 5.39 (d, 1H, *J* = 7.8

Hz), 4.13 (d, 2H, *J* = 6.8 Hz), 3.17(s, 3H), 2.06 (s, 3H), 1.44 (s, 9H), 1.14 (t, 3H, *J* = 6.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 171.32, 168.35, 154.93, 139.72, 137.21, 135.81, 132.86, 128.98, 127.84, 126.75, 122.47, 121.44, 118.92, 117.76, 117.58, 108.43, 81.46, 63.87, 53.96, 43.82, 29.04, 27.27, 11.10; Anal. ($C_{27}H_{31}FN_2O_6S$) C, H, N; MS-ESI: m/z 553.597 $[M+Na]^+$.

tert-Butyl (ethoxycarbonyl)-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]methyl carbamate (22b). Obtained following the same procedure as (**22a**), using (**21b**) as starting material. White solid (63 % yield), m.p. 105°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, *J* = 8.5 Hz), 7.20 (m, 4H), 7.05 (d, 2H, *J* = 8.5 Hz), 6.51 (s, 1H), 5.67 (d, 1H, *J* = 7.8 Hz), 5.40 (d, 1H, *J* = 7.8 Hz), 4.15 (q, 2H, *J* = 6.8 Hz), 3.15 (s, 3H), 2.05 (s, 3H), 1.45 (s, 9H), 1,13 (t, 3H, *J* = 6.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 171.21, 163.43, 158.20, 140.28, 139.37, 135.81, 132.96, 128.87, 127.93, 126.75, 122.47, 121.34, 118.92, 117.55, 81.68, 65.79, 54.20, 44.25, 28.61, 27.22, 11.20; Anal. ($C_{27}H_{31}FN_2O_6S$) C, H, N; MS-ESI: m/z 553.597 $[M+Na]^+$.

***N-tert*-Butyl-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-*1H*-pyrrol-3-yl]-2-amino-acetic acid (23a).**



22a

A solution of NaOH (0.005917) in water (3 mL) was added to a solution of the amino ester (**22a**) (0.002326 mol) in MeOH (15 mL), and the reaction mixture stirred for 17h. The mixture was then concentrated in vacuo, diluted with water (2mL) and the acid precipitated through the addition of HCl. The solid was collected to give the product as off white solid. White solid (64%

yield), m.p. 128 °C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 12.75 (s, 1H), 7.85 (d, 1H, *J* = 7.8 Hz), 7.72 (d, 2H, *J* = 8.4 Hz), 7.37 (m, 1H), 7.16 (d, 2H, *J* = 8.4 Hz), 7.12 (m, 1H), 6.90 (m, 1H), 6.64 (s, 1H), 5.10 (d, 2H, *J* = 7.8 Hz), 3,15 (s, 3H), 2.10 (s, 3H), 1.45 (s, 9H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 170.84, 169.17, 155.62, 140.23, 138.12, 136.04, 133.11, 129.01, 128.13, 126.88, 123.98, 122.21, 117.45, 117.32, 116.82, 108.99, 82.31, 63.74, 55.05, 42.46, 29.00, 18.26, 10.90; Anal. ($C_{25}H_{27}FN_2O_6S$) C, H, N; ESI-Mass: m/z 525.147 $[M+Na]^+$.

N-tert-Butyl-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]-2-amino-acetic acid (23b). Obtained following the same procedure as (**23a**), using (**22b**) as starting material. White solid (60% yield), m.p. 120°C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 12.70 (s, 1H), 7.81 (d, 1H, *J* = 7.8 Hz), 7.75 (d, 2H, *J* = 8.5 Hz), 7.35 (m, 4H), 7.15 (d, 2H, *J* = 8.5 Hz), 6.63 (s, 1H), 5.09 (d, 1H, *J* = 7.8 Hz), 3.17 (s, 3H), 2.02 (s, 3H), 1.43 (s, 9H); ^{13}C-NMR (100 MHz, DMSO-d6) δ (ppm): 170.23, 162.38, 157.75, 140.28, 139.77, 136.23, 133.06, 128.71, 127.86, 126.88, 121.98, 122.01, 119.32, 117.33, 82.01, 53.87, 44.15, 28.53, 11.11; Anal. ($C_{25}H_{27}FN_2O_6S$) C, H, N; MS-ESI: m/z 525.147 $[M+Na]^+$.

2-(Nitroxy)ethyl N-tert-butyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24a). Obtained following the same procedure as (**1a**), using (**23a**) as carboxylic acid partner. White solid (71% yield), m.p. 129°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.83 (d, 2H, *J* = 8.4 Hz), 7.41 (m, 1H), 7.11 (m, 3H), 6.94 (m, 2H), 6.59 (s, 1H), 5.55 (d, 1H, *J* = 7.8 Hz), 5.30 (d, 1H, *J* = 7.8 Hz), 4.73 (t, 2H, *J* = 7.0 Hz), 4.24 (t, 2H, *J* = 7.0 Hz), 3.05 (s, 3H), 2.17 (s, 3H), 1.40 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 170.27, 168.88, 154.23, 141.40, 138.07, 136.29, 134.52, 130.01, 128.13, 126.68, 123.12, 121.43, 117.32, 117.06, 118.11, 108.31, 81.93, 62.85, 60.56, 54.82, 43.83, 28.51, 27.31, 10.90; Anal. ($C_{27}H_{32}FN_3O_9S$) C, H, N; MS-ESI: m/z 614.14 $[M+Na]^+$.

3-(Nitroxy)propyl] N-tert-butyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24b). Obtained following the same procedure as (**1a**), using (**23a**) and (**19b**) as coupling partners. White solid (71% yield),

m.p. 114°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.83 (d, 2H, *J* = 8.4 Hz), 7.40 (m, 1H), 7.12 (m, 3H), 6.90 (m, 2H), 6.65 (s, 1H), 5.55 (d, 1H, *J* = 7.8 Hz), 5.30 (d, 1H, *J* = 7.8 Hz), 4.71 (t, 2H, *J* = 7.0 Hz), 4.30 (t, 2H, *J* = 7.0 Hz), 3.05 (s, 3H), 2.17 (s, 3H), 1.99 (m, 2H), 1.40 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 170.27, 168.88, 154.23, 141.40, 138.07, 136.29, 134.52, 130.01, 128.13, 126.68, 123.12, 121.43, 117.32, 117.06, 118.11, 108.31, 81.93, 62.85, 60.56, 54.82, 43.83, 28.51, 27.31, 10.90; Anal. ($C_{27}H_{32}FN_3O_9S$) C, H, N; MS-ESI: m/z 628.174 $[M+Na]^+$.

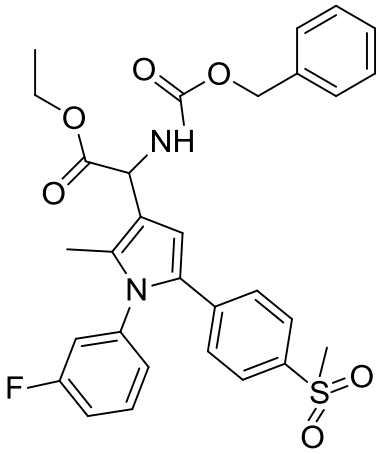
4-(Nitroxy)butyl N-tert-butyl-(ethoxycarbonyl)-2-[1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]-2-amino-acetate (24c). Obtained following the same procedure as (**1a**), using (**23a**) and (**19c**) as coupling partners. White solid, m.p. 118°C (68% yield) 1H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.83 (d, 2H, *J* = 8.5 Hz), 7.40 (m, 1H), 7.12 (m, 3H), 6.90 (m, 2H), 6.65 (s, 1H), 5.55 (d, 1H, *J* = 7.8 Hz), 5.30 (d, 1H, *J* = 7.8 Hz), 4.70 (t, 2H, *J* = 7.6 Hz), 4.11 (t, 2H, *J* = 7.6 Hz), 3.05 (s, 3H), 2.17 (s, 3H), 1.71 (m, 2H), 1.63 (m, 2H), 1.40 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 169.82, 167.54, 153.90, 142.04, 138.75, 135.81, 133.81, 129.85, 128.00, 127.41, 122.31, 123.56, 117.20, 117.06, 117.33, 107.02, 79.68, 65.79, 62.09, 54.82, 44.28, 27.94, 26.82, 22.54, 12.03; Anal. ($C_{28}H_{34}FN_3O_9S$) C, H, N; MS-ESI: m/z 642.190 $[M+Na]^+$.

2-(Nitroxy)ethyl N-tert-butyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24d). Obtained following the same procedure as (**1a**), using (**23b**) as carboxylic acid partner. White solid (76% yield), m.p. 131°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75 (d, 2H, *J* = 8.4 Hz), 7.35 (m, 4H), 7.13 (d, 2H, *J* = 8.4 Hz), 6.59 (s, 1H), 5.59 (d, 1H, *J* = 6.8 Hz), 5.33 (d, 1H, *J* = 6.8 Hz), 4.69 (t, 2H, *J* = 7.0 Hz), 4.25 (t, 2H, *J* = 7.0 Hz), 3.09 (s, 3H), 2.11 (s, 3H), 1.46 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 171.24, 168.72, 155.24, 138.72, 135.83, 134.88, 132.50, 129.21, 127.17, 126.74, 123.02, 123.33, 118.86, 117.32, 81.97, 64.05, 61.34, 52.94, 44.03, 29.50, 27.82, 11.24; Anal. ($C_{27}H_{32}FN_3O_9S$) C, H, N; MS-ESI: m/z 614.15 $[M+Na]^+$.

3-(Nitroxy)propyl N-tert-butyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24e). Obtained following the same procedure as (**1a**), using (**23b**) and (**19b**) as coupling partners. White solid (73% yield), m.p. 112°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75 (d, 2H, *J* = 8.4 Hz), 7.35 (m, 4H), 7.15 (d, 2H, *J* = 8.4 Hz), 6.63 (s, 1H), 5.59 (d, 1H, *J* = 6.8 Hz), 5.33 (d, 1H, *J* = 6.8 Hz), 4.64 (t, 2H, *J* = 7.0 Hz), 4.28 (t, 2H, *J* = 7.0 Hz), 3.05 (s, 3H), 2.15 (s, 3H), 1.98 (m, 2H), 1.46 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 171.24, 168.72, 155.24, 138.72, 135.83, 134.88, 132.50, 129.21, 127.17, 126.74, 123.02, 123.33, 118.86, 117.32, 81.97, 64.05, 61.34, 52.94, 44.03, 29.50, 27.82, 11.24; Anal. ($C_{27}H_{32}FN_3O_9S$) C, H, N; MS-ESI: m/z 628.174 $[M+Na]^+$.

4-(Nitroxy)butyl N-tert-butyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (24f). Obtained following the same procedure as (**1a**), using (**23b**) and (**19c**) as coupling partners. White solid (75% yield), m.p. 114°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75 (d, 2H, *J* = 8.5 Hz), 7.36 (m, 4H), 7.15 (d, 2H, *J* = 8.5 Hz), 6.65 (s, 1H), 5.59 (d, 1H, *J* = 6.8 Hz), 5.33 (d, 1H, *J* = 6.8 Hz), 4.65 (t, 2H, *J* = 7.6 Hz), 4.10 (t, 2H, *J* = 7.6 Hz), 3.05 (s, 3H), 2.15 (s, 3H), 1.73 (m, 2H), 1.58 (m, 2H), 1.46 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 170.82, 169.04, 156.23, 139.01, 135.82, 134.65, 132.46, 129.02, 126.89, 125.38, 124.21, 123.13, 118.15, 116.85, 109.95, 81.88, 65.82, 63.00, 53.28, 44.03, 25.24, 23.82, 10.98; Anal. ($C_{28}H_{34}FN_3O_9S$) C, H, N; MS-ESI: m/z 642.19 $[M+Na]^+$.

_N_-Benzyl-(ethoxycarbonyl)-[1-(3-fluorophenyl)-2-methyl-5-[4 (methylsulfonyl)phenyl]-1H-pyrrol-3-yl] methylcarbamate (25a).



25a

To a biphasic solution of amino ester (**21a**) (0.002326 mol) in DCM (10 mL), sodium carbonate (0.0046 mol) and benzyl chloroformate (0.002352 mol) were added at 0 °C, the reaction mixture was allowed to warm up to rt and stirred for further 4h. The mixture was then diluted with water and the two phases separated. The organic fraction was washed with water (50 mL × 2), dried over sodium sulfate and evaporated to give the crude product. The residue was purified by column chromatography (silica gel) eluting with EtOAc/hexane (1:20) (v/v) to afford the desired product as white solid. White solid (61% yield), m.p. 97°C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 8.01 (d, 1H, *J* = 7.8 Hz), 7.89 (d, 2H, *J* = 8.5 Hz), 7.59 (m, 6H), 7.46 (m, 3H), 7.06 (m, 2H), 6.48 (s, 1H), 5.18 (d, 1H, *J* = 7.8 Hz), 5.15 (s, 2H), 4.16 (q, 2H, *J* = 6.8 Hz), 3.15 (s, 3H), 2.04 (s, 3H), 1.17 (t, 3H, J = 6.8 Hz); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 170.23, 169.27, 155.08, 140.24, 139.23, 137.21, 136.03, 132.78, 130.58, 129.05, 128.04, 127.70, 127.12, 126.65, 124.55, 123.09, 119.25, 118.33, 117.67, 107.28, 67.98, 64.77, 54.05, 44.66, 15.62, 12.03; Anal. ($C_{30}H_{29}FN_2O_6S$) C, H, N; MS-ESI: m/z 587.163 $[M + Na]^+$.

***N*-Benzyl-(ethoxycarbonyl)-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]methylcarbamate (25b).** Obtained following the same procedure as (**25a**), using (**24b**) as starting material. White solid (65% yield), m.p. 95°C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.89 (d, 1H, *J* = 7.8 Hz), 7.74 (d, 2H, *J* = 8.5 Hz), 7.51 (m, 5H), 7.19 (m, 6H), 6.50 (s, 1H), 5.16 (d, 1H, *J* = 7.8 Hz), 5.08 (s, 2H), 4.11 (q, 2H, *J* = 6.9 Hz), 3.10 (s, 3H), 2.00 (s, 3H), 1.15 (t, 3H, *J* = 6.9 Hz); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 168.45, 159.71, 156.04, 142.96, 132.28, 138.86, 137.12, 135.98, 133.27, 130.34, 127.96, 126.51, 126.23, 125.38, 123.91, 122.54, 118.07, 117.44, 67.45, 64.31, 54.11, 43.89, 15.46, 12.01; Anal. ($C_{30}H_{29}FN_2O_6S$) C, H, N; MS-ESI: m/z 587.163 $[M+Na]^+$.

N-(Benzyloxy)carbonyl-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]-2-amino-acetic acid (26a). Obtained following the same procedure as (**23a**), using (**25a**) as starting material. White solid (>95% yield), m.p. 127°C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 12.81 (s, 1H), 7.89 (d, 1H, *J* = 7.8 Hz), 7.70 (d, 2H, *J* = 8.6 Hz), 7.52 (m, 1H), 7.47 (m, 7H), 7.32 (d, 2H, *J* = 8.6 Hz), 7.21 (m, 1H), 6.64 (m, 1H), 5.11 (d, 1H, *J* = 7.8 Hz), 5.09 (s, 2H), 3.15 (s, 3H), 2.09 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 171.22, 168.97, 155.85, 141.02, 139.18, 138.12, 136.58, 132.93, 128.56, 128.02, 127.90, 126.76, 126.54, 126.00, 124.21, 124.00, 117.11, 116.50, 115.99, 106.97, 63.74, 55.05, 42.46, 11.02; Anal. $C_{28}H_{25}FN_2O_6S$ (C, H, N); MS-ESI: m/z 559.132$[M+Na]^+$.

N-(Benzyloxy)carbonyl-2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]-2-amino-acetic acid (26b). Obtained following the same procedure as (**23a**), using (**25b**) as starting material. White solid (>95% yield), m.p. 130°C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 12.81 (s, 1H), 7.81 (d, 1H, *J* = 7.8 Hz), 7.79 (d, 2 H, *J* = 8.5 Hz), 7.68-7.37 (m, 9H), 7.16 (d, 2H, *J* = 8.5 Hz), 6.63 (s, 1H), 5.09 (d, 1H, *J* = 7.8 Hz), 5.06 (s, 2H), 3.21 (s, 3H), 2.03 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 171.03, 169.27, 156.98, 143.08, 140.23, 136.91, 134.21, 133.09, 130.88, 127.97, 127.34, 126.32, 124.55, 122.91, 119.00, 118.23, 117.43, 106.99, 63.21, 53.92, 43.71, 11.18; Anal. ($C_{28}H_{25}FN_2O_6S$) C, H, N; MS-ESI: m/z 559.13 $[M+Na]^+$.

2-(Nitroxy)ethyl N-benzyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino acetate (27a). Obtained following the same procedure as (**1a**), using (**26a**) as carboxylic acid partner. White solid (66% yield), 115 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, *J* = 8.5 Hz), 7.52-7.46 (m, 1H), 7.46 (m, 7H), 7.30 (d, 2H, *J* = 8.5 Hz), 7.21 (m, 1H), 6.64 (m, 1H), 5.52 (d, 1H, *J* = 7.8 Hz), 5.29 (d, 1H, *J* = 7.8 Hz), 5.10 (s, 2H), 4.69 (t, 2H, *J* = 6.9 Hz), 4.29 (t, 2H, *J* = 6.9 Hz), 3.00 (s, 3H), 2.20 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 170.83, 169.04, 155.28, 142.47, 142.06, 138.07, 136.29, 134.31, 130.83, 128.79, 127.64, 127.32, 127.13, 126.40, 124.08, 121.31, 117.51, 117.08, 116.92, 108.09, 67.20, 63.04, 60.93, 54.82, 43.83, 28.51, 10.90; Anal. ($C_{30}H_{28}FN_3O_9S$) C, H, N; MS-ESI: m/z 648.17 $[M+Na]^+$.

3-(Nitroxy)propyl N-benzyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino acetate (27b). Obtained following the same procedure as (**1a**), using (**26a**) and (**19b**) as coupling partners. White solid (66% yield), 115 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, *J* = 8.5 Hz), 7.52 (m, 1H), 7.47 (m, 7H), 7.32 (d, 2H, *J* = 8.5 Hz), 7.21 (m, 1H), 6.64 (m, 1H), 5.50 (d, 1H, *J* = 7.8 Hz), 5.29 (d, 1H, *J* = 7.8 Hz), 5.10 (s, 2H), 4.67 (t, 2H, *J* = 6.9 Hz), 4.29 (t, 2H, *J* = 6.9 Hz), 3.00 (s, 3H), 2.20 (s, 3H), 1.95 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 170.83, 169.04, 155.28, 142.47, 142.06, 138.07, 136.29, 134.31, 130.83, 128.79, 127.64, 127.32, 127.13, 126.40, 124.08, 121.31, 117.51, 117.08, 116.92, 108.09, 67.20, 63.04, 60.93, 54.82, 43.83, 28.51, 10.90; Anal. ($C_{31}H_{30}FN_3O_9S$) C, H, N; MS-ESI: m/z 662.158 $[M+Na]^+$.

4-(Nitroxy)butyl N-benzyl-(ethoxycarbonyl)-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino acetate (27c). Obtained following the same procedure as (**1a**), using (**26a**) and (**19c**) as coupling partners. White solid (64% yield), 119 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, *J* = 8.4 Hz), 7.55 (m, 1H), 7.47 (m, 7H), 7.29 (d, 2H, *J* = 8.4 Hz), 7.21 (m, 1H), 6.64 (s, 1H), 5.48 (d, 1H, *J* = 7.7 Hz), 5.31 (d, 1H, *J* = 7.7 Hz), 5.12 (s, 2H), 4.60 (t, 2H, *J* = 7.0 Hz), 4.07 (t, 2H, *J* = 7.0 Hz), 3.05 (s, 3H), 2.10 (s, 3H), 1.75 (m, 2H),

1.60 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 170.92, 169.00, 155.20, 142.45, 142.21, 138.07, 136.29, 134.31, 130.83, 128.79, 127.64, 127.32, 127.03, 126.41, 124.08, 121.89, 117.51, 117.08, 116.97, 108.09, 67.20, 63.44, 61.45, 54.82, 43.83, 27.33, 28.51, 10.91; Anal. ($C_{32}H_{32}FN_3O_9S$) C, H, N; MS-ESI: m/z 676.18 $[M+Na]^+$.

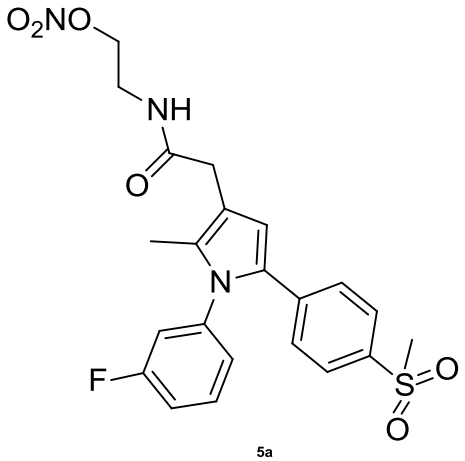
2-(Nitroxy)ethyl N-benzyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (27d). Obtained following the same procedure as (**1a**), using (**26b**) as carboxylic acid partner. White solid (62% yield), 118 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.70 (d, 2 H, *J* = 8.4 Hz), 7.54-7.49 (m, 9H), 7.16 (d, 2 H, *J* = 8.4 Hz), 6.64 (s, 1H), 5.54 (d, 1H, *J* = 7.7 Hz), 5.32 (d, 1H, *J* = 7.7 Hz), 5.10 (s, 2H), 4.69 (t, 2H, *J* = 6.8 Hz), 4.29 (t, 2H, *J* = 6.8 Hz), 3.00 (s, 3H), 2.20 (s, 3H); ^{13}C-NMR (100 MHz, CDCl3) δ (ppm): 170.83, 168.72, 154.98, 143.23, 139.01, 135.82, 134.65, 132.43, 131.80, 127.98, 127.49, 127.29, 127.17, 126.89, 123.45, 123.22, 119.05, 116.92, 115.88, 66.82, 66.24, 60.45, 44.03, 27.80, 11.24; Anal. ($C_{30}H_{28}FN_3O_9S$) C, H, N; MS-ESI: m/z 648.17 $[M+Na]^+$.

3-(Nitroxy)propyl N-benzyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino-acetate (27e). Obtained following the same procedure as (**1a**), using (**26b**) and (**19b**) as coupling partners. White solid, 118 °C (62% yield); 1H-NMR (400 MHz, CDCl3): δ (ppm) 7.69 (d, 2 H, J = 8.4 Hz), 7.55 (m, 9H), 7.16 (d, 2 H, J = 8.4 Hz), 6.64 (s, 1H), 5.54 (d, 1H, J = 7.7 Hz), 5.32 (d, 1H, J = 7.7 Hz), 5.10 (s, 2H), 4.66 (t, 2H, J = 6.8 Hz), 4.39 (t, 2H, J = 6.8 Hz), 3.00 (s, 3H), 2.20 (s, 3H), 1.95 (m, 2H). 13C-NMR (100 MHz, CDCl3): δ (ppm) 170.83, 168.72, 154.98, 143.23, 139.01, 135.82, 134.65, 132.43, 131.80, 127.98, 127.49, 127.29, 127.17, 126.89, 123.45, 123.22, 119.05, 116.92, 115.88, 66.82, 66.24, 60.45, 44.03, 27.8, 11.24. ESI-Mass: m/z 662.158 [M + Na]+; Anal. C31H30FN3O9S (C, H, N).

4-(Nitroxy)butyl N-benzyl-(ethoxycarbonyl)-2-(1-(4-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)-2-amino acetate (27f). Obtained following the same procedure as (**1a**), using (**26b**) and (**19c**) as coupling partners. White solid (61% yield), 121 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.65 (d, 2 H, *J* = 8.5 Hz), 7.53 (m, 9H), 7.17 (d, 2 H, *J* =

8.5 Hz), 6.64 (s, 1H), 5.55 (d, 1H, *J* = 7.6 Hz), 5.34 (d, 1H, *J* = 7.6 Hz), 5.12 (s, 2H,), 4.65 (t, 2H, *J* = 7.0 Hz), 4.11 (t, 2H, *J* = 7.0 Hz), 3.05 (s, 3H), 2.15 (s, 3H), 1.73 (m, 2H), 1.58 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 171.00, 168.66, 154.99, 143.21, 139.34, 135.04, 134.09, 132.40, 131.81, 128.22, 127.39, 127.21, 127.07, 126.09, 123.31, 123.20, 118.95, 117.55, 116.09, 66.67, 66.04, 60.05, 44.03, 27.81, 27.55, 11.21; Anal. ($C_{32}H_{32}FN_3O_9S$) C, H, N; MS-ESI: m/z 676.18 $[M+Na]^+$.

N-[2-(Nitroxy)ethyl] 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-*1H*-pyrrol-3yl]acetamide (5a).



5a

Nitroxyethylamine (**29a**, nitrate salt) (0.3 mmol), DMAP (0.1 mmol), and EDCI (0.2 mmol) were added in sequence to a solution of 1,5-diarylpyrrole-3-acetic acid (**17b**) (0.1 mmol) in dichloromethane (5 mL), under nitrogen atmosphere. An excess of triethylamine (TEA, 0.35 mmol) was added dropwise and the reaction was stirred at rt for 3h. Then the mixture was quenched with water and extracted with chloroform. The organic layer was washed with 1N HCl, $NaHCO_3$ saturated solution, brine and dried over Na_2SO_4. After filtration and concentration of the organic phase a crude material was obtained. The material was then purified by chromatography on silica gel/alumina (1:1) using petroleum ether/chloroform/ethyl acetate, 4:4:1 (v/v/v), as the eluent to give the desired product in good yield. Yellow powder (yield 80%), mp 133°C.; FT-IR (neat, cm^{-1}): 3300, 1640, 1595, 1520, 1278, 865; ^{1}H-NMR (400 MHz,

$CDCl_3$) δ (ppm): 7.71 (d, 2H, *J* = 8.6 Hz), 7.18 (m, 6H), 6.40 (s, 1H), 6.12 (s broad, 1H), 4.59 (t, 2H, *J* = 7.6 Hz), 3.63 (t, 2H, *J* = 78.6 Hz), 3.49 (s, 2H), 3.02 (s, 3H), 2.04 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.89, 32.21, 38.41, 44.61, 69.77, 113.06, 113.44, 115.90, 120.71, 125.59, 127.10, 130.66, 130.71, 131.30, 131.83, 136.12, 138.23, 169.45. Anal. ($C_{22}H_{22}FN_3O_6S$) C, H, N; MS-ESI: m/z 475.12 $[M+H]^+$.

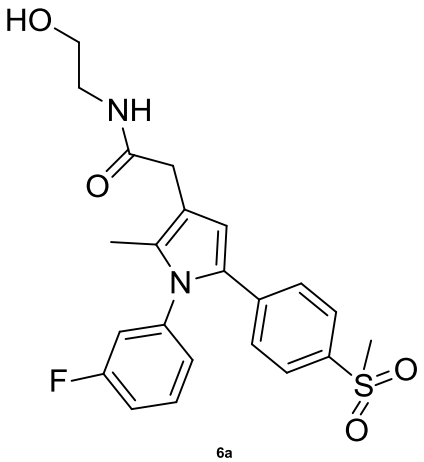
N-[3-(Nitroxy)propyl] 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (5b). Obtained with the same procedure as (**5a**), using (**29b**) as coupling partner. Yellowish powder (yield 76%), m.p. 125 °C; FT-IR (neat, cm^{-1}): 3310, 1642, 1597, 1515, 1280, 899; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.73 (d, 2H, *J* = 8.6 Hz), 7.20 (m, 6H), 6.40 (s, 1H), 6.12 (s broad, 1H), 4.59 (t, 2H, *J* = 7.6 Hz), 3.63 (t, 2H, *J* = 78.6 Hz), 3.49 (s, 2H), 3.02 (s, 3H), 2.04 (s, 3H), 1.88 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.78, 31.10, 32.20, 38.35, 44.55, 69.70, 113.09, 113.50, 115.81, 120.66, 125.43, 127.17, 130.46, 130.21, 131.270, 131.80, 136.32, 138.41, 169.49; Anal. ($C_{23}H_{24}FN_3O_6S$) C, H, N; MS-ESI: m/z 489.14 $[M+H]^+$.

N-[2-(Nitroxy)ethyl] 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (5c). Obtained with the same procedure as (**5a**), using (**17c**) as coupling partner. Yellowish powder (yield 81%), mp 135 °C; FT-IR (neat, cm^{-1}): 3300, 1640, 1520, 1278, 1595, 865; ^{1}H-NMR (400 MHz, $CDCl_3$) ppm: 7.70 (d, 2H, *J* = 8.6 Hz), 7.23 (m, 6H), 6.43 (s, 1H), 6.12 (s broad, 1H), 4.59 (t, 2H, *J* = 7.6 Hz), 3.63 (t, 2H, *J* = 7.6 Hz), 3.49 (s, 2H), 3.02 (s, 3H), 2.09 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.77, 32.31, 38.27, 44.59, 69.60, 112.81,113.66, 115.71, 120.28, 124.45, 130.09, 130.69, 131.33, 135.07, 136.80, 138.110, 139.35, 169.60; Anal. ($C_{22}H_{22}FN_3O_6S$) C, H, N; MS-ESI: m/z 475.12 $[M+H]^+$.

N-[3-(Nitroxy)propyl] 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (5d). Obtained with the same procedure as (**5a**), using (**17c**) and (**29b**) as coupling partners. Yellowish powder, m.p. 125 °C (yield 76%). FT-IR cm-1: 3310, 1642, 1597, 1515, 1280, 899. ^{1}H-NMR (400 MHz, $CDCl_3$) ppm: 7.67 (d, 2H, *J* = 8.6 Hz), 7.20 (m, 6H), 6.44 (s, 1H), 6.15 (s broad, 1H), 4.61 (t, 2H, *J* = 7.6 Hz), 3.65 (t, 2H, *J* = 7.6 Hz), 3.47 (s, 2H), 3.00 (s, 3H),

2.07 (s, 3H), 1.89 (m, 2H). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.80, 30.15, 32.21, 38.25, 44.61, 69.60, 112.81, 113.66, 115.71, 120.25, 124.45, 130.12, 130.69, 131.30, 135.11, 136.81, 138.11, 139.30, 169.57; Anal. ($C_{23}H_{24}FN_3O_6S$) C, H, N; MS-ESI: m/z 489.14 $[M+H]^+$.

N-[2-(Hydroxy)ethyl] 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (6a).



6a

Ethanolamine (**28a**) (1.0 mmol), HOBt (0.9 mmol), and EDCI (1.2 mmol) were added in sequence to a solution of 1,5-diarylpyrrole-3-acetic acid (**17b**) (0.7 mmol) in dichloromethane (20 mL), under nitrogen atmosphere. An excess of TEA (2.0 mmol) was added dropwise and the reaction was stirred at rt for 3h. Then the mixture was quenched with water and extracted with chloroform. The organic layer was washed with 1N HCl, $NaHCO_3$ saturated solution, brine and dried over Na_2SO_4. After filtration and concentration of the organic phase a crude material was obtained. The material was then purified by chromatography on silica gel using petroleum ether/chloroform/ethyl acetate, 3:2:1 (v/v/v), as the eluent to give the desired product in good yield. Yellowish powder (77% yield), mp 97 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.69 (d, 2H, *J* = 8.6 Hz), 7.40 (m, 1H), 7.19 (d, 2H, *J* = 8.6 Hz), 7.13 (m, 1H), 6.96 (m, 1H), 6.87 (m, 1H), 6.47 (s, 1H), 6.40 (s broad, 1H), 3.70 (t, 2H, *J* = 7.4 Hz), 3.48 (s, 2H), 3.40 (m, 2H), 3.00 (s, 3H), 2.70 (s broad, 1H), 2.04 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.89, 32.21, 36.41, 44.61, 60.17,

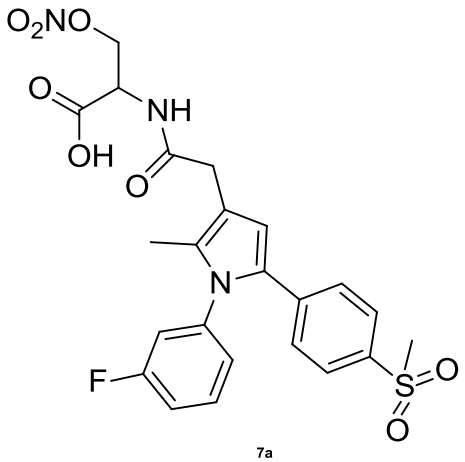
113.08, 113.54, 115.88, 120.31, 125.61, 127.12, 130.56, 130.44, 131.37, 131.85, 136.32, 138.63, 169.47; Anal. ($C_{22}H_{23}FN_2O_4S$) C, H, N; MS-ESI: m/z 430.14 [M+H$^+$].

N-[3-(Hydroxy)propyl] 2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (6b). Obtained with the same procedure as (**6a**), using (**28b**) as coupling partner. Yellowish powder (yield 76%), m.p. 99 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, *J* = 8.6 Hz), 7.17 (m, 6H), 6.45 (s, 1H), 6.37 (s broad, 1H), 3.67 (t, 2H, *J* = 7.4 Hz), 3.50 (m, 2H), 3.49 (s, 2H), 3.00 (s, 3H), 2.00 (s, 3H), 1.79 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.67, 31.10, 32.00, 38.27, 44.55, 60.11, 113.09, 113.53, 115.73, 120.56, 125.48, 127.27, 130.46, 130.21, 131.270, 131.56, 136.33, 138.40, 169.39; Anal. ($C_{23}H_{25}FN_2O_4S$) C, H, N; MS-ESI: m/z 444.15 [M+H]$^+$.

N-[2-(Hydroxy)ethyl] 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (6c). Obtained with the same procedure as (**6a**), using (**17c**) as coupling partner. Yellowish powder (yield 81%), mp 102 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.76 (d, 2H, *J* = 8.6 Hz), 7.20 (m, 6H), 6.40 (s, 1H), 6.33 (s broad, 1H), 3.69 (t, 2H, *J* = 7.4 Hz), 3.49 (s, 2H), 3.40 (m, 2H), 3.02 (s, 3H), 2.09 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.77, 32.21, 38.20, 44.39, 60.06, 112.77, 113.46, 116.01, 121.18, 124.50, 130.10, 130.70, 131.43, 135.17, 136.11, 138.18, 139.35, 169.41; Anal. ($C_{22}H_{23}FN_2O_4S$) C, H, N; MS-ESI: m/z 430.15 [M+H]$^+$.

N-[3-(Hydroxy)propyl] 2-[1-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3yl]acetamide (6d). Obtained with the same procedure as (**6a**), using (**17c**) and (**28b**) as coupling partners. Yellowish powder (yield 76%), m.p. 105 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68 (d, 2H, *J* = 8.6 Hz), 7.20 (m, 6H), 6.41 (s, 1H), 6.35 (s broad, 1H), 3.68 (t, 2H, *J* = 7.6 Hz), 3.47 (s, 2H), 3.40 (m, 2H), 3.00 (s, 3H), 2.07 (s, 3H), 1.79 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.69, 30.15, 32.01, 38.25, 44.61, 60.02, 112.85, 113.70, 115.82, 120.33, 124.50, 130.23, 130.59, 131.70, 135.10, 136.81, 138.11, 139.40, 169.47; Anal. ($C_{23}H_{25}FN_2O_4S$) C, H, N; MS-ESI: m/z 444.15 [M+H]$^+$.

(*R,S*)-2-[2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-nitroxypropanoic acid (7a).



7a

PyBop (0.93 mmol) and TEA (3 mmol) are added in sequence to a solution of (**17b**) (0.78 mmol) in THF (20 mL) at rt and the mixture stirred for 1h. Then (**31a**) (1.5 mmol) was added over 5 minutes. After 4 h the reaction is concentrated in vacuo and the oily residue is treated with KOH 1N (10 mL). The solution is filtered through a paper filter. The liquid is collected, cooled to 0 °C and HCl 5N was added dropwise till a white precipitate is formed. The solid obtained is filtered and dried. After recrystallization from ethanol/petroleum ether **7a** is obtained in quite good yield. Yellow powder (yield 80%), mp 110°C; FT-IR (neat, cm^{-1}): 3310, 1734, 1640, 1595, 1520, 1278, 865; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.13 (s broad, 1H), 7.70 (d, 2H, *J* = 8.5 Hz), 7.20 (m, 6H), 6.73 (s broad, 1H), 6.50 (s, 1H), 4.79 (m, 1H), 4.62 (m, 2H), 3.51 (s, 2H), 3.10 (s, 3H), 2.09 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.80, 32.21, 38.41, 55.71, 70.18, 113.22, 113.43, 115.98, 120.56, 125.59, 127.00, 130.66, 130.71, 131.30, 131.80, 136.13, 138.50, 169.67; Anal. ($C_{23}H_{22}FN_3O_8S$) C, H, N; MS-ESI: m/z 519.17 $[M+H]^+$.

(R,S)-2-[2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-nitroxybutanoic acid (7b). Obtained with the same procedure as (**7a**), using **31b** as coupling partner. Yellowish powder (79% yield), m.p. 112 °C; FT-IR (neat, cm^{-1}): 3320, 1735, 1642, 1589, 1524, 1282, 871; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.13 (s broad, 1H), 7.70 (d,

2H, *J* = 8.6 Hz), 7.30 (m, 4H), 7.23 (m, 2H), 6.70 (s broad, 1H), 6.51 (s, 1H), 4.80 (m, 1H), 4.67 (m, 2H), 3.54 (s, 2H), 3.01 (s, 3H), 2.07 (s, 3H), 1.79 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.80, 30.19, 33.01, 38.49, 55.79, 70.08, 113.22, 113.43, 115.98, 120.56, 125.59, 127.11, 130.67, 130.71, 131.34, 131.75, 136.13, 138.54, 169.60; Anal. ($C_{24}H_{24}FN_3O_8S$) C, H, N; MS-ESI: m/z 533.21 $[M+H]^+$.

(R,S)-2-[2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-nitroxypropanoic acid (7c). Obtained with the same procedure as (**7a**), using **17c** as coupling partner. Yellowish powder (81% yield), mp 109 °C; FT-IR (neat, cm^{-1}): 3323, 1735, 1642, 1589, 1524, 1282, 871; ^{1}H-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.10 (s broad, 1H), 7.70 (d, 2H, *J* = 8.6 Hz), 7.23 (m, 6H), 6.50 (s, 1H), 4.79 (m, 1H), 4.62 (m, 2H), 3.51 (s, 2H), 3.10 (s, 3H), 2.09 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.66, 32.31, 38.27, 55.59, 70.60, 112.67, 113.34, 115.19, 120.39, 124.45, 130.32, 130.19, 131.36, 135.11, 136.80, 138.01, 139.39, 169.57; Anal. ($C_{23}H_{22}FN_3O_8S$) C, H, N; MS-ESI: m/z 519.17 $[M+H]^+$.

(R,S)-2-[2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-nitroxybutanoic acid (7d). Obtained with the same procedure as (**7a**), using **17c** and **31b** as coupling partners. Yellowish powder (76% yield), m.p. 116 °C; FT-IR (neat, cm^{-1}): 3310, 1642, 1597, 1515, 1280, 899; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.14 (s broad, 1H), 7.60 (d, 2H, *J* = 8.6 Hz), 7.20 (m, 6H), 6.72 (s broad, 1H), 6.51 (s, 1H), 4.80 (m, 1H), 4.67 (m, 2H), 3.55 (s, 2H), 3.03 (s, 3H), 2.07 (s, 3H), 1.79 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.77, 30.22, 33.17, 38.51, 55.839, 70.10, 112.80, 113.57, 115.70, 120.27, 124.55, 130.12, 130.48, 131.27, 135.10, 136.80, 138.11, 139.30, 169.50; Anal. ($C_{24}H_{24}FN_3O_8S$) C, H, N; MS-ESI: m/z 533.21 $[M+H]^+$.

(R,S)-2-[2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-hydroxypropanoic acid (8a). Obtained with the same procedure as (**7a**), using **30a** as coupling partner. Yellow powder (80% yield), mp 118°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.17 (s broad, 1H), 7.71 (d, 2H, *J* = 8.5 Hz), 7.23 (m, 6H), 6.78 (s broad, 1H),

6.51 (s, 1H), 4.77 (m, 1H), 4.12 (m, 2H), 3.51 (s, 2H), 3.10 (s, 3H), 2.76 (s broad, 1H), 2.09 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.84, 32.20, 38.41, 55.34, 65.25, 113.25, 113.40, 115.89, 120.45, 125.69, 127.01, 130.76, 130.70, 131.41, 131.77, 136.21, 138.50, 169.72; Anal. ($C_{23}H_{23}FN_2O_6S$) C, H, N; MS-ESI: m/z 474.12 $[M+H]^+$.

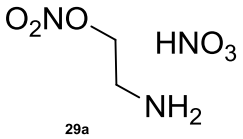
(R,S)-2-[2-[1-(3-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-hydroxybutanoic acid (8b). Obtained with the same procedure as (**7a**), using **30b** as coupling partner. Yellowish powder (79% yield), m.p. 128 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.18 (s broad, 1H), 7.73 (d, 2H, *J* = 8.6 Hz), 7.33 (m, 4H), 7.22 (m, 2H), 6.72 (s broad, 1H), 6.50 (s, 1H), 4.76 (m, 1H), 4.17 (m, 2H), 3.54 (s, 2H), 3.01 (s, 3H), 2.67 (s broad, 1H), 2.07 (s, 3H), 1.79 (m, 2H); ^{13}C-NMR (100 MHz, CDCl3) δ (ppm): 11.67, 30.22, 33.10, 38.38, 55.81, 60.08, 113.32, 113.29, 115.88, 120.51, 125.53, 127.14, 130.69, 130.70, 131.34, 131.70, 136.20, 138.55, 169.68; Anal. ($C_{24}H_{25}FN_2O_6S$) C, H, N; MS-ESI: m/z 488.14 $[M+H]^+$.

(R,S)-2-[2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-hydroxypropanoic acid (8c). Obtained with the same procedure as (**7a**), using (**17c**) and (**30a**) as coupling partners. Yellowish powder (81% yield), mp 113 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.10 (s broad, 1H), 7.73 (d, 2H, *J* = 8.6 Hz), 7.20 (m, 6H), 6.51 (s, 1H), 4.79 (m, 1H), 4.12 (m, 2H), 3.51 (s, 2H), 3.10 (s, 3H), 2.65 (s broad, 1H), 2.09 (s, 3H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.60, 32.30, 38.25, 55.44, 60.55, 112.60, 113.30, 115.09, 120.32, 124.40, 130.30, 130.29, 131.16, 135.10, 136.57, 138.07, 139.19, 169.49; Anal. ($C_{23}H_{23}FN_2O_6S$) C, H, N; MS-ESI: m/z 474.12 $[M+H]^+$.

(R,S)-2-[2-[1-(4-Fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide]-4-hydroxybutanoic acid (8d). Obtained with the same procedure as (**7a**), using **17c** and **30b** as coupling partners. Yellowish powder (76% yield), m.p. 126 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 11.14 (s broad, 1H), 7.60 (d, 2H, *J* = 8.6 Hz), 7.20 (m, 6H), 6.73 (s broad, 1H), 6.51 (s, 1H), 4.75 (m, 1H), 4.17 (m, 2H), 3.58 (s, 2H), 3.05 (s, 3H), 2.62 (s broad, 1H), 2.09 (s, 3H), 1.81 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 11.77, 30.20, 33.21, 38.44, 55.89, 60.10,

112.81, 113.66, 115.70, 120.27, 124.52, 130.10, 130.37, 131.27, 135.10, 136.81, 138.11, 139.33, 169.48; Anal. ($C_{24}H_{25}FN_2O_6S$) C, H, N; MS-ESI: m/z 533.21 $[M+H]^+$.

2-(Nitroxy)ethylamine nitrate salt (29a).



29a

Nitric acid (3 mL) was added to dichloromethane in a 100 mL round-bottomed flask. The solution was allowed to reach 0 °C and then ethanolamine (**28a**) (16 mmol) was added dropwise. After 50 minutes of stirring, acetic anhydride (2 mL) is added dropwise over 2 minutes. After 45 minutes a precipitate was formed and then filtered. The solid is then crystallised with hot chloroform/ethanol to give (**29a**) as nitrate salt. Off-white crystals (90% yield), mp 89 °C; FT-IR (neat, cm^{-1}): 1636, 889; ^{1}H-NMR (400 MHz, MeOH-d_4) δ (ppm): 4.80 (t, 2H, J = 7.4 Hz), 3.40 (t, 2H, J = 7.4 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 38.49, 69.17.

2-(Nitroxy)propylamonium nitrate (29b). Off-white crystals (80% yield), mp 90 °C; FT-IR (neat, cm^{-1}): 1641, 880; ^{1}H-NMR (400 MHz, MeOH-d_4) δ (ppm): 4.78 (t, 2H, J = 7.4 Hz), 3.60 (t, 2H, J = 7.4 Hz), 2.10 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 30.12, 38.44, 69.17.

2-Amino-3-(nitroxy)propanoic acid nitric salt (31a). Off-white crystals (80% yield), mp 109 °C; FT-IR (neat, cm^{-1}): 3160, 1723, 1630, 877; ^{1}H-NMR (400 MHz, MeOH-d_4) δ (ppm): 8.31 (broad s, 3H), 4.99-4.77 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 35.67, 68.97, 170.34.

2-Amino-4-(nitroxy)butanoic acid nitric salt (31b). Off-white crystals (88% yield), mp 104 °C; FT-IR (neat, cm^{-1}): 3165, 1730, 1625, 880; ^{1}H-NMR (400 MHz, MeOH-d_4) δ (ppm): 8.71 (broad s, 3H), 4.73 (m, 2H), 4.14 (m, 1H), 2.37 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 30.87, 35.67, 69.08, 170.21.

N-[2-(Nitroxy)ethyl] (R,S)-2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide (9a). Obtained following the same procedure as (**3a**). Yellowish powder (81% yield), m.p. 144°C; FT-IR (neat, cm^{-1}) v: 3330, 2890,

1654, 1590, 1300, 1147, 1102, 899; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.67 (d, 2H, *J* = 8.4 Hz), 7.28 (d, 2H, *J* = 8.4 Hz), 7.12 (m, 3H, *J* = 8.4 Hz), 7.04 (m, 1H), 6.78 (s broad, 2H), 6.51 (s, 1H), 4.67 (t, 2H, *J* = 7.4 Hz), 3.47 (m, 2H), 3.00 (s, 3H), 2.20 (s, 3H); ^{13}C-NMR (100 MHz, CDCl3): δ (ppm) 169.33, 159.91, 145.23, 144.51, 141.22, 138.00, 131.32, 129.55, 127.91, 126.19, 122.45, 121.20, 116.09, 68.34, 58.18, 43.07, 33.15, 11.06; Anal. ($C_{22}H_{23}FN_4O_6S$) C, H, N; MS-ESI: m/z 491.14 $[M+H]^+$.

N-[3-(Nitroxy)propyl] (R,*S*)-2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide (9b). Obtained following the same procedure as (**3a**). Yellowish powder (83% yield), m.p. 131°C; FT-IR (neat, cm-1) v: 3340, 2889, 1638, 1581, 1289, 1142, 1105, 877; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.68 (d, 2H, *J* = 8.4 Hz), 7.25 (d, 2H, *J* = 8.4 Hz), 7.11 (m, 3H, *J* = 8.4 Hz), 7.05 (m, 1H), 6.83 (s broad, 2H), 6.53 (s, 1H), 4.69 (t, 2H, *J* = 7.4 Hz), 3.45 (m, 2H), 3.08 (s, 3H), 2.17 (s, 3H), 1.88 (m, 2H); ^{13}C-NMR (100 MHz, CDCl3): δ (ppm) 169.53, 159.88, 145.64, 144.50, 141.23, 138.10, 131.38, 129.52, 127.90, 126.22, 122.15, 121.10, 116.03, 68.31, 58.17, 43.01, 33.12, 31.12, 11.00; Anal. ($C_{23}H_{25}FN_4O_6S$) C, H, N; MS-ESI: m/z 515.13 $[M+H]^+$.

N-[2-(Hydroxy)ethyl] (R,*S*)-2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide (10a). Obtained following the same procedure as (**13a**). Yellowish powder, m.p. 109°C (>95% yield); ^{1}H-NMR (400 MHz, DMSO-*d6*): δ (ppm) 7.65 (d, 2H, *J* = 8.4 Hz), 7.22 (d, 2H, *J* = 8.4 Hz), 7.10 (m, 3H, *J* = 8.4 Hz), 7.05 (m, 1H), 6.76 (s broad, 2H), 6.53 (s, 1H), 4.12 (t, 2H, *J* = 7.4 Hz), 3.45 (m, 2H), 3.02 (s, 3H), 2.15 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-*d6*): δ (ppm) 169.37, 159.63, 145.33, 144.78, 141.51, 138.00, 131.32, 129.25, 128.00, 126.20, 122.48, 121.53, 116.12, 62.10, 58.21, 44.07, 33.23, 11.06; Anal. ($C_{22}H_{24}FN_3O_4S$) C, H, N; MS-ESI: m/z 446.16 $[M+H]^+$.

N-[3-(Hydroxy)propyl] (R,*S*)-2-amino-2-[1-(3-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]-1H-pyrrol-3-yl]acetamide (10b). Obtained following the procedure as (**13a**). Yellowish powder, m.p. 115°C (>95% yield); ^{1}H-NMR (400 MHz, DMSO-*d6*):

δ (ppm) 7.66 (d, 2H, *J* = 8.4 Hz), 7.26 (d, 2H, *J* = 8.4 Hz), 7.09 (m, 3H, *J* = 8.4 Hz), 7.04 (m, 1H), 6.79 (s broad, 2H), 6.51 (s, 1H), 4.15 (t, 2H, *J* = 7.4 Hz), 3.48 (m, 2H), 3.09 (s, 3H), 2.17 (s, 3H), 192 (m, 2H); ^{13}C-NMR (100 MHz, DMSO-*d6*): δ (ppm) 169.40, 159.12, 145.31, 144.71, 141.49, 138.06, 131.44, 129.23, 127.00, 126.29, 122.42, 121.47, 116.10, 62.10, 58.11, 44.07, 33.23, 30.12, 11.06; Anal. ($C_{23}H_{26}FN_3O_4S$) C, H, N; MS-ESI: m/z 460.15 $[M+H]^+$.

N-[2-(Nitroxy)ethyl] (R,S)-2-[(tert-butyl)oxycarbonyl]amino-[2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)]acetamide (32a). Obtained following the same procedure as (**7a**), using (**23a**) as carboxylic acid partner. White solid (70% yield), m.p. 102°C; FT-IR (neat, cm^{-1}) v: 3330, 2895, 1688, 1645, 1579, 1228, 1155, 1101, 867; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.73 (d, 2H, *J* = 8.4 Hz), 7.26 (m, 4H), 7.15 (d, 2H, *J* = 8.4 Hz), 6.63 (s, 1H), 6.52 (s broad, 1H), 5.59 (m, 1H), 4.69 (t, 2H, *J* = 7.3 Hz), 4.19 (t, 2H, *J* = 7.3 Hz), 3.05 (s, 3H), 2.15 (s, 3H), 1.46 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 169.24, 168.70, 159.20, 138.70, 135.63, 134.18, 132.43, 129.18, 127.11, 126.66, 123.00, 123.13, 120.18, 117.33, 81.91, 60.05, 58.34, 44.03, 32.21, 30.50, 27.82, 11.24; Anal. ($C_{27}H_{31}FN_4O_8S$) C, H, N; MS-ESI: m/z 591.18 $[M+H]^+$.

N-[3-(Nitroxy)propyl] (R,S)-2-[(tert-butyl)oxycarbonyl]amino-[2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)]acetamide (32b). Obtained following the same procedure as (**7a**), using (**23b**) and (**29b**) as coupling partners. White solid (71% yield), m.p. 100°C; FT-IR (neat, cm^{-1}): 3335, 2893, 1687, 1646, 1577, 1225, 1152, 1100, 869; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, 2H, *J*= 8.4 Hz), 7.23 (m, 4H), 7.16 (d, 2H, J= 8.4 Hz), 6.60 (s, 1H), 6.52 (s broad, 1H), 5.59 (m, 1H), 4.67 (t, 2H, *J* = 7.3 Hz), 4.17 (t, 2H, *J* = 7.3 Hz), 3.05 (s, 3H), 2.15 (s, 3H), 1.46 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 170.27, 168.88, 154.23, 141.40, 138.07, 136.29, 134.52, 130.01, 128.13, 126.68, 123.12, 121.43, 117.32, 117.06, 118.11, 108.31, 81.93, 62.85, 60.56, 54.82, 43.83, 28.51, 27.31, 10.90; Anal. ($C_{28}H_{33}FN_4O_8S$) C, H, N; MS-ESI: m/z 615.17 $[M+H]^+$.

N-[2-(Nitroxy)ethyl] (R,S)-[(benzyloxy)carbonyl]amino-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)acetamide (33a). Obtained following the same procedure as (**7a**), using (**26a**) as carboxylic acid partner. White solid (75% yield), 138 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.71 (d, 2H, *J* = 8.4 Hz), 7.30 (m, 9H), 7.16 (d, 2H, *J* = 8.4 Hz), 6.88 (s broad, 1H) 6.60 (s, 1H), 5.54 (d, 1H, *J* = 7.7 Hz), 5.32 (d, 1H, *J* = 7.7 Hz), 5.10 (s, 2H), 4.70 (t, 2H, *J* = 6.8 Hz), 3.49 (m, 2H), 3.03 (s, 3H), 2.14 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 169.23, 168.66, 159.78, 143.23, 139.01, 135.82, 134.65, 132.43, 131.80, 127.98, 127.49, 127.29, 127.17, 126.89, 123.45, 123.22, 119.05, 116.92, 115.88, 70.82, 60.09, 58.32, 44.03, 32.80, 11.04; Anal. ($C_{30}H_{29}FN_4O_8S$) C, H, N; MS-ESI: m/z 625.16 $[M+H]^+$.

N-[3-(Nitroxy)propyl] (R,S)-[(benzyloxy)carbonyl]amino-2-(1-(3-fluorophenyl)-2-methyl-5-(4-(methylsulfonyl)phenyl)-1H-pyrrol-3-yl)acetamide (33b). Obtained following the same procedure as (**7a**), using (**26b**) and **(29b**) as carboxylic acid partner. White solid (70% yield), 135 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.71 (d, 2H, *J* = 8.4 Hz), 7.30 (m, 9H), 7.16 (d, 2H, *J* = 8.4 Hz), 6.88 (s broad, 1H) 6.60 (s, 1H), 5.54 (d, 1H, *J* = 7.7 Hz), 5.32 (d, 1H, *J* = 7.7 Hz), 5.10 (s, 2H), 4.70 (t, 2H, *J* = 6.8 Hz), 3.49 (m, 2H), 3.03 (s, 3H), 2.14 (s, 3H), 193 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 169.5, 168.67, 159.71, 143.20, 139.21, 135.12, 134.51, 132.31, 131.83, 127.78, 127.52, 127.29, 127.37, 126.91, 123.44, 123.31, 117.05, 116.92, 115.88, 70.82, 60.09, 58.32, 44.03, 32.80, 30.12, 11.04; Anal. ($C_{31}H_{31}FN_4O_8S$) C, H, N; MS-ESI: m/z 639.17 $[M+H]^+$.

Biology and Pharmacology.

In Vitro Anti-Inflammatory Study. The *in vitro* profiles of all the compounds were evaluated through a cell-based assay employing murine monocyte/macrophage J774 cell lines. The cell line was grown in DMEM supplemented with 2 mM glutamine, 25 mM HEPES, 100 units/mL penicillin, 100 μg/mL streptomycin, 10% fetal bovine serum (FBS), and 1.2% sodium pyruvate. Cells were plated in 24-well culture plates at a density of 2.5 x 10^5 cells/mL or in 60 mm

diameter culture dishes ($3x10^6$ cells per 3mL per dish) and allowed to adhere at 37 °C in 5% CO_2 for 2 h. Immediately before the experiments, culture medium was replaced with fresh medium and cells were stimulated as described previously.[44] The evaluation of COX-1 inhibitory activity was achieved by pre-treating cells with test compounds (10 μM) for 15 min and then incubating them at 37 °C for 30 min with 15 μM arachidonic acid to activate the constitutive COX. For the compounds with COX-1 % inhibition higher than 50% (at 10 μM), the cells were treated also with lower concentrations (0.01-1 μM). At the end of the incubation, the supernatants were collected for the measurement of prostaglandin E_2 (PGE_2) levels by a radioimmunoassay (RIA). To evaluate COX-2 activity, cells were stimulated for 24 h with *Escherichia coli* lipopolysaccharide (LPS, 10 μg/mL) to induce COX-2, in the absence or presence of test compounds (0.01-10 μM). Celecoxib was utilized as a reference compound for the selectivity index. The supernatants were collected for the measurement of PGE_2 by means of RIA. Throughout the time the experiments lasted, triplicate wells were used for the various conditions of treatment. Results are expressed as the mean, for three experiments, of the percent inhibition of PGE_2 production by test compounds with respect to control samples. The IC_{50} values were calculated with GraphPad Instat, and the data fit was obtained using the sigmoidal dose-response equation (variable slope) (GraphPad).

Ex Vivo Vasorelaxing Activity. All the experimental procedures were carried out following the guidelines of the European Community Council Directive 86-609. The effects of the compounds were tested on isolated thoracic aortic rings of male normotensive Wistar rats (250-350 g). After a light ether anaesthesia, rats were sacrificed by cervical dislocation and bleeding. The aortae were immediately excised, freed of extraneous tissues and the endothelial layer was removed by gently rubbing the intimal surface of the vessels with a hypodermic needle. Five mm wide aortic rings were suspended, under a preload of 2 g, in 20 mL organ baths, containing Tyrode solution (composition of saline in mM: NaCl 136.8; KCl 2.95; $CaCl_2$ 1.80; $MgSO_4$ 1.05; NaH_2PO_4 0.41; $NaHCO_3$ 11.9; Glucose 5.5), thermostated at 37 °C and continuously gassed with a

mixture of O_2 (95%) and CO_2 (5%). Changes in tension were recorded by means of an isometric transducer (Grass FTO_3), connected with a computerised system (Biopac). After an equilibration period of 60 minutes, the endothelium removal was confirmed by the administration of acetylcholine (ACh) (10 μM) to KCl (30 mM)-precontracted vascular rings. A relaxation < 10% of the KCl-induced contraction was considered representative of an acceptable lack of the endothelial layer, while the organs, showing a relaxation ≥ 10% (i.e. significant presence of the endothelium), were discarded. From 30 to 40 minutes after the confirmation of the endothelium removal, the aortic preparations were contracted by a single concentration of KCl (30 mM) and when the contraction reached a stable plateau, 3-fold increasing concentrations of compounds (1nM-10 μM) were added. Preliminary experiments showed that the KCl (30 mM)-induced contractions remained in a stable tonic state for at least 40 minutes. The same experiments were carried out also in the presence of a well-known GC inhibitor: ODQ 1 μM which was incubated in aortic preparations after the endothelium removal confirmation. The vasorelaxing efficacy was evaluated as maximal vasorelaxing response (Emax), expressed as a percentage (%) of the contractile tone induced by KCl 30 mM. When the limit concentration 10 μM (the highest concentration, which could be administered) of the tested compounds did not reach the maximal effect, the parameter of efficacy represented the vasorelaxing response, expressed as a percentage (%) of the contractile tone induced by KCl 30 mM, evoked by this limit concentration. The parameter of potency was expressed as pIC_{50}, calculated as negative logarithm of the molar concentration of the tested compounds evoking a half reduction of the contractile tone induced by KCl 30 mM. The pIC_{50} could not be calculated for those compounds showing an efficacy parameter lower than 50%. The parameters of efficacy and potency were expressed as mean ± standard error, for 6-10 experiments. Two-way ANOVA was selected as statistical analysis, $P < 0.05$ was considered representative of significant statistical differences. Experimental data were analysed by a computer fitting procedure (software: GraphPad Prism 4.0).

Ex vivo determination of the formation of nitrites and nitrates, in rat liver homogenate. The experimental procedures were carried out following the guidelines of the European Community Council Directive 86-609. The liver homogenates were obtained from male normotensive Wistar rats (250–350 g). After a light ether anesthesia, rats were sacrificed by cervical dislocation and bleeding. The portal vein was immediately cannulated and the liver was perfused with 4 °C-cold homogenation buffer (composition: K_2HPO_4 100 mM; EDTA 1 mM; KCl 15 mM; sucrose 0.25 M and Ethanol 0.1%, pH 7.4). After about 5 min of slow perfusion, the liver was minced with scissors and washed with cold homogenation buffer. Then, it was dried with filter paper, weighted and resuspended (1:5 w/v) in cold homogenation buffer. The sample was finally homogenated in Turrax. In a vial, 400 µl of the above homogenate were mixed with 500 µl of assay buffer (composition: K_2HPO_4 100mM; EDTA 1mM; pH 7.4), containing glutathione, NADH and NADPH (all at the final concentration of 1mM). The vials were then thermostated at 37 °C, and 100 µl of a DMSO solution of the tested *NO*-donor were added (final concentration of the tested compound = 1 mM), allowing the release of NO, which in turn is rapidly converted to the stable inorganic metabolites, i.e. nitrites and nitrates. Aliquots (100 µl) of the above medium were collected at selected intervals (5, 15, 30, 60 and 120 min) and added into a beaker containing 1.9 ml of an aqueous solution of H_2SO_4 (0.1 M) and KI (0.1 M). This solution allows nitrite ions to be exhaustively and instantaneously reduced to *NO*, which is amperometrically titrated by a *NO*-selective electrode connected to an Apollo 4000 free radical analyzer (World Precision Instrument), allowing us to determine the concentration of the nitrites previously formed in the biological sample. In parallel experiments, a Nitrate Reductor (World Precision Instrument) was constantly kept immerged into the vial. This tool allowed nitrates to be constantly converted to nitrites. The concentration of nitrites in these sample is defined as NOx, which reflects the sum of all the nitrites and nitrates previously derived from NO. Opportune calibration curves were previously obtained with standard solutions of sodium nitrite.

Ex vivo Human Whole Blood (HWB) Assay. Three healthy volunteers (2 females and 1 male, aged 29 ±3 years) were enrolled to participate in the study after its approval by the Ethical Committee of the University of Chieti. Informed consent was obtained from each subject. Compounds 11c (0.05 -50 mM) and 12c (0.05 - 50 mM) were dissolved in DMSO. Aliquots of the solutions (2 μL) or vehicle were pipetted directly into test tubes to give final concentrations of 0.1 - 100 μM in whole blood samples. To evaluate COX-2 activity, 1 mL aliquots of peripheral venous blood samples containing 10 IU of sodium heparin were incubated in the presence of LPS (10 μg/mL) or saline for 24 h at 37 °C, as previously described.[36] The contribution of platelet COX-1 was suppressed by pretreating the subjects with aspirin (300 mg, 48 h) before sampling. Plasma was separated by centrifugation (10 min at 2000 rpm) and kept at -80 °C until assayed for PGE_2 as an index of monocyte COX-2 activity. Moreover, peripheral venous blood samples were drawn from the same donors when they had not taken any NSAID during the 2 weeks preceding the study. Aliquots (1 mL) of whole blood were immediately transferred into glass tubes and allowed to clot at 37 °C for 1 h. Serum was separated by centrifugation (10 min at 3000 rpm) and kept at -80 °C until assayed for TXB_2. Whole blood TXB_2 production was measured as a reflection of maximally platelet COX-1 activity in response to endogenously formed thrombin.[45] Analysis of PGE_2 and TXB_2. PGE_2 and TXB_2 concentrations were measured by previously described and validated radioimmunoassays.[45] Unextracted plasma and serum samples were diluted in the standard diluent of the assay (0.02 M phosphate buffer, pH 7.4) and assayed in a volume of 1.5 mL at a final dilution of 1:50-1:30000. [3H]PGE_2 or [3H]TXB_2 (3000 cpm, specific activity >100 Ci/mmol, 1:100000 dilution) and anti-TXB2 (1:120000 dilution) sera were used. The least detectable concentration was 1-2 pg/mL for both prostanoids.

In Vivo Analgesic and Anti-Inflammatory Study. Male Swiss albino mice (23-25 g) and Sprague-Dawley or Wistar rats (150-200 g) were used. The animals were fed with a standard laboratory diet and tap water *ad libitum* and kept at 23 (1 °C with a 12 h light/dark cycle, light on at 7 a.m.) The paw pressure test was performed by inducing an inflammatory process by the intraplantar

(ipl) carrageenan administration 4 h before the test. in the administered intraplantar (ipl) carrageenan 4 h before the test. The carrageenan-induced paw edema test was also performed, evaluating the paw volume of the right hind paw 4 h after the injection of carrageenan and comparing it with saline/carrageenan-treated controls. The analgesic activity of compounds was also assessed by performing the abdominal constriction test, using mice into which a 0.6% solution of acetic acid (10 mL/kg) had been injected intra-peritoneal (ip). The number of stretching movements was counted for 10 min, starting 5 min after administration.

Solubility and stability assessment.

Solubility assessment in phosphate buffered saline (PBS) and simulated gastric fluid (SGF). Stock solutions of test compounds (20 mM) were prepared in DMSO. A standard phosphate buffered saline (PBS) 0.01 M (pH 7.4) was used to determine the solubility in neutral pH conditions; a simulated gastric fluid (SGF) 0.05 M solution without pepsin (pH was adjusted to 1.5 using concentrated HCl) was instead used to check the solubility of compounds in acid medium. Seven point calibration standards (1, 5, 10, 25, 50, 100 and 200 μM) were prepared from each 20 mM solution stock by serial dilution. 2 μL of each test compound (stock solution 20 mM in DMSO) were added to 198 μL of PBS and SGF in two duplicate wells in multiscreen solubility filter plate, covered and shaken for 90 minutes at 300 rpm using a Multi-pulse Vortexer (Glas-Col). The sample was filtered using a Millipore manifold filter assembly and collected in an acceptor plate and then analysed on HPLC together with the standards solutions (HPLC-Waters Separations Module 2695 with photodiode array detector).

Stability assessment in phosphate buffer saline (PBS), simulated gastric fluid (SGF) and rat plasma. Stock solutions of test compounds (1mM) were prepared in DMSO. A standard phosphate buffered saline (PBS) 0.01 M (pH 7.4) was used to determine the stability in neutral pH conditions; a simulated gastric fluid (SGF) 0.05M solution without pepsin (pH was adjusted to 1.5 using concentrated HCl) was instead used to check the stability of compounds in acid medium. Rat plasma (Sprague Dawley rat) was used to underline the sensitivity of test

compounds to plasma enzymes; in this case, Na_2EDTA dehydrate was used as anticoagulant for the experiment. An aliquot of each medium of 588 µL is taken and stored in a 2 mL eppendorf vial. Afterwards, 12 µL of 1 mM solution of each test compound is added to each medium. The samples are incubated in an hybridisation oven. At each time point (0, 30, 60, 120 min.), a 100 µL aliquote is taken and quenched immediately with 300 µL of 100% ice cold acetonitrile (containing 60 ng/mL of internal standard, i.e. phenacetine) and stored at -80 °C. All the sample then are centrifuged at 14000 rpm for 5 min. The supernatant is transferred into an HPLC vial and analysed by LC-MS/MS (Shimadzu-API4000).

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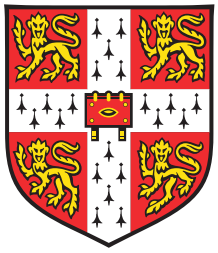
Chapter 2

A Machine-Assisted Flow Procedure for Meclinertant, a Neurotensin Receptor Probe

(Under the supervision of Professor Steven V. Ley and Professor Ian R. Baxendale)



Introduction

Flow chemistry. The term flow chemistry refers to a method of performing chemical reactions as a progressively flowing process.[1] Although batch chemistry is still widely used, automated platforms where a reaction is operated as a flow-through process are becoming increasingly popular due to the distinctive advantages offered by this mode of working (**Figure 1**).

Figure 1. Uniqsis FlowSyn and Vapourtec R2/R4+ flow platforms

A machine-assisted flow process can offer several benefits including improved safety, automation, better real-time control over a range of parameters, enhanced mass and heat transfer, the ability to increase reaction temperatures far above the solvent's boiling point and easier integration with diagnostic techniques (**Figure 2**).[2-8]

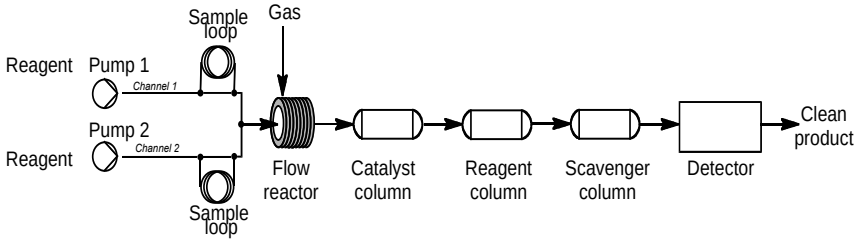


Figure 2. Integration of a flow platform.

Multi-step processes can be arranged in a continuous sequence,[9-12] allowing *in-situ* generation and use of unstable, toxic, or air-sensitive intermediates.[13] Furthermore, multi-phase reactions can be easily elaborated[14-17] and scale-up of a proven reaction can be easily achieved by simply operating the reactor for a longer period of time.[18]

The application of flow chemistry in research laboratories has been an exponentially growing phenomenon which has stimulated the development of much custom-designed and commercially available equipment. Modular flow systems built from components based upon flow chips, mixers, capillaries, packed bed columns, and/or dedicated reactors that perform certain types of chemistry or specific unit operations allow a broad range of transformations. Flow reactors are constructed from a variety of materials including metal, glass, silica, ceramics, and polymers, and their designs range from simple capillaries to complex micro-structured devices. These laboratory scale flow reactors generally involve the processing of solution phase chemistry through channels ≤1 mm i.d., such that reactions are carried out continuously on small scale. One of most important aspects of bench top flow platforms is that they can allow rapid scale up of developed reactions, reducing the requirement for routes to be redesigned before entering production. Indeed, the micro-environment inside a flow reactor can be seen as a steady state scenario where reaction parameters such as temperature, concentration and composition of reactants established for a small scale flow process can be directly transferred to larger flow reactors, without substantial alteration.

The discovery and development of new drugs for human diseases is a major scientific priority of modern society. However, the process of discovery and development is impeded by challenges that make it difficult, expensive and protracted. One of the most addressed issues in the pharmaceutical field is represented by key synthetic challenges of performing scalable, reliable and robust processes. Over the years chemists have tremendously improved methodology (transition-metal catalysis, asymmetric catalysis, etc.) but in contrast the technology of performing chemistry has remained more or less the same. Although process chemists are well aware of most of the aspects of performing chemical reactions, (such as heat and mass transfer, mixing and thermodynamic phaenomena) these concepts are quite underestimated in small research laboratories. In this context, the pharmaceutical industry is searching for new technologies to speed up the whole process of drug discovery and development.[19-21]

Neurotensin. Neurotensin (NT) (**Figure 3**) is a tridecapeptide highly distributed throughout the central nervous system (CNS), especially in the hypothalamus, amygdala and nucleus accumbens, which was first isolated from bovine hypothalamii in 1973.[22]

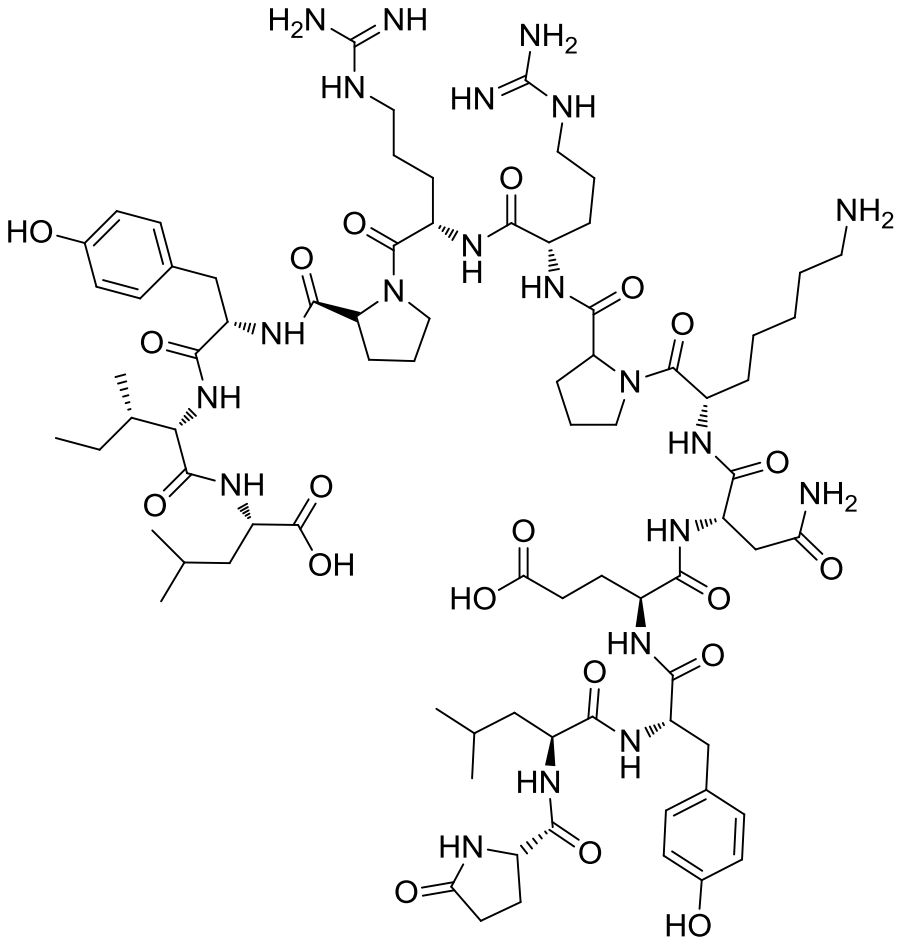


Figure 3. Neurotensin

Numerous studies have shown that NT is likely to be involved in cancer and neurodegenerative diseases,[23-28] thus a deeper understanding of its functions and downstream effects related to the activation of its receptors is urgently required. This process has been aided by the development of small molecular probes. Investigations employing NT itself are hampered by a number of challenges. NT is a peptide and is, therefore, extremely susceptible to metabolism; its size also makes the absorption into the brain troublesome. Antagonists targeting these receptors are also required to build a complete picture.

Meclinertant. In the early 90's Sanofi-Aventis researchers identified SR-45398 (**1**), a compound able to bind selectively to NT receptor 1 (NTR1) in the Guinea pig brain (IC_{50} = 40 uM) (**Figure 4**).

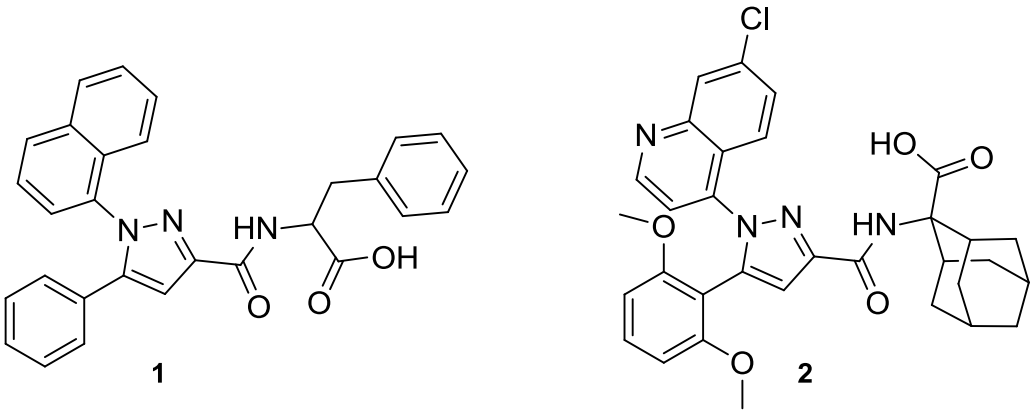
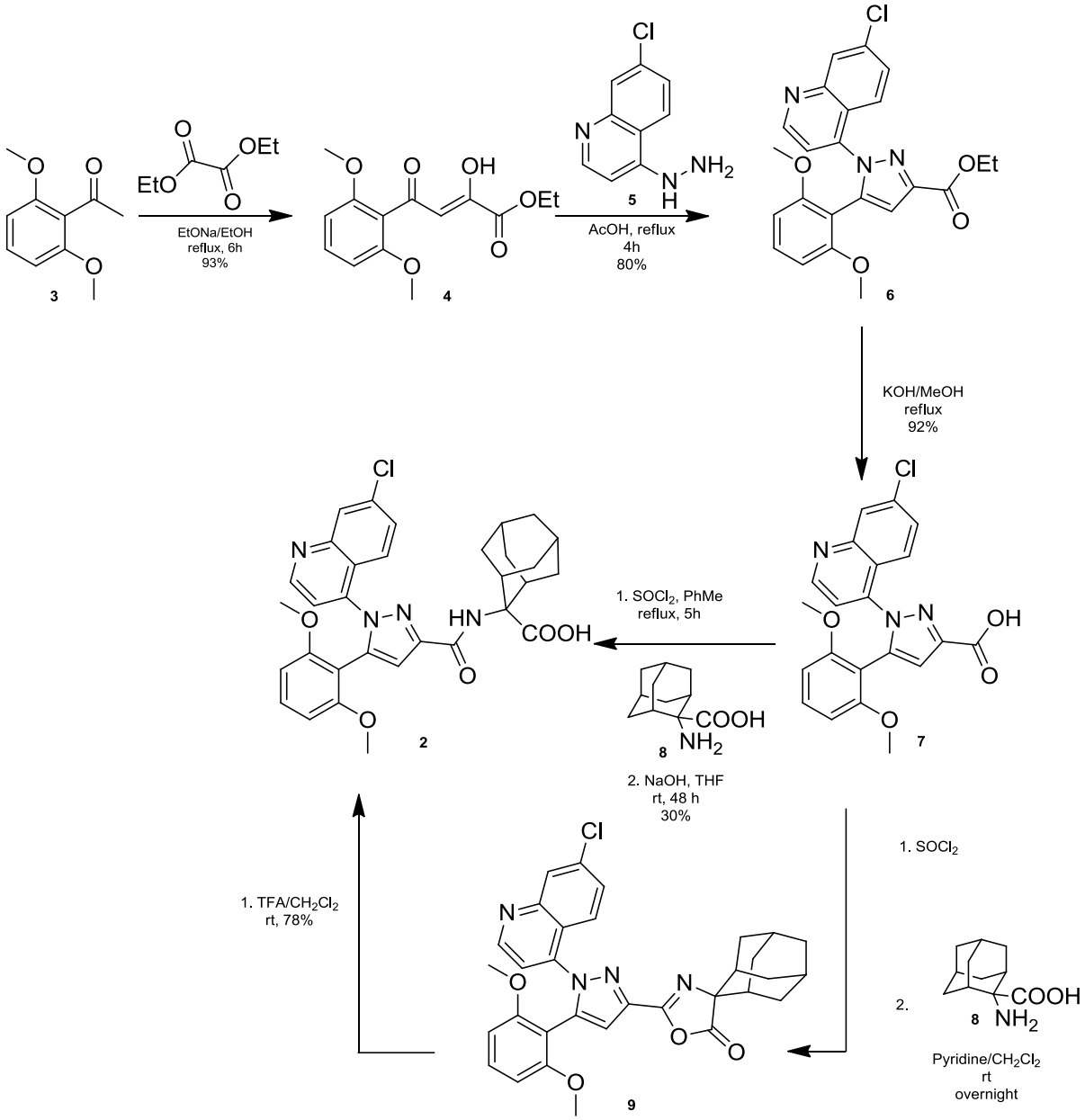


Figure 4. Molecular structure of SR-45398 (**1**) and SR-48692 (**2**)

In an effort to develop a novel and more active molecule, they generated SR-48692 or Meclinertant (**2**), an antagonist with outstanding binding properties towards NTR1 (Ki = 2.6±0.2 nM) but also capable of binding to NTR2 (Ki = 418±82.2 nM) and NTR3 (Ki = 238±46 nM) at higher concentrations. The research laboratories of Sanofi Aventis reported two main routes to SR-48692.[29,30]

In the first approach, a Claisen condensation between ketone (**3**) and diethyl oxalate gave access to the tricarbonyl compound (**4**). This was followed by a condensation of (**4**) and hydrazine (**5**) and subsequent saponification of the resultant ester (**6**) to yield acid (**7**). Activation of the acid and coupling with amino acid (**8**) afforded the desired product Meclinertant (**2**) (**Scheme 1**).

Scheme 1. Routes to Meclinertant reported by Sanofi Aventis



Their second route affords Meclinertant (**2**) through activation of acid (**7**) and coupling with amino acid (**8**) using pyridine: DCM as the solvent system and then treating the intermediate with TFA:DCM. The publication gives very limited experimental procedures and data, making reproduction of this work extremely difficult. Previous studies on the batch synthesis of Meclinertant (carried out by Dr Matthew O. Kitching, University of Cambridge) highlighted that one the most problematic part of the process is the synthesis of the amino acid (**8**) (2-

aminoadamantane-2-carboxylic acid).[31,32] In addition, all the steps characterising the synthesis of Meclinertant were poorly reproducible, especially when scaling up to multi-gram scale. For these reasons, we decided to pursue a reliable, robust, machine assisted flow procedure for the synthesis of Meclinertant. Work began with the development of a novel procedure of the amino acid (**8**).[20,33] Since the idea was to develop the whole process in flow, we envisaged that ketone (**5**) could be obtained from cheap starting materials using enabling technologies, and then the following stages could be adapted to the flow platform to achieve automation and reproducibility on a multi-gram scale.[34]

The synthesis of 2-aminoadamantane-2-carboxylic acid

(Battilocchio, C.; Baxendale, I.R.; Biava, M.; Kitching, M.O.; Ley, S.V. *Org. Process Res. Dev.* **2012**, 16(5), 798-810. *Ref. 18*; Battilocchio, C.; Baxendale, I.R.; Biava, M.; Ley, S.V. *Org. Syn.* Submitted for publication. Ref. 33)

A novel approach to amino acid (**8**), based on a five-step sequence, was investigated. We anticipated that each step would be both high yielding and readily scalable under flow conditions. The sequence commences with a Grignard addition to 2-adamantanone (**10**) followed by conversion of the resulting propargylic alcohol (**11**) to the acyl-propargylamide (**12**) *via* a Ritter reaction. Next, a 5-enol-exo-dig cyclisation furnishes the oxazolidine intermediate (**13**), which is transformed to the azalactone (**14**)[35] by ozonolysis and then hydrolytically cleaved to the desired amino acid (**8**) (**Figure 6**).[36]

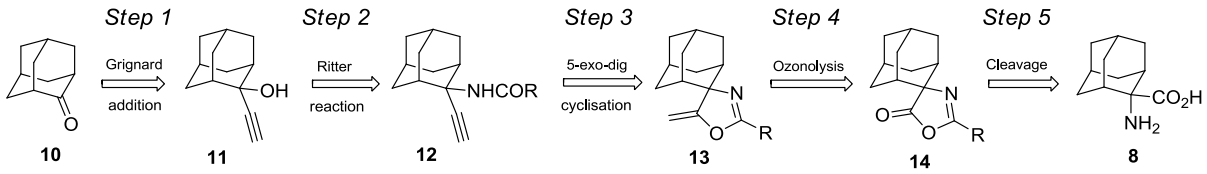


Figure 6. Novel five-step sequence to the amino acid (**8**)

The reversibility of the Grignard addition under basic conditions was anticipated so a screen of cryogenic conditions was conducted in batch.[37] It was observed that upon scale-up under batch conditions, substantial quantities of the ketone starting material were isolated when the propargylic alcohol anion was quenched at temperatures above -10 °C. The reaction was therefore run at -20 °C employing a cryo-flow reactor ("Polar Bear" reactor, **Figure 7**) equipped with a 52 mL PFA (perfluoroalkoxy polymer) reactor coil and two 1 mL PFA pre-cooling loops.[38] A 0.5 M solution of 2-adamantanone (**10**) in THF and a 0.5 M THF solution of ethynylmagnesium bromide, each delivered at a flow rate of 0.2 $mLmin^{-1}$ through the pre-cooling loops, were combined in a T-piece and then passed through the reactor coil.

Figure 7. Polar Bear reactor developed by Cambridge Reactor Design (CRD) and connected to a Uniqsis FlowSyn platform, used to run reaction under cryogenic conditions

Under this set-up, an approximate 40% yield was obtained when processing 10 mmol of the substrate (**Figure 8**). Further attempt to optimise the reaction (increasing the residence time or altering the relative stoichiometries of the reagents) failed to increase the yield.

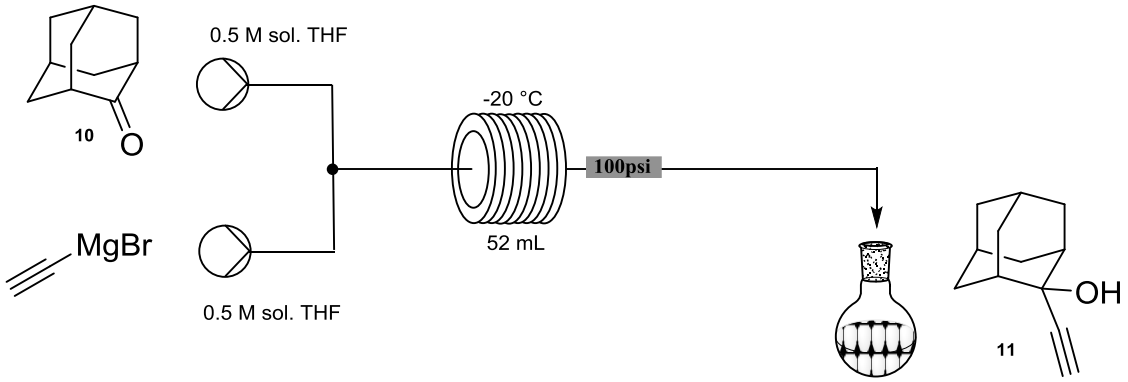


Figure 8. Grignard reaction in flow under cryogenic conditions

Eventually it was found that the cryogenic conditions were actually detrimental to the reaction progress under flow processing. Indeed, carrying out the reaction at a higher temperature of 40 °C using a 14 mL reactor coil on a Uniqsis FlowSyn platform (0.5 M solution of 2-adamantanone (**5**) in THF at 0.18 mL min-1 and 0.5 M solution of ethynylmagnesium bromide in THF at 0.2 $mLmin^{-1}$), resulted in an 80% isolated yield of the 2-ethynyl-2-adamnatanol on a 300 mmol scale after a standard aqueous extraction (**Figure 9**).

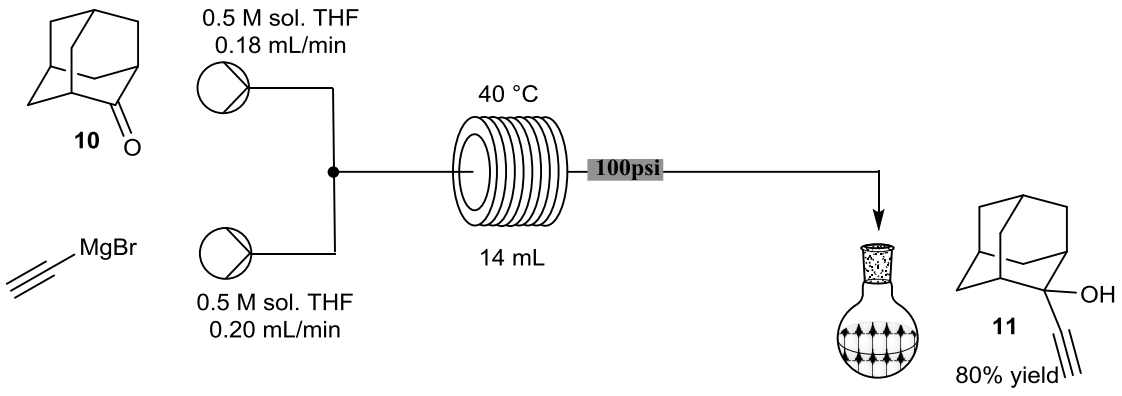


Figure 9. Grignard reaction in flow at 40 °C on a 300 mol scale

This difference was attributed to the improved stability of the intermediate in the flow processing regime where the ionic concentration of the reaction mixture remains relatively constant. In the batch scenario the pH of the reaction media steadily increases eventually favoring the retro reaction. This was observed in batch reactions where higher relative conversion to the desired product was achieved using lower stoichiometries of the Grignard. Also higher final isolated yields were obtained when the reaction was worked up immediately following the Grignard addition. Since the product has the potential to liberate acetylene, we investigated the stability of this key alkyne intermediate (**11**). A differential scanning calorimetry (DSC) study was used to determine the behavior of this compound (**Figure 10**).[39]

Figure 10. DSC machine from Mettler-Toledo

An exothermic event was observed at temperatures above 160 °C which was thought to be due to liberation of acetylene. The identity of the suspected degradation product, 2-adamantanone

(**10**), was confirmed repeating the scan on the same sample (following cooling). The results of this scan convincingly matched the profile obtained from authentic 2-adamantanone (**10**). It was concluded that the high temperature heating caused the de-ethynylation of 2-ethynyladamantan-2-ol (**11**) (**Figure 11**).

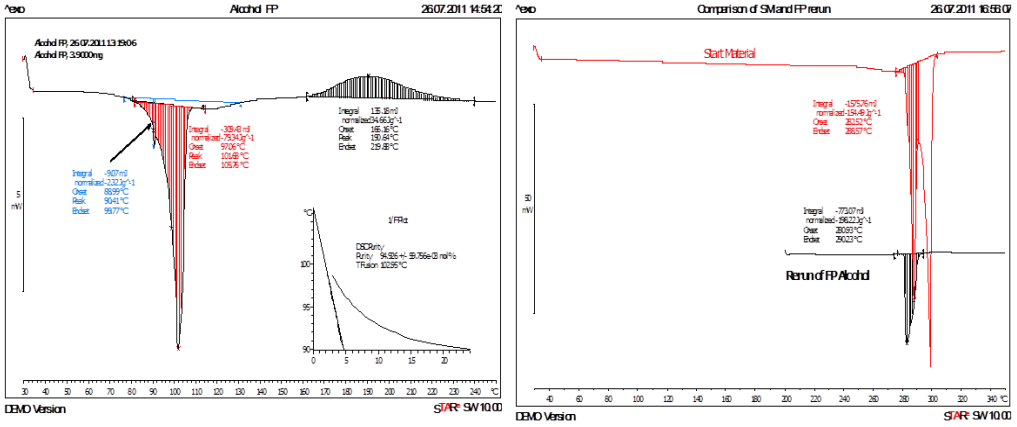


Figure 11. DSC of 2-ethynyladamantan-2-ol (left), 2-adamantanone and degradation product of 2-ethynyladamantan-2-ol (right)

Due to the inherent reversibility of the prop-2-yn-1-olate formation and knowledge that the acidic work-up of this anion is exothermic, it was reasoned that the use of flow micro-mixing could prevent any overheating when conducting the quenching step. Consequently, a straightforward modification of the original set-up was made to incorporate an in-line quenching step using a saturated solution of NH_4Cl. This was achieved by the incorporation of a second T-piece. However, this initially proved problematic due to the rapid precipitation of magnesium salts which accumulated over time in the T-connector and eventually resulted in the blockage of the reactor. A simple solution to this problem was to use pulsed sonication where the T-piece connector and a short length (10 cm) of the subsequent tubing were submerged in the water bath of a standard laboratory sonicator.[40] This completely eliminated build-up of precipitates enabling steady-state operation. The product could then be processed continuously (longest run 660 mol) giving an improved isolated yield of 90% (**Figure 12**).

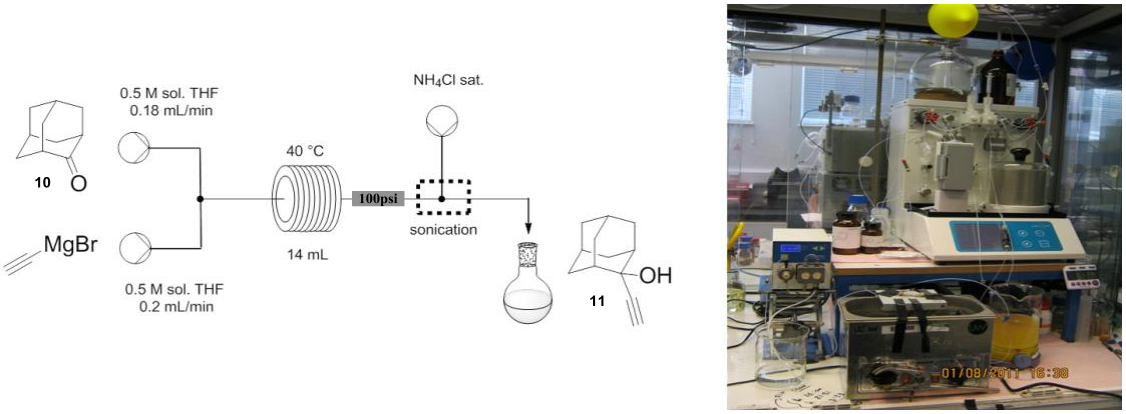


Figure 12. Scheme of the set-up for the optimised Grignard reaction in flow with in-line quenching (left) and a picture of the set-up itself (right)

Ritter reactions in microfluidic flow devices have already been convincingly described by Wirth and coworkers, demonstrating that even at mild temperatures, ranging from 45-85 °C depending on the substrate, high conversions could be realized in only 10 minutes residence time.[41] Building upon these results the conditions for the adamantane substrate (**11**) were rapidly established. Two different nitriles were chosen, namely benzonitrile and acetonitrile, to generate amides with differing electronic natures thereby influencing the subsequent cyclisation and also the potential ease of their eventual deprotection at the final stage. The reaction set up was made possible due to the use of an acid resistant Uniqsis FlowSyn platform.

The preliminary reaction set-up employed two injection loops (3 mL internal volume each), one filled with a mixture of the alcohol (**11**) along with the nitrile, the other containing neat concentrated sulfuric acid (>95%). These were simultaneously switched into the main flow stream (glacial acetic acid/acetic anhydride 10/1 [v/v]) combining at a T-piece before progressing into a 14 mL PFA continuous flow coil (CFC). It was quickly determined that the temperature was a crucial parameter; all attempts to perform the reaction at temperatures exceeding 40 °C gave substantial byproduct formation. This compound was later identified as arising from a Meyer-Schuster rearrangement followed by a subsequent 1,2-hydride migration (Wagner-Meerwein rearrangement) (**Figure 13**).

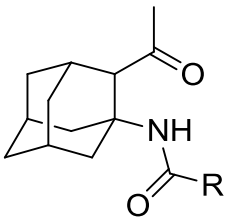
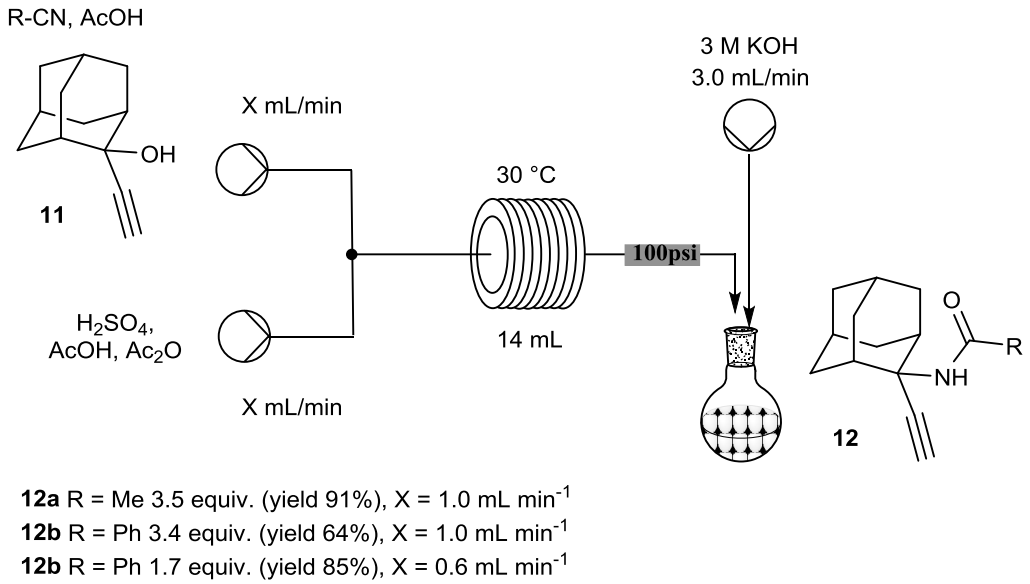


Figure 13. Byproduct obtained when running the Ritter reaction at temperatures above 40 °C

At an operating temperature of 30 °C this byproduct was only present in trace amounts allowing the desired adduct to be isolated in yields of around 85% on a 3 mmol scale. Since the use of large volumes of concentrated sulfuric acid resulted in work up issues, especially when scaling up, we further investigated the performance of the reaction using different solvent compositions and additives. The best results were obtained using a mixture of H_2SO_4/AcOH/Ac_2O 5:11:5 (v/v/v). Under these optimized conditions a solution of nitrile/AcOH/(**11**) were introduced to the system at a flow rate of 1.0 $mLmin^{-1}$. A corresponding solution of H_2SO_4/AcOH/Ac_2O also at a flow rate of 1.0 $mLmin^{-1}$ was directed to meet the first stream at a mixing-T piece prior to entering a 14 mL PFA CFC maintained at 30 °C. The output stream was collected into a continuously stirred tank reactor which was continuously quenched and neutralized by an auxiliary feed line delivering a solution of aqueous KOH (3 M). The flask was periodically exchanged and the solution extracted with DCM allowing isolation of the Ritter product (**12**) (**Figure 14**).



12a R = Me 3.5 equiv. (yield 91%), X = 1.0 mL min^{-1}
12b R = Ph 3.4 equiv. (yield 64%), X = 1.0 mL min^{-1}
12b R = Ph 1.7 equiv. (yield 85%), X = 0.6 mL min^{-1}

Figure 14. Ritter reaction set-up in flow

The lower overall yield obtained in the case of amide (**12b**) is a consequence of its problematic separation. Determination of the crude conversion to benzamide (**12b**) indicated > 95% by ^{1}H-NMR and LC-MS. However, upon scale-up the excess benzonitrile and its resulting hydrolysis by-product (benzamide) created considerable issues with regards to the efficient and clean extraction of the product from the reaction media. Some success was eventually realized by reducing the excess of benzonitrile (to 1.7 equiv.) and increasing the residence time, thereby allowing isolation of an improved 85% of the desired amide product (**12b**). Unfortunately, isolation still remained a significant issue at larger scales (i.e. > 25 g) with no satisfactory alternative work-up conditions being readily identified.

In order to rapidly scope out the potential options for the formal 5-enol-exo-dig cyclisation we evaluated a series of batch conditions in parallel. Attempts to catalyze the cyclisation of the propargyl amides (**12**) with various metal salts such as silver and copper were only partially successful (**Table 1**).[42]

Table 1. Investigations of metal salts for the catalytic 5-enol-exo-dig cyclisation to (**13**)

Entry	Catalyst	Solvent/additive	T(°C), Time	Conversion
1	CuI	Benzene/TEA (1:1)	reflux, 12 h	-
2	$AgNO_3$	n-butyl acetate	reflux, 20 h	20%
3	$AgSbF_6$	dichloromethane	rt, 30 min	65%
4	NaOH	Toluene/water /TBACl*	reflux, 6 h	50%

*tetrabutylammonium chloride.

The best results were obtained using silver hexafluoroantimonate (DCM, rt) leading to a 65% conversion although we were discouraged from its use at scale due to its cost (**Table 1**, entry 3). The use of gold was avoided for the same reason. Interestingly, using sodium hydroxide in a biphasic toluene/water mixture in the presence of tetrabutylammonium chloride as a phase transfer catalyst (PTC) allowed isolation of the product (**13**) in a 50% yield. Further optimisation of the reaction by screening various bases, concentrations, temperatures and reaction times highlighted a combination of potassium hydroxide in ethanol as an improved medium. It was found that when a ~ 3:1 molar ratio of KOH/starting material (**12**) in EtOH (1.3 M) was microwave irradiated at 100 °C for 2 h, a high isolated yield of the desired products could be obtained (R = Me; **13a**, 91% yield and R = Ph; **13b**, 92% yield). At higher temperatures the reaction produced larger quantities of an unidentified by-product (**Figure 15**). Interestingly, the tailored conditions of the flow procedure allowed the use of an elevated temperature window with a correspondingly shorter reaction time without observing any byproduct formation.

Figure 15. Relationship between yield and temperature in batch reactions

Under the optimised conditions, the flow cyclisation of the benzoyl propargylamide derivative (**12b**) occurred within a residence time of 24 min at 120 °C (1:16 molar ratio amide (**12b**) to KOH). The corresponding acetyl derivative (**12a**) was less amenable to cyclisation and so required a prolonged residence time (48 min) to achieve complete conversion to the desired product (**13a**) under the same reaction conditions (**Figure 16**).

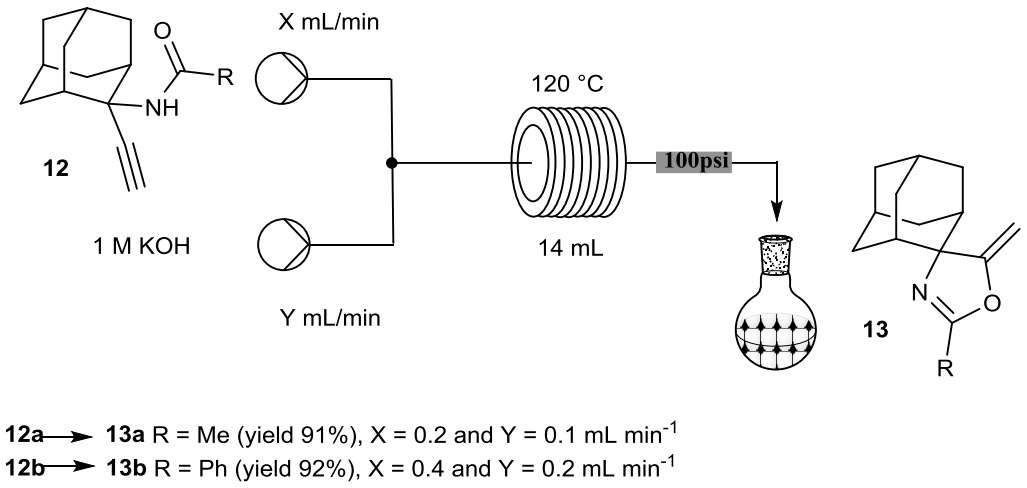
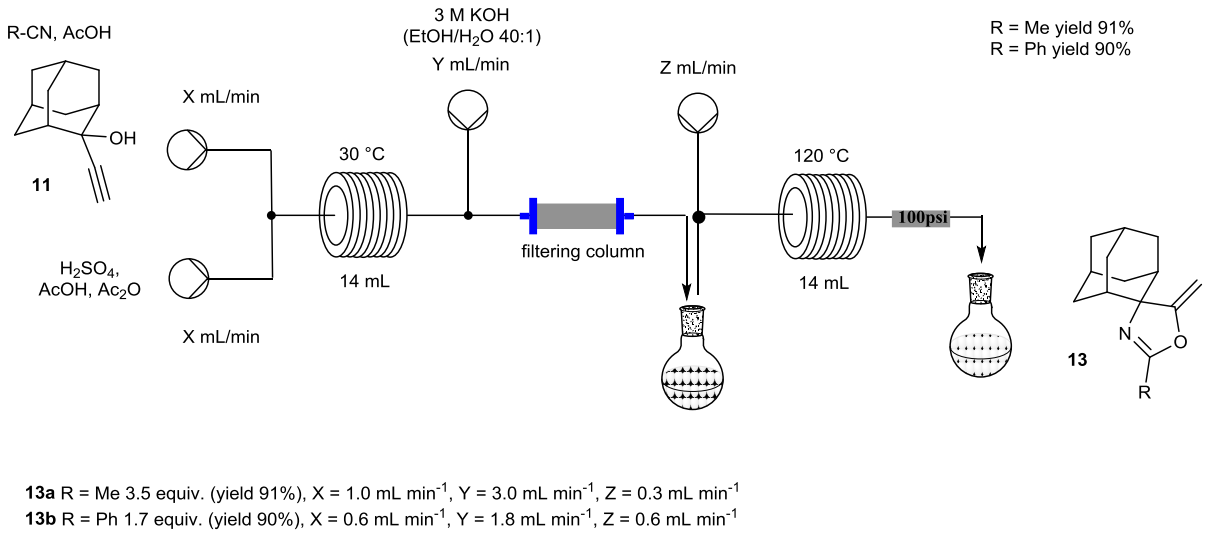


Figure 16. Flow set-up for the 5-enol-exo-dig cyclisation

In both cases an overall high yield of the cyclised products could be realised following a very trivial organic extraction of the reactor output. It was reasoned that the basic quenching step used in the Ritter process (**Figure 14**) could be adapted to deliver the material directly through to the subsequent cyclisation step (**Figure 16**) as a single integrated flow process. In addition, to shorten the sequence and reduce the manual workup it was anticipated that the basic conditions could facilitate easier extraction of the final product. In the Ritter reaction the major problem was due to issues of extraction and isolation of the product from any residual nitrile (due to the excess used in the Ritter reaction, especially for (**12b**)) and the corresponding

partially hydrolysed by-product amide (acetamide or benzamide). This was particularly troublesome when benzonitrile was used at scale. However, under the strongly basic conditions and higher reaction temperature of the cyclisation step, complete hydrolysis of the nitrile (and amide) to the carboxylate should occur making extraction of (**13**) far easier. The flow reactor set-up established to test this process is shown in (**Figure 17**).



13a R = Me 3.5 equiv. (yield 91%), X = 1.0 mL min^{-1}, Y = 3.0 mL min^{-1}, Z = 0.3 mL min^{-1}
13b R = Ph 1.7 equiv. (yield 90%), X = 0.6 mL min^{-1}, Y = 1.8 mL min^{-1}, Z = 0.6 mL min^{-1}

Figure 17. Telescoped Ritter and 5-enol-exo-dig cyclisation in flow

Under the telescoped conditions, a solution of nitrile/AcOH/(**11**) and a corresponding mixture of H_2SO_4/AcOH/Ac_2O were combined (at a T-piece) and introduced into a 14 mL PFA CFC maintained at 30 °C. The exiting flow stream was coupled with a second T-connector supplying a solution of KOH in EtOH/H_2O (3 M, 40:1 v/v). Any resulting precipitate was directly removed by in-line filtration (glass wool) and the solution collected into a storage vessel. Following a suitable delay (dependent on the flow rate of the two steps) the eluent was progressed using a second HPLC pump through a heated PFA CFC (120 °C). The solution from the reactor was readily extracted to give the final product in good yield and excellent purity. This abridged process proved particularly effective for processing larger quantities of material significantly reducing the work-up burden.

The oxidizing properties of ozone are well-known, especially for its use in industrial applications spanning chemical synthesis to waste treatment and water purification. Although it has a minimal environmental impact and low cost, there are safety issues relating to the generation of unstable and highly energetic ozonide intermediates.[43] Consequently, any system using ozone should be simple, high-performance and safe for the processing of larger quantities of substrate.[44] We established a flow based ozonolysis device of our own creating. The first flow set-up (Figure 14) used a Knauer K100 HPLC pump to deliver a sprayed stream of the substrate (0.2 M solution in DCM) into the gas flow (O_2/O_3, 500 mL min-1) *via* an infusion T-piece (**Figure 18**).

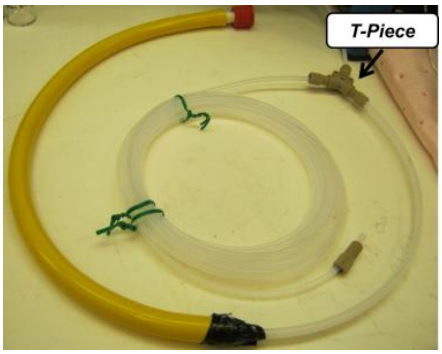


Figure 18. Infusion T-piece and coil

The combined stream then progressed through a reaction coil (20 mL, 2.5 mm i.d.) with a residence time of 5 min (**Figure 18** and **19**). The resulting output stream was collected into a vented chamber with the excess ozone being eliminated by gently flushing argon into the solution. The purged solution was then pumped through a packed bed cartridge containing an excess of polymer-supported thiourea inducing reductive cleavage of the ozonide.[45,46] The output stream was periodically tested for residual ozone (Ozone Test Kit OZ-2 – Camlabs Part No. HH/20644-00) and the presence of unreacted ozonide (via LC-MS by Ph_3P oxidation against an internal standard of 2,3-dimethoxynaphthalene). The output solution was finally evaporated under reduced pressure to give the desired compound (**14**).

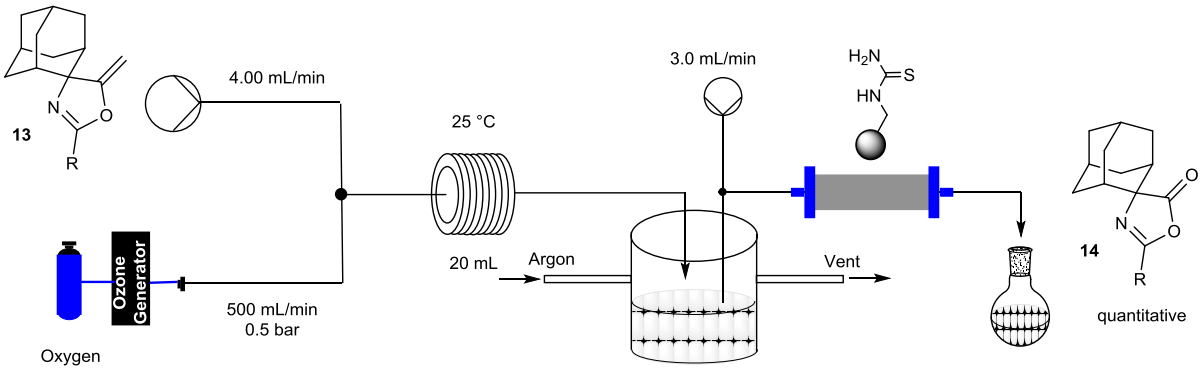


Figure 19. Ozonolysis system and quenching reactor (polymer-supported thiourea)

During the oxidative process we tried to identify the flow pattern in order to help with the optimisation of the reaction conditions: the very first portion of the reactor coil (5 cm) was characterized by formation of small liquid droplets along the inner surface of the tubing. This was accompanied by an instantaneous bleaching of the solution (from light yellow to colourless) and a sharp drop in temperature (rt→4 °C as detected by IR measurements at the outer surface of the reactor). Then a wavy/annular pattern was observed along the reactor tubing (**Figure 20**). It was speculated that the endothermic process occurring was related to the "spraying effect" (latent heat of evaporation) that occurred as the liquid phase entered the fast moving gas flow.

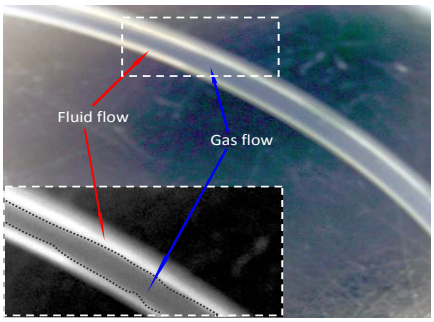


Figure 20. Flow pattern observed during the ozonolysis

Having observed the immediate colour change at the start of the reactor we wanted to determine the composition of the reaction mixture at this stage. Sampling the flow stream after only 18 cm of tubing (1 mL internal volume) showed total consumption of the starting material which upon treatment with the thiourea resin gave the desired product (**14**) in quantitative

conversion. Wishing to take advantage of this "spraying" phenomenon we envisaged the assembly of a short path reactor unit with a residence time of only 15 s. In addition to facilitate rapid reaction processing and self-controlled cooling the spray dispersion phenomenon would also assist flushing excess ozone from the reaction stream by showering the output feed into an argon (or nitrogen) blanketed purge vessel (**Figure 21**).

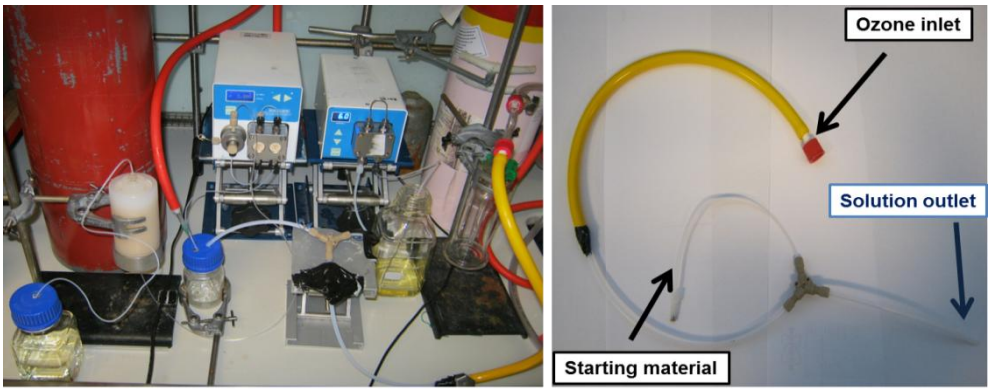


Figure 21. Picture of the ozonolysis set-up (left) and the 1-mL reactor for ozonolysis (right)

At this stage a secondary pump could then be used to advance the ozonide solution through the previously developed resin quenching process. This set-up was readily achieved and successfully used to process larger quantities of material (**Figure 22**).

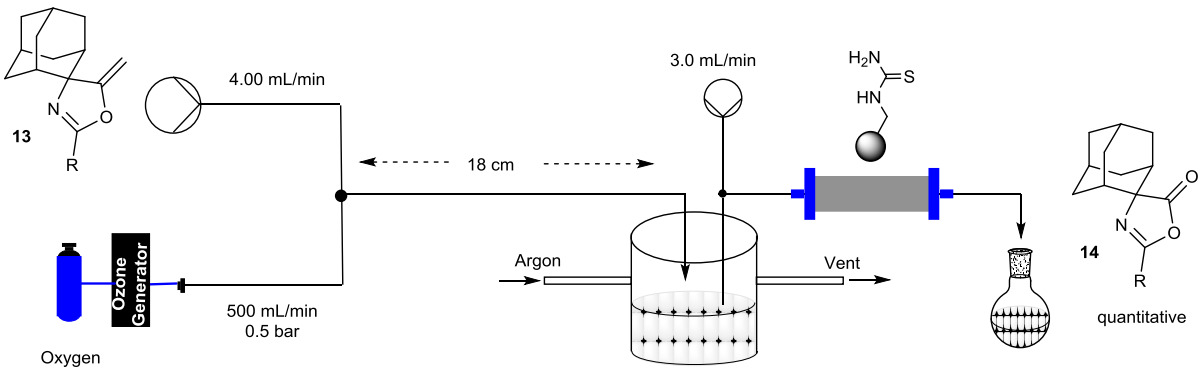


Figure 22. Final scheme for the ozonolysis in flow.

Another useful feature of this set-up was the very small stoichiometric excess of ozone required, ranging from only 1.5 to 2 equivalents. Under optimised conditions it was possible to achieve a

throughput of 6.6 g/h, equating to over 200 g of product per day when processing in a continuous fashion.

Next applying the hydrolysis conditions used by Edward *et al*. in batch[32] the phenyl derivative **14b** was quantitatively converted to the corresponding amino acid **8** after 80 h at reflux in 70% isolated yield on a 10 mmol scale. Surprisingly, a much faster reaction was observed for the corresponding methyl derivative **14a** which required only 28 h (96 % isolated yield). However, in each case the product obtained as its hydrochloride salt was a match for the authentic material. The transferal of the general hydrolysis procedure to flow was quite straightforward. The first set-up employed two injection loops (3 mL per loop), one loaded with the azalactone (**14**) in acetic acid and the other containing a mixture of AcOH/H_2O/HCl 9/1.8/1 (v/v/v). The two channels were simultaneously switched into the main flow stream of AcOH/HCl 40/1 (v/v) and combined at a T-piece before progressing into a 14 mL PFA CFC. Once again temperature was found to be a principle parameter: reaction temperatures below 130 °C gave substantial quantities of the *N*-acylamino acid derivatives (**15a**) and (**15b**) (**Figure 23**).

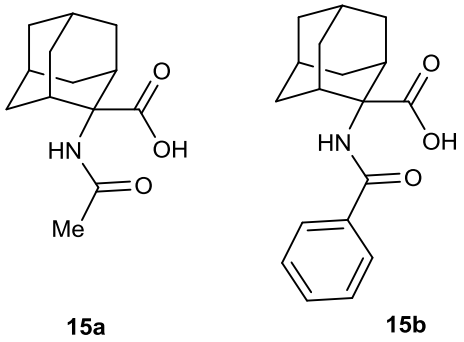
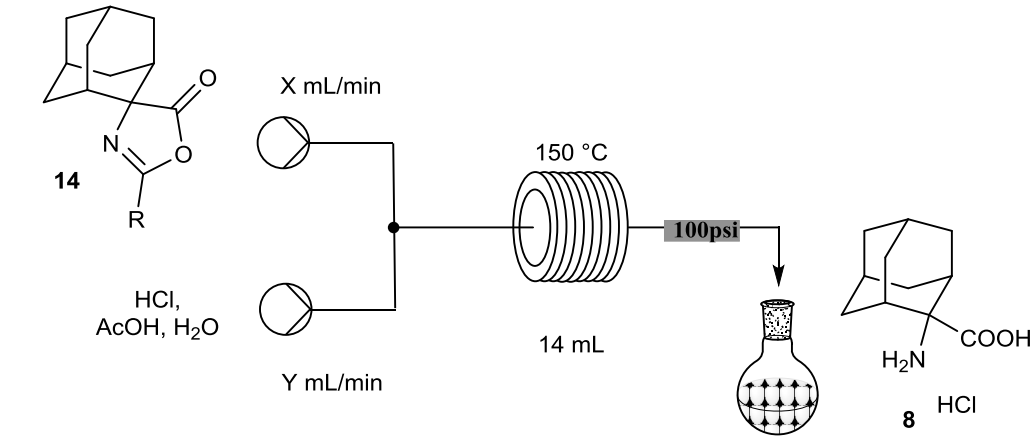


Figure 23. Structure of **15a** and **15b**.

Any attempt to increase the flow rate beyond 0.80 mLmin^{-1} per channel (residence time 9 min) gave incomplete hydrolysis. The final optimised conditions involved processing a solution of the azalactone (**14**) in AcOH continuously introduced to the system at a fixed flow rate (0.40 mLmin^{-1} for (**14a**); 0.25 mLmin^{-1} for (**14b**) equating to residence times of 18 min and 28 min respectively) to meet at a standard T-connector with a solution of HCl/AcOH/H_2O (1/1.8/9

v/v/v equal flow rate). The combined solution was then directed into a 14 mL PFA CFC heated at 150 °C (**Figure 24**).



14a → **1** R = Me (yield 94%), X = 0.40 and Y = 0.40 mL min^{-1}
14b → **1** R = Ph (yield 87%), X = 0.25 and Y = 0.25 mL min^{-1}

Figure 24. Final set-up for the cleavage in flow

The exiting reaction mixture was collected and concentrated *in vacuo* to obtain an off-white solid which was first triturated with hot Et_2O. Bulk recrystallization from methanol (0 °C, overnight), gave 2-aminoadamantane-2-carboxylic acid (**8**) as the hydrochloride salt in very good yield (94% from (**14a**) and 87% from (**14b**)).

It must be noted that the isolation at each stage of the process has been achieved without recourse to column chromatography, in high yield and giving quality material ready for telescoping into the subsequent step. The overall yield for the route to the methyl derivative (**14a**) was 76% whereas a lower final yield of 70% was attained for the phenyl derivative (**14b**) (**Figure 25**).[20,30]

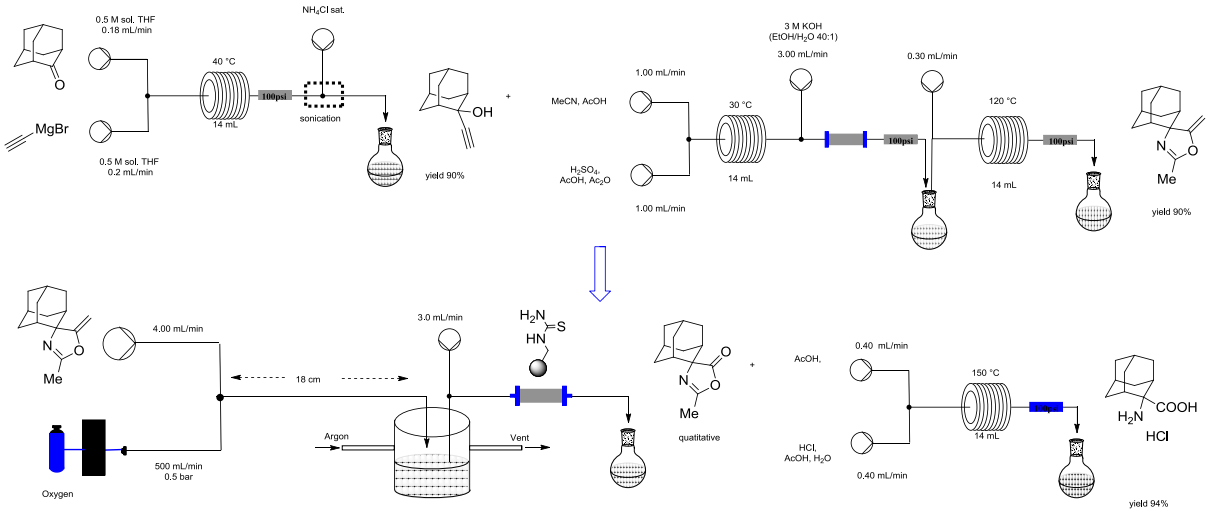


Figure 25. Flow sequence to the amino acid (**8**).

Meclinertant in flow

(Battilocchio, C.; Deadman, B.J.; Nikbin, N.; Ley, S.V.; Baxendale, I.R. *Chem. Eur. J.* Submitted for publication Ref. 34)

Synthesis of 2,6-dimethoxyacetophenone. The approach to the development of the machine-assisted route to 2,6-dimethoxyacetophenone (**3**) progressed through a four-step synthesis. It was envisaged that beginning from 1,3-cyclohexadione (**16**) an iodination/aromatisation/methylation tandem reaction would yield the intermediate (**17**) and, subsequently, a Sonogashira coupling (**18**) followed by deprotection (**19**) and hydration would afford the desired acetophenone (**3**) (**Figure 26**).

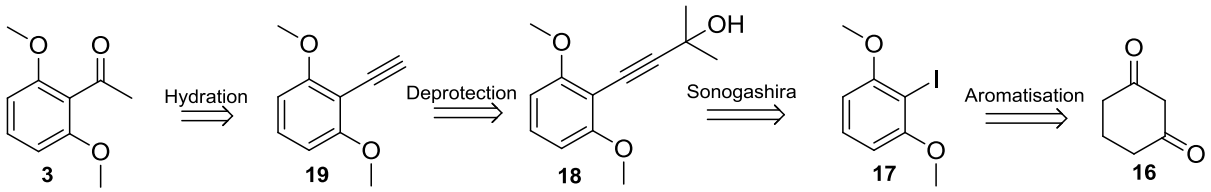


Figure 26. Envisaged route to the acetophenone **3**

The batch reaction for the initial step showed that the selection of the solvent was a crucial parameter. Indeed, reacting iodine and compound (**16**) in the presence of an excess of trimethyl orthoformate (TMOF), as reported in the literature,[47] gave only a 20-30% yield on a 1 mmol scale. After screening many different reaction conditions, it was noticed that addition of methanol, as solvent, improved the conversion of the starting material to the product to 90%. Increasing the temperature above 30 °C led to the formation of several by-products, the two principle identified by-products are shown in (**Figure 27**).

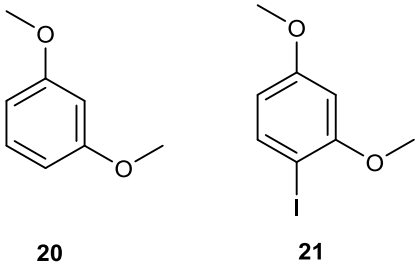


Figure 27. By-products generated when raising the temperature above 30 °C

When the reaction was transferred to the flow platform it was found that the micro-mixing effect, in those conditions, was not beneficial at all. Indeed, when a solution of 1,3-cyclohexadione (**16**) in methanol and a methanol solution of iodine were delivered at a flow rate of 0.10 mL/min and then combined (T-connector) thereafter in the reactor coil (14 mL) at rt, only a 50% conversion was obtained (when processing 10 mmol of substrate). Attempts to optimise the reaction by increasing the residence time or changing the temperature failed to appreciably change the resulting yield. We speculate this is the result of multiple competing equilibria that in a micro-environment are controlled by reversible processes. The next step was to evaluate other iodonium donors. *N*-iodosuccinimide (NIS) is a fairly-known iodinating agent and it is known to generate *in situ* iodonium ions in the presence of an activating agent (usually a Brønsted acid).[48] Indeed, using NIS as iodonium source we were able to isolate the final product in a very good yield (87%). Consequently a solution of 1,3-cyclohexadione (**16**) in methanol and a solution of NIS and *para*-toluene sulfonic acid (PTSA) in methanol were delivered at a flow rate of 0.10 mL/min and combined thereafter in a reactor coil (14 mL) via a T-connector at rt. Attempts to increase the temperature and reduce the reaction time were detrimental to the reaction yield, giving access to almost quantitative conversion to the non-iodinated derivative **20** (**Figure 27** and **Figure 28**).

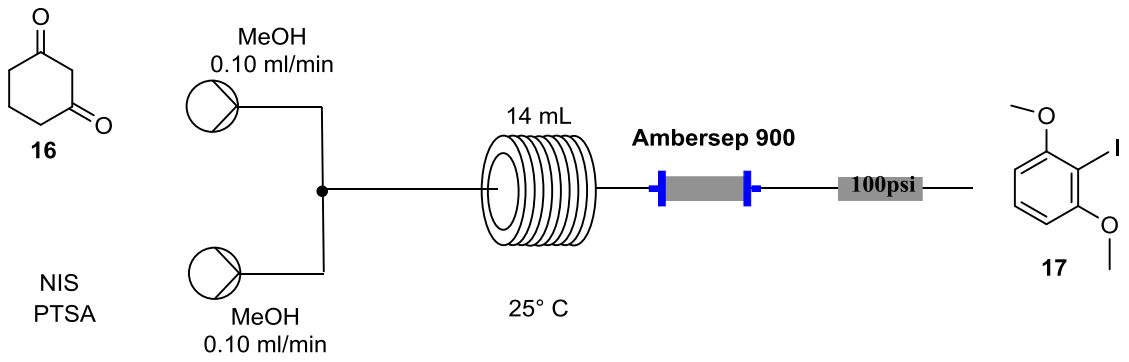


Figure 28. Aromatisation/Iodination with NIS

The succinimide by-product was easily removed from the reaction stream by passing the mixture through a cartridge packed with strongly basic polymer supported beads (Ambersep-OH 900 strongly basic, purchased from Sigma Aldrich), which after removal of the solvent *in vacuo*, gave access to compound (**17**) (**Figure 29**).

Figure 29. Succinimide trapped on Ambersep 900 (strong basic ion-exchange resin).

Having successfully prepared the iodo intermediate we next turned our attention to the coupling sequence. A series of different reaction conditions for the Sonogashira cross-coupling were first screened in batch.[49] The reactions highlighted that the use of polymer supported catalysts (Pd En-cat, Cu on Quadrapure, etc.) was not ideal for these particular pairings of substrates with yields ranging between 10% to 30%.[50] After screening different Pd and Cu sources, optimised conditions employing *bis*(triphenylphosphine) palladium chloride and copper iodide in methanol with tetramethylguanidine as base gave full conversion to the product (**18**) after 1 h under microwave irradiation at 100 °C (**Figure 30**).

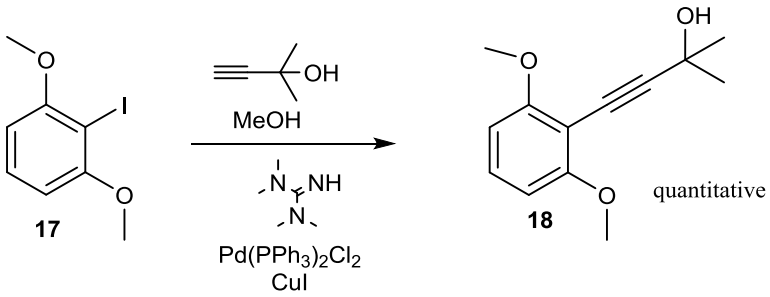


Figure 30. Sonogashira-coupling

The flow set-up was easily arranged with a nearly quantitative conversion to (**18**) being attained. Since we wanted to use the product directly in the next stage, we investigated an in-

line workup using polymer supported scavengers. Unfortunately, this scavenging process proved to be detrimental to the reaction yields dropping them to 30-45% (**Figure 31**).

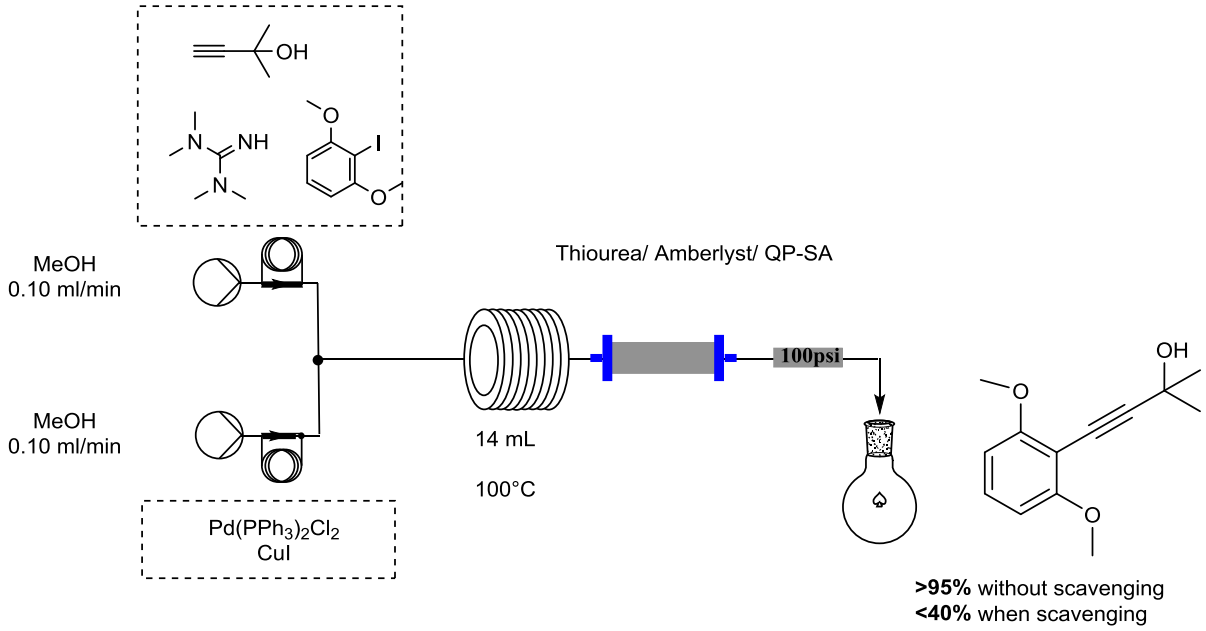


Figure 31. Sonogashira-coupling in flow

While the route had been successful up to this point, there were few concerns about its cost-effectiveness, especially when scaling-up the procedure. To overcome this, an alternative route based on a four step sequence was proposed. The synthesis commences with an *O*-acylation of the cyclohexadione (**16**) followed by rearrangement of (**22**) to the triketone derivative (**23**). A subsequent aromatisation with iodine in methanol would furnish the mono-methoxyacetophenone (**24**). A final methylation step using dimethyl carbonate and 1,2-dimethylimidazole would give access to the product (**3**) (**Figure 32**).

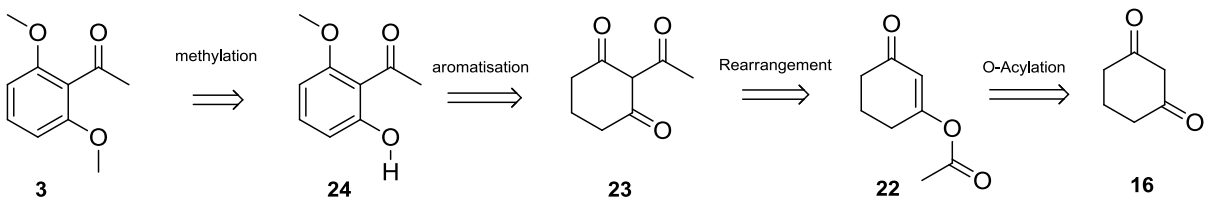


Figure 32. Second route retrosynthesis.

A series of batch reactions for the *O*-acylation were evaluated in parallel and the best conditions found to be using acetyl chloride and diisopropylamine. Pleasingly, the optimised conditions for the corresponding flow procedure allowed for a very short reaction time (0.54 mL/min per channel, residence time 14 min) and high yield (92% isolated) without the need for further purification. We then turned our attention to the rearrangement step from *O*-acyl (**22**) to the corresponding *C*-acyl (**23**). In the literature this kind of reaction is usually carried out with the use of Lewis acid (especially $AlCl_3$).[51] However, most Lewis acids are poorly soluble in most organic solvents and there were safety issues that could be a problem to overcome. Prompted by the need to come up with a different approach to this rearrangement, we then focused on the use of dimethylaminopyridine (DMAP) as catalyst.[52] The use of molecular DMAP in batch gave complete conversion to the *C*-acyl product (**23**) (1 mmol scale with 20 mol% of catalyst) when the reaction was run overnight at rt. It was therefore decided to use a polymer supported version of DMAP catalyst which would be amenable to recycling, especially if applied in a flow process. Running the reaction in toluene at 120 °C we obtained a complete conversion in 4 hours (20 mol% of catalyst using a polymer with loading of 3-6 mmol/g of polymer) on a 2 mmol scale. Nonetheless, as expected with polymer catalyzed reactions in batch, we found that increasing the amount of starting material prolonged the reaction times: starting from 100 mmol of *O*-acyl (**22**) derivative, complete conversion to the *C*-acyl (**23**) was only obtained after 5 days. This was seen as an opportunity to use flow chemistry to reduce the reaction time. A first set of reactions were set up packing the polymer in an glass column and heating at different temperatures ranging from 50 to 120 °C. Unfortunately, the conversion was low (less than 30%) at temperature below 80 °C, even with very slow flow rates. Increasing the temperature led to blockages of the system due to the swelling of the polymer. Attempts to reduce the density of the packing gave similar results. Unfortunately this approach seemed unlikely to be successful.

However, the use of monolithic reactors has proven to be beneficial in expediting flow process and overcoming synthetic problems such as waste disposal and byproduct formation. A monolith is a single continuous piece of macroporous material prepared by precipitation

polymerisation within a column or cartridge. These systems have a permanent porous structure and can be made at a range of shapes and sizes (**Figure 33**).[53]

Figure 33. Picture of a monolith.

Their porosity can be tailored to allow proper flow of solvents and reagents at reasonable pressures (up to 25 bar) through the system. Monolithic polymers do not exhibit the same levels of swelling and shrinking when treated with different solvents, so the generation of a monolithic polymer containing a DMAP functional catalyst was evaluated. The resulting synthesis of the monomer was easily achieved through a simple single step reaction (**Figure 34**).[54]

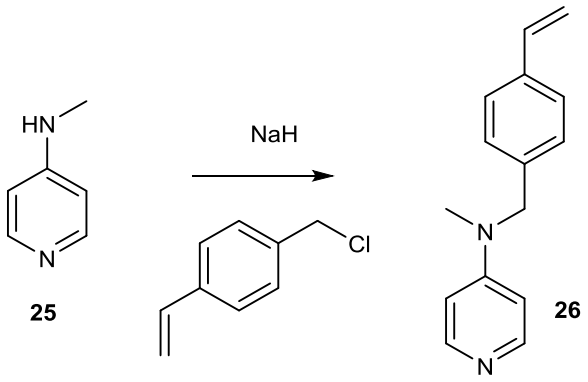


Figure 34. Reaction for the synthesis of the DMAP monomer

The monolith was prepared using a mixture of divinylbenzene (15%), styrene (15%) and monomer (**26**) (10%) in the presence of dodecanol (60%) as porogen and aza-

bis(cyclohexanecarbonitrile) (ACHC, 2 mol% of the monomer). Using this method a monolith with very good physical properties for use in a flow process was obtained.

Utilizing this structure in the flow transformation of starting material (**22**) to compound (**23**) was next evaluated. Under optimised flow conditions, two grams of the precursor (**22**) were processed with the column heated at 100 °C, delivering the solution of (**22**) at a flow rate of 50 μLmin^{-1} (residence time of 20 min), achieving full conversion to (**23**). In order to telescope the material from the very first step, each step must be performed in the same solvent. Having found that the use of toluene as solvent is of extreme importance for the rearrangement using the DMAP monolith, we were pleased to find that the *O*-acylation reaction could also be run in toluene. After the *O*-acylation step, the reaction output stream was dispensed onto a filtering surface to eliminate the eventual solids formed and the solution directed to a glass column containing the DMAP monolith. Collection and concentration of the solution gave the triketone (**23**) as a reddish liquid, in 94% yield on a 20 mmol scale (**Figure 35**).

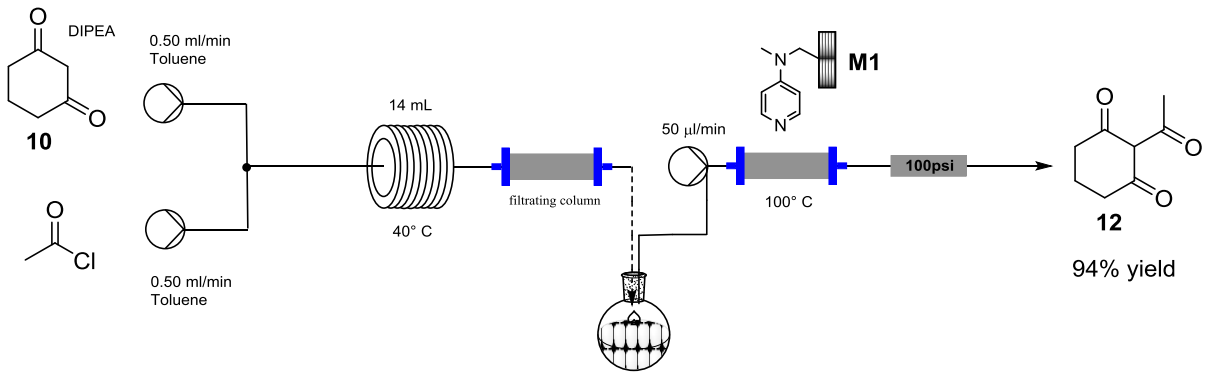


Figure 35. Telescoped-flow acylation/rearrangement using the DMAP monolith

Having successfully obtained the triketone (**23**) *via* a flow reaction, we turned our attention to the aromatization and methylation steps.

After screening various modified conditions based upon the original iodination/aromatisation chemistry described above, a flow set-up was established that used a solution of compound (**23**)

in methanol and a methanolic solution of iodine, each stream delivered at a flow rate of 0.20 mLmin^{-1} and combined at a T-connector. Thereafter the solution passed into a CFC reactor (14 mL) which was maintained at 80 °C; the output stream was then directed to a glass column packed with a mixture of calcium carbonate to scavenge the hydroiodic acid formed and sand as a packing agent. The flow stream was then directed to second packed column containing thiosulfate beads mixed with Celite to scavenge any excess of iodine in solution. The output was collected and concentrated *in vacuo* to obtain the mono-methoxyacetophenone (**24**) in 68 % yield (**Figure 36**). The reduced yield was speculated to be a result of the interaction of the triketone (**23**) or the product (**24**) with the calcium carbonate, since no other material was identified in the outlet. Alternative strategies to remove the hydroiodic acid formed during the reaction proved detrimental, leading to further decrease of the yield. This step is still under investigation.

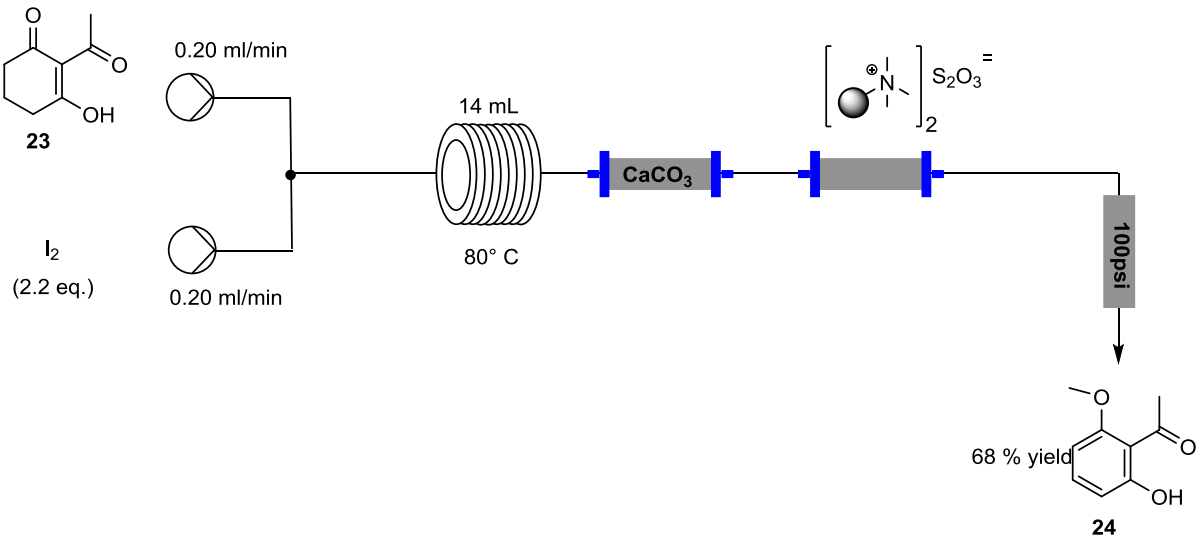


Figure 36. Flow synthesis of the mono-methyl intermediate.

The final step of the sequence was carried out using a recently reported procedure, employing dimethylcarbonate (DMC) and 1,2-dimethylimidazole (DMI).[55] A solution of acetophenone (**24**) in DMF (flow rate 1.00 mLmin^{-1}) and a solution of DMI in DMC (flow rate 1.00 mLmin^{-1}) were combined in a T-piece and then directed into a CFC reactor (14 mL) heated at 150 °C to obtain (**3**) in quantitative yield after trivial extraction procedures. We found that DMC decomposes in

the presence of strong acid and DMI can be scavenged by strongly acid media an immobilized acid scavenger allowed a complete in-line workup. Mindful of our objective to telescope the flow process further, it was noticed that methanol proved to be as effective in the reaction as DMF. Indeed, when performing the reaction in this solvent a quantitative conversion was achieved by reducing the flow rate, each channel being 0.7 mL/min (**Figure 37**). The solution stream was then directed to a QP-SA containing cartridge to scavenge the imidazole and decompose DMC successfully.

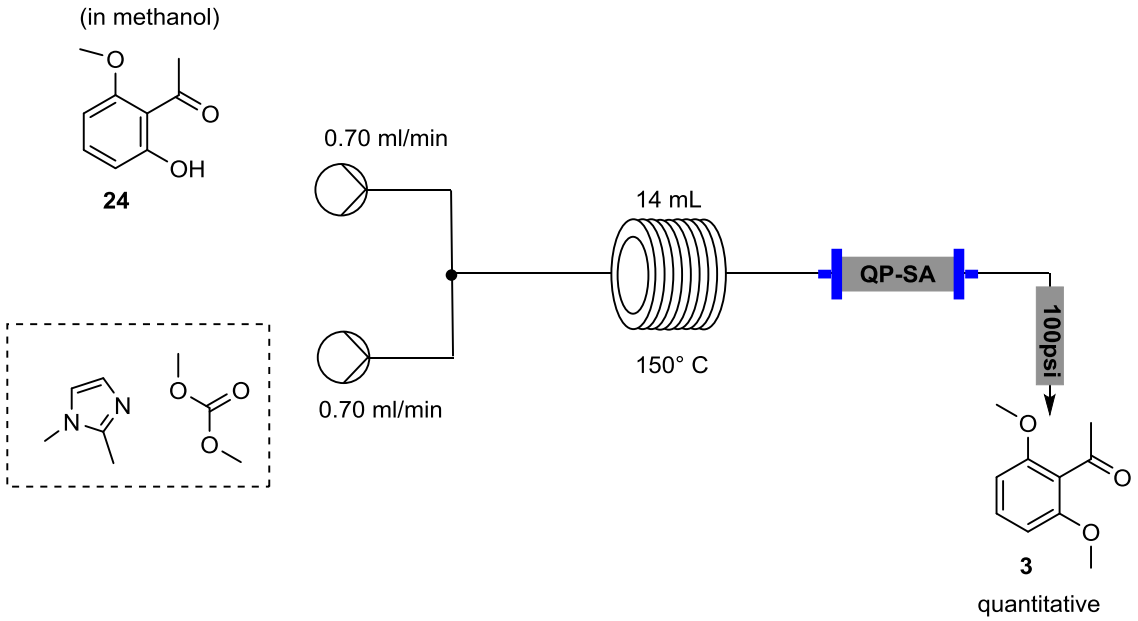


Figure 37. Final step for the flow synthesis of the final product

Following the procedure previously outlined, the material (a solution of **24** in methanol) from the aromatisation step was then directed in a second reactor were it was combined with the solution of dimethylcarbonate and 1,2-dimethylimidazole. Eventually, we were able to achieve a 63% overall yield of **3** (two steps), employing a flow rate (for the final methylation step) of 0.80 mL/min (**Figure 38**).

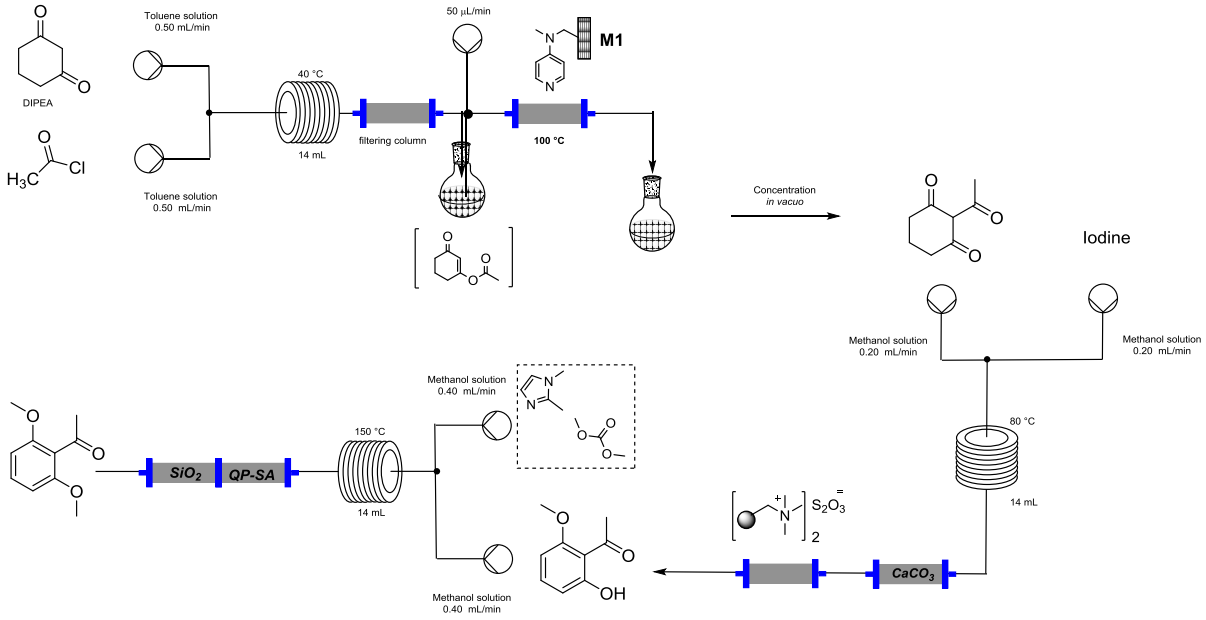


Figure 38. Telescoped flow aromatisation/methylation.

The pyrazole core. With the acetophenone (**3**) in hand, we began to investigate the Claisen condensation[31,32] to obtain the 1,3-dicarbonyl compound (**4**) (this step of the work has been done in collaboration with Benjamin J. Deadman, University of Cambridge). Screening a number of conditions under microwave irradiation indicated that the reaction could be driven to completion after just 30 min at 80 °C, using ethanol as solvent. However, the set-up for the flow reaction proved quite troublesome, due to blockages caused by precipitation of the product. It was identified that with higher concentrations of sodium ethoxide these problems were amplified, but at lower concentration the reaction proved to be sluggish. A compromise was chosen whereby the concentration of the reagent input was lowered and the solvent system was changed to ethanol/THF. Under these conditions, a solution of acetophenone and diethyl oxalylester in a mixture of ethanol/THF (1:1, v/v) was combined in a T-piece with a 0.25 M solution of sodium ethoxide in ethanol. Both solutions were delivered at a flow rate of 0.40 mL/min, and reacted at 60 °C in a 25 mL stainless steel coil. The product was obtained in a sufficient yield (around 50%) without blockages, but did not justify the use of flow technology in this instance. Indeed, the batch reaction under these conditions indicated a 70% yield. After

various test reactions in batch, we came up with the solution whereby reducing the proportion of THF in the solvent mixture and increasing the temperature to 100 °C gave a quantitative transformation of the starting material. At this point the challenge was to set up a platform which could handle suspensions under high pressure. This was overcome by employing a gas-pressurised system (developed previously within the group) instead of using a 1mm i.d. BPR, which we thought would be the primary cause of blockages, and a wider PFA coil (10 mm i.d.) to prevent the solid building up inside the reactor (**Figure 39**).

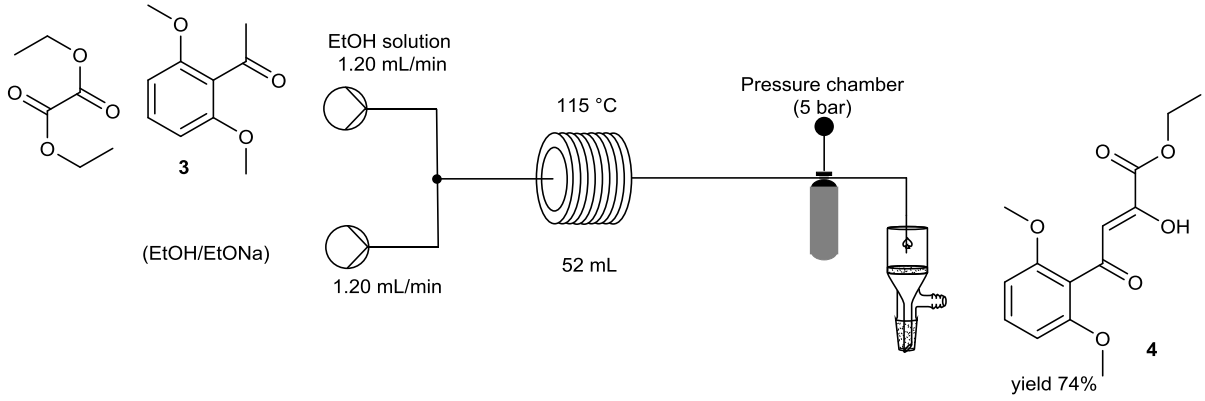


Figure 39. Flow step for the Claisen condensation.

The pressure chamber applied a pressure of 5 bar to the system and this allowed the reaction to be easily run without any problem. Additionally, we wrapped an electric heating system around the chamber which helped to keep the material in solution. Under these optimized conditions, a solution of acetophenone and diethyl oxalyl ester in ethanol (flow rate 1.20 mL/min) was combined in a T-piece with a solution 1M of sodium ethoxide in ethanol (flow rate 1.20 mL/min) and heated to at 115 °C in a 52 mL PFA coil (10 mm i.d.) (**Figure 40**).

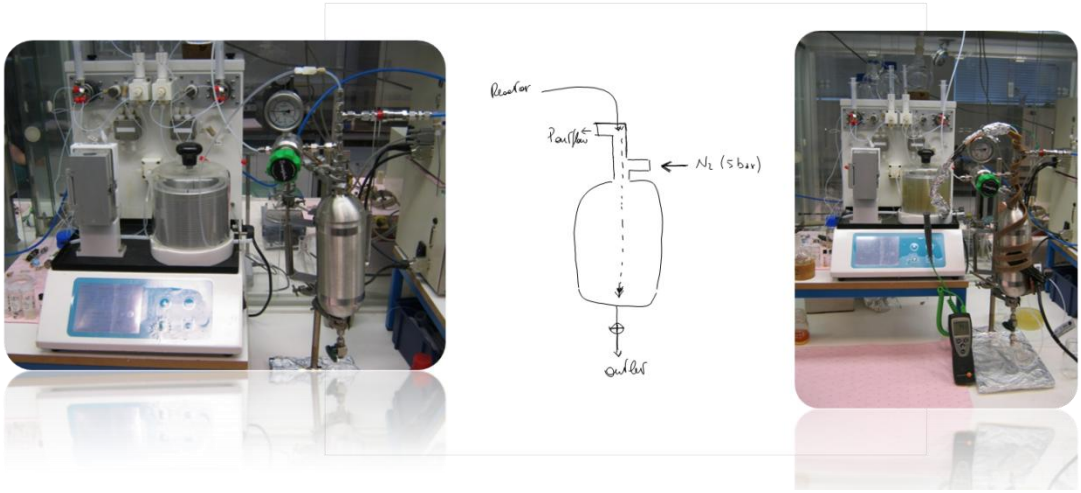


Figure 40. Claisen reaction using the pressure chamber.

The material was then telescoped to the following stage where cyclisation with a substituted hydrazine gave access to the pyrazole core (**6**). Investigations of the reaction conditions under microwave irradiation led to the choice of ethanol as solvent (condition identified by Dr Matthew O. Kitching, University of Cambridge). Adapting these conditions to a flow system proved troublesome due to the insolubility of both compounds (**4**) and (**5**). We therefore went back to the microwave conditions to screen different solvents that could solve the solubility issue. The best choice of solvent turned out to be DMF. Using this solvent, the product could be synthesised under microwave conditions by heating the mixture at 140 °C for 1.5 h. Under flow conditions, a mixture of (**4**) and (**5**) in DMF were delivered to a T-piece to meet a solution of sulfuric acid (conc.) in DMF; afterwards the mixture was heated in a 52 mL PFA coil (10 mm i.d.) at 140 °C. Collection, extraction and concentration of the output afforded the pyrazole ester **6** in a very good yield (89%) without requiring chromatography (**Figure 41**).

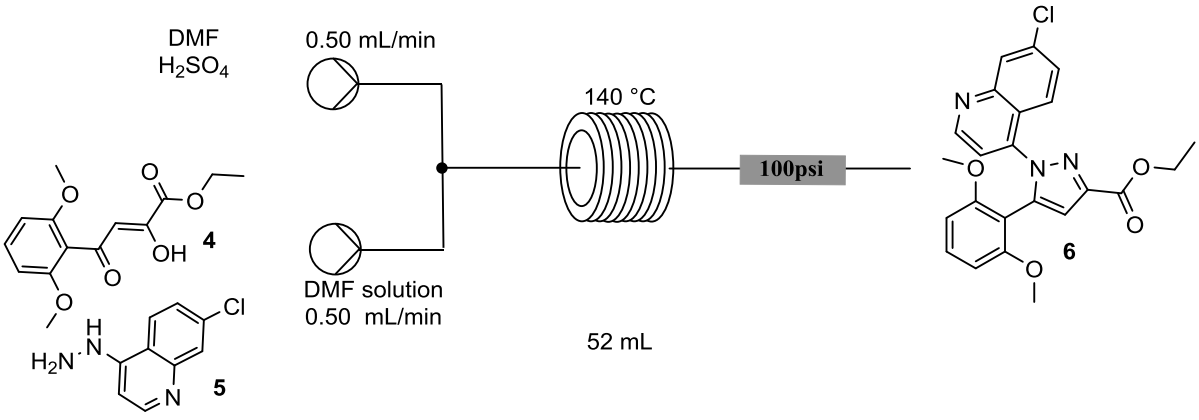


Figure 41. Flow step for the pyrazole ring formation.

Penultimately hydrolysis of the pyrazole ester was achieved by reacting a THF solution of (**6**) with a solution of KOH (3 M). Two delivery channels were used set at a flow rate of 0.5 $mLmin^{-1}$ to combine at a T-piece, the mixture was then progressed through a 14 mL PFA CFC which was maintained at 140 °C. The reactor output was collected and THF was removed *in vacuo*; then HCl was used to precipitate the acid (**7**) (pH 3) (**Figure 42**).

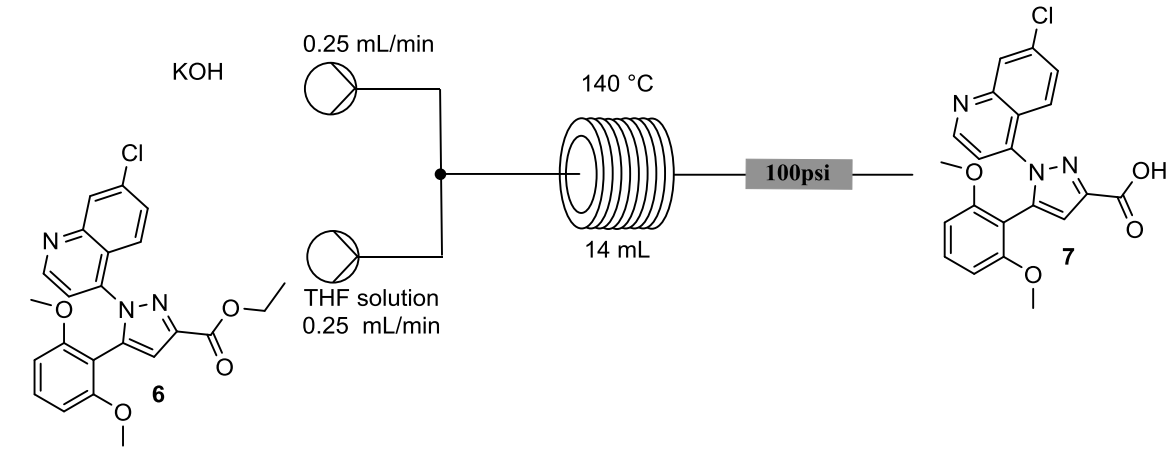


Figure 42. Flow set-up for the hydrolysis.

The final coupling. The flow end-game to Meclinertant was achieved using a strategy previously described for other applications.[40b] The carboxylic acid (**7**) was transformed to the corresponding acid chloride (**27**) using the Ghosez reagent. Under the optimised conditions, a solution of acid (**7**) in DCM was reacted with a solution of the Ghosez reagent also in DCM. The two solutions were combined at a flow rate of 0.10 mLmin^{-1} and passed into a 14 mL CFC reactor at 80 °C. The solution was directly taken into the next stage where it reacted with a solution of (**8**) in pyridine/DCM. Each flow channel was set at 0.10 mLmin^{-1} with the solutions combining at a T-connector and passing into a heated (80 °C) PFA CFC. After collection and extraction, Meclinertant was obtained in a satisfactory yield of 20% (over the last two steps and after crystallization from ethanol/hexanes) (**Figure 43**).[31]

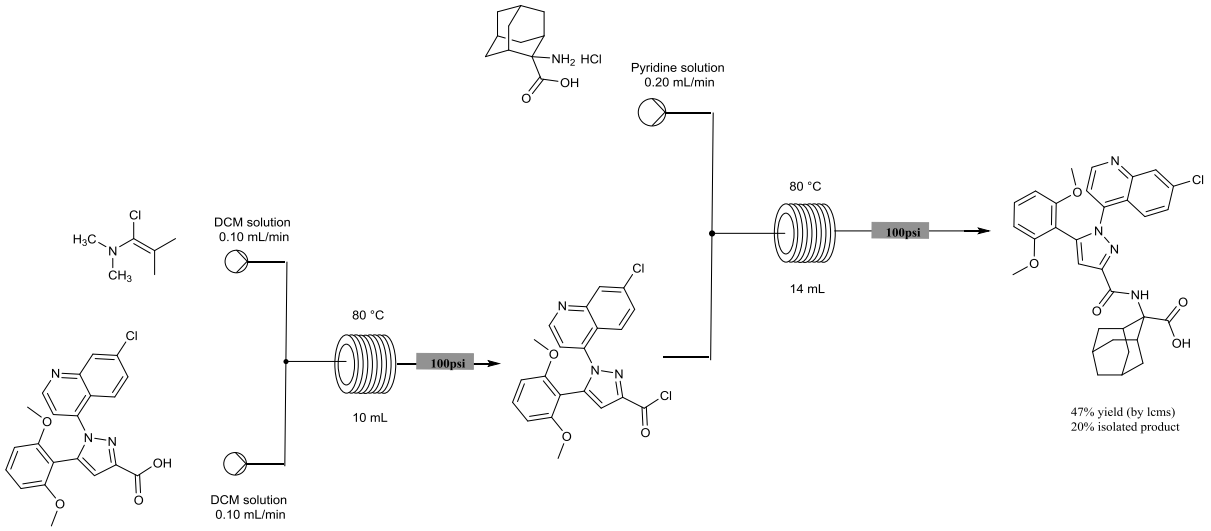


Figure 43. End-game to Meclinertant.

Conclusions

To date only one poorly documented synthesis of Meclinertant has been reported. In this study we have succeded in developing a novel machine-assisted synthesis of this molecule.

This process allowed the preparation of Meclinertant without the need for any chromatography procedures for the intermediates. The process proved to be robust, reliable and amenable to scaling up and it represents a valuable example of a flow synthesis of a commercially significant molecule. We have demonstrated that flow chemistry procedures and concepts have gone beyond the proof of concept stage and can now be used to solve synthetic challenges.

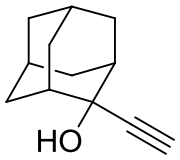
Further work to fully automate the flow platform and integrating a screening device would enable Meclinertant, and any analogue, to be synthesised and screened in a single process.[56]

Experimental procedures

Chemistry. ^{1}H-NMR spectra were recorded on a Bruker Avance DPX-400 spectrometer with the residual solvent peak as the internal reference (CDCl3 = 7.26 ppm, d6-DMSO = 2.50 ppm). 1H resonances are reported to the nearest 0.01 ppm. ^{13}C-NMR spectra were recorded on the same spectrometers with the central resonance of the solvent peak as the internal reference (CDCl3 = 77.16 ppm, d6-DMSO = 39.52 ppm). All 13C resonances are reported to the nearest 0.1 ppm. DEPT 135, COSY, HMQC, and HMBC experiments were used to aid structural determination and spectral assignment. The multiplicity of 1H signals are indicated as: s = singlet, d = doublet, t = triplet, m = multiplet, br. = broad, or combinations of thereof. Coupling constants (J) are quoted in Hz and reported to the nearest 0.1 Hz. Where appropriate, averages of the signals from peaks displaying multiplicity were used to calculate the value of the coupling constant. Infrared spectra were recorded neat on a PerkinElmer Spectrum One FT-IR spectrometer using Universal ATR sampling accessories. Letters in parentheses refer to the relative absorbency of the peak: w = weak, less than 40% of the most intense peak; m = medium, ca. 41-69% of the most intense peak; s = strong, greater than 70% of the most intense peak. Unless stated otherwise, reagents were obtained from commercial sources and used without purification. Laboratory reagent grade EtOAc, petroleum ether 40-60, and DCM were obtained from Fischer Scientific and distilled before use. Unless stated otherwise, heating was conducted using standard laboratory apparatus. The removal of solvent under reduced pressure was carried out on a standard rotary evaporator, a Genevac EZ-2 Plus personal evaporator, or a Vapourtec V-10 evaporator. Melting points were performed on either a Stanford Research Systems MPA100 (OptiMelt) automated melting point system and are uncorrected. High resolution mass spectrometry (HRMS) was performed using a Waters Micromass LCT Premier™ spectrometer using time of flight with positive ESI, or conducted by Mr Paul Skelton on a Bruker BioApex 47e FTICR spectrometer using positive ESI or EI at 70ev to within a tolerance of 5 ppm of the theoretically calculated value. LC-MS analysis was performed on an Agilent HP 1100 series chromatography (Mercury Luna 3u C18 (2) column) attached to a Waters ZQ2000 mass

spectrometer with ESCi ionization source in ESI mode. Elution was carried out at a flow rate of 0.6 mL min-1 using a reverse phase gradient of acetonitrile and water containing 0.1% formic acid. Retention time (Rt) is given in min to the nearest 0.1 min and the m/z value is reported to the nearest mass unit (m.u.). X-ray crystal structures were determined by Dr John Davies at the Department of Chemistry, University of Cambridge. CIF numbers are reported as part of compound characterization. Elemental analyses within a tolerance of ±0.3% of the theoretical values were determined by Mr Alan Dickerson and Mrs Patricia Irele in the microanalytical laboratories at the Department of Chemistry, University of Cambridge.

2-Ethynyl adamantan-2-ol (11).



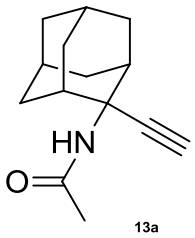
11

Batch reaction. A solution of ethynyl magnesium bromide (0.5 M in THF, 100 mL) was added dropwise to a stirred solution of adamantanone (**10**) (0.042 mol, 6.25 g in 300 mL of THF) at -78 °C in a dry ice/acetone bath. After 2 h the dry ice bath was removed and the reaction further stirred for 16 h at room temperature. The reaction was quenched gently with NH_4Cl solution at 0 °C and the two phases separated; the water solution was washed twice with Et_2O and the organic layer dried over sodium sulfate. After concentration, the solid obtained was crystallized from hexane to obtain 2-ethynyl adamantan-2-ol (**11**) as white solid (0.033 mol, 5.81 g, yield 79.2 %).

Flow reaction. A solution of 2-adamantanone (**10**) (100.0 g, 660 mmol, 0.5 M in THF, flow rate 0.18 mL min^{-1}, pump A) and a solution of ethynyl magnesium bromide (0.5 M in THF, flow rate 0.20 mL min^{-1}, pump B) were combined at a T-piece using a Uniqsis Flowsyn (residence time 37 min) and passed through a 14 mL PFA CFC heated at 40 °C. After exiting the CFC the flow stream was quenched with a saturated solution of NH_4Cl solution (flow rate 0.18 mL min^{-1}, pump C)

mixed at a second T-piece. The T-piece was sonicated to avoid blockages due to formation of insoluble magnesium salts. The combined biphasic output was collected and the two phases were separated; the water phase was washed once with Et_2O and then the organic solution obtained dried over sodium sulfate. Evaporation of the solvent and subsequent crystallization from hexane furnished the desired product 2-ethynyl adamantan-2-ol (**11**) as a white solid (105.5 g, 590 mmol, yield 90 %, mp 101.7 °C); LC-MS: retention time 4.63 min, m/z $[M+H]^+$ = 159.00. FT-IR (neat, cm-1) v: 3366 (m), 3181 (m), 2898 (m), 2159 (w), 754 (m); ^{1}H-NMR (400 MHz, CDCl3): δ/ppm = 2.53 (s, 1H), 2.18-2.13 (m, 4H), 1.96-1.96 (m, 3H), 1.82-1.77 (m, 4H), 1.70 (br. s, 2H), 1.55-1.58 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ/ppm = 88.51, 72.70, 72.50, 39.31, 37.60, 35.37, 31.54, 26.40, 26.23. HRMS: calculated for $[C_{12}H_{17}O]^+$ 177.2550, found 177.2558.

2-Ethynyl-2-acetamido adamantane (12a).



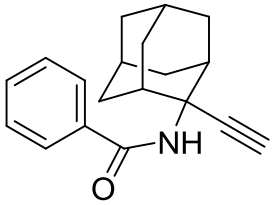
13a

Batch reaction. To a cooled (0 °C) solution of 2-ethynyl adamantan-2-ol (**11**) (1.5 g, 8.5 mmol), MeCN (5 mL) and acetic acid (3 mL), sulfuric acid (1.7 mL, 95% v/v) is added dropwise over 1 minute. Subsequently, the reaction is allowed to reach room temperature and stirred for further 2 h. Then the mixture is cooled again (sub-zero temperature) and quenched gently with NaOH solution 1M (60 mL). The suspension obtained is extracted with Et2O (4 × 100 mL) and washed with brine. The organic layer is dried over sodium sulfate and concentrated to obtain a crude material that after trituration with hexane gave the desired 2-ethynyl-2-acetylamino adamantane (**12a**) as a white solid (1.54 g, 7.0 mmol, yield 83 %).

Flow reaction. EXPT A (small scale). A solution of 2-ethynyl adamantan-2-ol (**11**) (0.3 g, 1.7 mmol), MeCN (1.6 mL) and AcOH (2 mL), loaded in a 3mL injection loop (A), and neat conc. sulfuric acid (3 mL), loaded in a 3 mL injection loop (B), were simultaneously switched into the

main flow stream of glacial acetic acid/acetic anhydride 10/1 (v/v) (flow rate of 1.00 mL min^{-1} per channel) combining at a T-piece before progressing into a 14 mL PFA CFC heated at 30 °C (residence time 7 min). After exiting the CFC this flow stream was quenched with a third stream containing KOH solution (3 M, pump C at a flow rate 3 mL min^{-1}) and the salts formed were filtered and washed with DCM. After separation of the two phases the organic layer was dried over sodium sulfate and concentrated to obtain a crude material that after trituration with hexane gave the title compound (**12a**) as a white solid (0.3 g, 1.4 mmol, yield 86 %); EXPT B (scale-up). A solution of 2-ethynyl adamantan-2-ol (**11**) (20.0 g, 113.6 mmol, pump A), MeCN (80 mL) and AcOH (88 mL) and a mixture of conc. sulfuric acid (40 mL, pump B), acetic acid (88 mL) and acetic anhydride (40 mL) were pumped using a Uniqsis Flowsyn at a flow rate of 1.00 mL min^{-1} per channel (residence time 7 min) through a 14 mL PFA CFC heated at 30 °C. After exiting the CFC this flow stream was quenched with a third stream containing KOH solution (3 M, pump C at a flow rate 3 mL min^{-1}) and the salts formed were filtered and washed with DCM. After separation of the two phases the organic layer was dried over sodium sulfate and concentrated to obtain a crude material that after trituration with hexane gave the title compound (**12a**) as a white solid (21.8 g, 99.0 mmol, yield 91 %, mp 147 °C); LC-MS: retention time 4.39 min, m/z $[M+H]^+$ = 218.14. FT-IR (neat, cm-1) v: 3287 (m), 2903 (m), 2166 (w), 1645 (s), 1537 (s), 670 (m); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm = 5.41 (br. s, 1H), 2.46 (s, 1H), 2.40 (br. s, 2H), 2.35 (br. s, 1H), 2.31 (br. s, 1H), 2.01 (s, 3H), 1.82-1.77 (m, 4H), 1.70 (br. s , 2H), 1.55-1.58 (m, 3H); ^{13}C-NMR (400 MHz, $CDCl_3$): δ/ppm = 169.12, 86.18, 71.73, 56.47, 37.64, 35.06, 34.37, 32.18, 27.24, 26.76, 24.23. HRMS: calculated for $[C_{14}H_{20}NO]+$ 218.1545, found 218.1548.

2-Ethynyl-2-benzamido adamantane (12b).



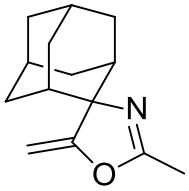
12b

Batch reaction. To a cooled (0 °C) solution of 2-ethynyl adamantan-2-ol (**11**) (1.42 g, 8.0 mmol), benzonitrile (2.22 mL) and acetic acid (3 mL), sulfuric acid (1.7 mL, 95% v/v) is added dropwise over 1 min. Subsequently, the reaction is allowed to reach room temperature and stirred for further 4 h. Then the mixture is cooled again (sub-zero temperature) and quenched gently with NaOH solution 1 M (60 mL). The suspension obtained is extracted with Et_2O (4 × 100 mL) and washed with brine. The organic layer is dried over sodium sulfate and concentrated to obtain a crude material that after trituration with hexane gave the desired product (**12b**) as a white needles (1.8 g, 6.5 mmol, yield 81 %).

Flow reactions. EXPT A (small scale). A solution of 2-ethynyl adamantan-2-ol (**11**) (0.3 g, 1.7 mmol), benzonitrile (0.8 mL, 7.8 mmol, 4.6 equiv.) and AcOH (2.1 mL), loaded in a 3mL injection loop (A), and neat conc. sulfuric acid (3 mL), loaded in a 3 mL injection loop (B), were simultaneously switched into the main flow stream of glacial acetic acid/acetic anhydride 10/1 (v/v) (flow rate of 1.00 mL min^{-1} per channel) combining at a T-piece before progressing into a 14 mL PFA-CFC heated at 30 °C (residence time 7 min). After exiting the CFC this flow stream was quenched with a third stream containing KOH solution (3 M, pump C at a flow rate 3 mL min^{-1}) and the salts formed were filtered and washed with DCM. After separation of the two phases the organic layer was dried over sodium sulfate and concentrated to obtain a crude material that after trituration with hexane gave the title compound (14b) as a white solid (0.38 g, 1.3 mmol, yield 77 %); *EXPT B (scale-up).* A solution of 2-ethynyl adamantan-2-ol (**11**) (50.0 g, 280 mmol, pump A), benzonitrile (98 mL, 0.95 mol, 3.4 equiv.) and acetic acid (196 mL) and a mixture of concentrated sulfuric acid (98 mL, pump B), acetic acid (215 mL) and acetic

anhydride (98 mL) were pumped using a Uniqsis FlowSyn at a flow rate of 1.00 mL min^{-1} per channel (residence time 7 min) through a 14 mL PFA CFC maintained at 30 °C. After exiting the CFC this flow stream was quenched with 3 M aqueous KOH solution (pump C, flow rate 3 mL min^{-1}) and the salts formed were filtered off washing with DCM. After separation of the phases the organic one is dried over sodium sulfate and concentrated to obtain a crude oily material that after trituration with hexane gave the title compound (**12b**) as white solid (50.1 g, 174 mmol, yield 64 %); *EXPT C (scale-up modified conditions).* A solution of 2-ethynyl adamantan-2-ol (**11**) (50.0 g, 0.28 mol, pump A), benzonitrile (49 mL, 0.475 mol, 1.7 equiv.) and acetic acid (196 mL) and a mixture of concentrated sulfuric acid (98 mL, pump B), acetic acid (215 mL) and acetic anhydride (98 mL) were pumped using a Uniqsis FlowSyn at a flow rate of 0.60 mL min^{-1} per channel (residence time 11.6 min) through a 14 mL PFA CFC maintained at 30 °C. After exiting the CFC this flow stream was quenched with 3 M aqueous KOH solution (pump C, flow rate 1.8 mL min^{-1}) and the salts formed were filtered off washing with DCM. After separation of the phases the organic one is dried over sodium sulfate and concentrated to obtain a crude oily material that after trituration with hexane gave the title compound (**12b**) as white needles (66.5 g, 231 mmol, yield 85 %, mp 189 °C); LC-MS: retention time 4.98 min, m/z $[M+H]^+$ = 280.15. FT-IR (neat, cm^{-1}) v: 3400 (m), 3281 (m), 2905 (m), 1645 (s), 1537 (s), 690 (s); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm = 7.96 (d, 2H, J = 6.8 Hz), 7.49 (m, 1H), 7.40 (m, 2H), 6.14 (br. s, 1H), 2.56 (s, 2H), 2.49 (s, 1H), 2.40 (d, 2H, *J* = 11.2 Hz), 2.00 (d, 2H, *J* = 11.2 Hz), 1.89-1.70 (m, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ/ppm = 166.26, 135.27, 131.47, 128.60, 126.94, 86.04, 71.99, 56.63, 37.66, 35.28, 34.06, 32.01, 26.92, 26.48. HRMS: calculated for $[C_{19}H_{22}NO]^+$ 280.1701, found 280.1691.

2-Methyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (13a).



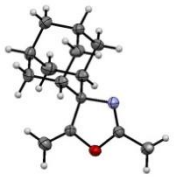
13a

Batch reaction. A solution of 2-ethynyl 2-acetylamino adamantane (**12a**) (0.640 g, 3 mmol) and KOH (0.45 g, 8 mmol) in EtOH (6 mL) was heated at 100 °C for 2 h employing a microwave apparatus system. Then the mixture was quenched with brine (100 mL), extracted with DCM (4 x100 mL) and washed with brine (100 mL). Then the organic layer obtained was dried over sodium sulfate and concentrated to obtain the title compound (**13a**) as a yellowish solid (0.58 g, 2.6 mmol, yield 91 %).

Flow reaction. A solution of 2-ethynyl 2-acetylamino adamantane (**12a**) (9.0 g, 41.3 mmol, flow rate 0.20 mL min-1, pump A) in EtOH (400 mL) and a solution of potassium hydroxide (13.0 g, flow rate 0.10 mL min^{-1}, pump B) in EtOH (200 mL) were pumped using a Uniqsis Flowsyn (residence time 47 min) through a 14 mL PFA CFC heated at 125 °C using a 100 psi BPR. After exiting the CFC this flow stream was collected and the solution concentrated in vacuo. The solid obtained was quenched with distilled water (ice bath to avoid any retro-reaction) and extracted with DCM. After drying over sodium sulfate, the solvent was evaporated and the title compound (**13a**) isolated as a yellowish solid (8.2 g, 37.6 mmol, yield 91 %, mp 61.5 °C).

Telescoped Ritter/5-enol exo-dig cyclisation flow procedure. A solution of 2-ethynyl adamantan-2-ol (**11**) (2.0 g, 11 mmol, pump A), MeCN (8.0 mL) and AcOH (9 mL) and a mixture of conc. sulfuric acid (4.0 mL, pump B), acetic acid (9 mL) and acetic anhydride (4.0 mL) were pumped using a Uniqsis Flowsyn at a flow rate of 1.00 mL min^{-1} per channel (residence time 7 min) through a 14 mL PFA CFC heated at 30 °C. After exiting the CFC the flow stream was directed to a T-piece to meet a further solution of KOH (3M) in EtOH/H_2O 40:1 (v/v) (pump C, flow rate 3.0

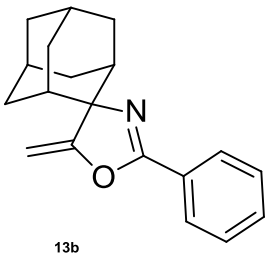
mL min^{-1}) then filtered in-line (column packed with glass wool) to remove any precipitate (column packed with glass wool) to remove any precipitate (eluent collected in a filter flask). The resultant solution was pumped into a PFA CFC and heated to 120 °C (residence time 48 min, flow rate 0.30 mL min^{-1}). After exiting the CFC the flow stream was collected and the solution concentrated in vacuo. The solid obtained was quenched with distilled water (ice bath to avoid any retro-reaction), extracted with DCM, dried over sodium sulfate and the solvent evaporated. The title compound (**13a**) was obtained as a yellowish solid (2.2 g, 10 mmol, yield 91 %). LC-MS: retention time 3.70 min, m/z $[M+H]^{protonated}$ = 219.05; time 4.49 min, m/z $[M+H]^+$ = 218.07. FT-IR (neat, cm^{-1}) v: 2906 (s), 1686 (s), 1654 (s), 1262 (s), 1199 (s), 1063 (s), 965 (s), 834 (s). 1H-NMR (400 MHz, $CDCl_3$): δ/ppm = 4.79 (d, 1H, *J* = 2.4 Hz), 4.52 (d, 1H, *J* = 2.4 Hz), 2.44 (d, 2H, *J* = 12.4 Hz), 2.21 (d, 2H, *J* = 12.4 Hz), 2.03 (s, 3H), 1.86 (broad s, 3H), 1.74 (s , 2H), 1.69-1.55 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ/ppm = 166.00, 158.66, 87.91, 76.02, 38.70, 37.08, 34.06, 31.40, 28.03, 26.76, 14.43. HRMS: calculated for $[C_{14}H_{20}NO]^+$ 218.1545, found 218.1541.



CCDC860429

The structure was unambiguously confirmed by single X-ray crystallography and deposited at Cambridge University (Crystallographic Data Centre) with the unique reference *CCDC860429*; space group P21/c; a = 6.6834 (2), b = 7.0945 (2), c = 23.8587 (8), β = 90.449 (2).

2-Phenyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (13b).



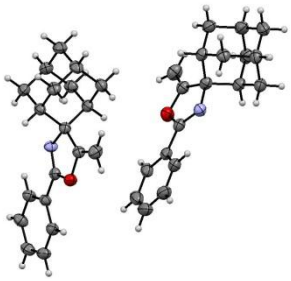
13b

Batch reaction. A solution of 2-ethynyl 2-benzamidoadamantane (**12b**) (0.56 g, 2 mmol) and KOH (0.29 g, 5.2 mmol) in EtOH (4 mL) was heated at 100 °C for 2 h employing a microwave apparatus system. Then the mixture was quenched with brine (100 mL), extracted with DCM (4 x100 mL) and washed with brine (100 mL). Then the organic layer obtained was dried over sodium sulfate and concentrated to obtained 2-phenyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (**13b**) as a yellowish solid (0.53 g, 1.9 mmol, yield 95 %).

Flow reaction. A solution of 2-ethynyl 2-benzamido adamantane (**12b**) (17.5 g, 63 mmol, flow rate 0.40 mL min-1, pump A) in EtOH (800 mL) and a solution of potassium hydroxide (20.0 g, flow rate 0.20 mL min^{-1}, pump B) in EtOH (400 mL) were pumped using a Uniqsis Flowsyn (residence time 24 min) through a 14 mL PFA CFC heated at 120 °C using a 100 psi BPR. After exiting the CFC this flow stream was collected and the solution concentrated in vacuo. The solid obtained was quenched with distilled water (ice bath to avoid any retro-reaction) and extracted with DCM. After drying over sodium sulfate and evaporating the solvent, the title compound (**13b**) was obtained as a yellowish solid (16.2 g, 53 mmol, yield 92 %, mp 67 °C).

Telescoped Ritter/5-enol exo-dig cyclisation flow procedure. A solution of 2-ethynyl adamantan-2-ol (**11**) (5.0 g, 28 mmol, pump A), benzonitrile (4.9 mL, 47.5 mmol, 1.7 equiv.) and acetic acid (20 mL) and a mixture of concentrated sulfuric acid (9.8 mL, pump B), acetic acid (21.5 mL) and acetic anhydride (9.8 mL) were pumped using a Uniqsis FlowSyn at a flow rate of 0.60 mL min^{-1} per channel (residence time 11.6 min) through a 14 mL PFA CFC maintained at 30 °C. After

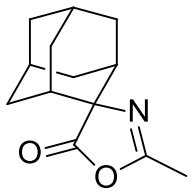
exiting the CFC the flow stream was directed to a T-piece to meet a further solution of KOH (3M) in EtOH/H2O 40:1 (v/v) (pump C, flow rate 1.8 mL min^{-1}) then filtered in-line (column packed with glass wool) to remove any precipitate (eluent collected in a filter flask). The resultant solution was pumped into a PFA CFC and heated to 120 °C (residence time 24 min, flow rate 0.60 mL min^{-1}). After exiting the CFC the flow stream was collected and the solution concentrated in vacuo. The solid obtained was quenched with distilled water (ice bath to avoid any retro-reaction), extracted with DCM, dried over sodium sulfate and the solvent evaporated. The title compound (**13b**) was obtained as a yellowish solid (7.0 g, 25 mmol, yield 90 %). LC-MS: retention time 6.05 min, m/z $[M+H]^+$ = 280.08. FT-IR (neat, cm^{-1}) v: 2899 (s), 1666 (s), 1644 (s), 1056 (s). ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm = 8.01 (d, 2H, *J* = 6.8 Hz), 7.49-7.40 (m, 3 H), 4.99 (d, 1H, *J* = 2.4 Hz), 4.65 (d, 1H, *J* = 2.4 Hz), 2.63 (d, 2H, *J* = 11.2 Hz), 2.29 (d, 2H, *J* = 11.2 Hz), 1.95 (m, 2H), 1.74 (s, 2H), 1.69-1.55 (m, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ/ppm = 166.04, 157.63, 131.98, 128.81, 128.12, 126.97, 88.45, 77.14, 38.73, 37.68, 34.14, 31.51, 27.96, 26.54. HRMS: calculated for $[C_{19}H_{22}NO]^+$ 280.1701, found 218.1704.



CCDC860430

The structure was unambiguously confirmed by single X-ray crystallography and deposited at Cambridge University (Crystallographic Data Centre) with the unique reference *CCDC860430*; space group Pbca: a = 20.3254 (3), b = 12.2093 (1), c = 23.7513 (3).

2-Methyl-4-(adamantane-2'-spiro)-oxazolin-5-one (14a).

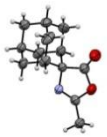


14a

Batch reaction. EXPT A. A solution of 2-methyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (**13a**) (0.436 g, 2 mmol) in DCM (20 mL) was cooled to -78 °C with acetone/dry ice bath and then ozone was bubbled inside the mixture till the solution turns to light blue. Afterwards the excess of ozone was removed from the solution blowing argon and then the reaction was quenched employing supported polymer thiourea. After filtering off the polymer and concentrating the solution, 2-methyl-4-(adamantane-2'-spiro)-oxazolin-5-one (**14a**) was obtained as a white solid (0.418 g, 1.90 mmol, yield 95 %); EXPT B. Ozone was bubbled (20 min) inside a solution of 2-methyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (**13a**) (0.44 g, 2 mmol) in DCM (20 mL) at room temperature until the solution turned colourless. Afterwards the excess of ozone was removed from the solution blowing argon and then the reaction was quenched employing polymer-supported thiourea. After filtering off the polymer and concentrating the solution, the title compound (**14a**) was obtained as a white solid (0.410 g, 1.86 mmol, yield 93 %).

Flow reaction. A solution of 2-methyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (**13a**) (9.0 g, 41.3 mmol, flow rate 4.00 mL min^{-1}, pump A) in DCM (400 mL) and a stream of ozone (0.5 bar, flow rate 500 mL min^{-1}) were combined in a T-piece and directly through a 1 mL PTFE tube reactor (180 mm, 2.5 mm i.d., ~ 15 s residence time). After exiting the tube this flow stream was collected and the excess of ozone was blown out with argon. The solution was then directly delivered to a scavenger cartridge containing QP-TU (3.00 mL min^{-1}, pump B). The collection of the output stream and the concentration of the solution gave the title compound (**14a**) as a

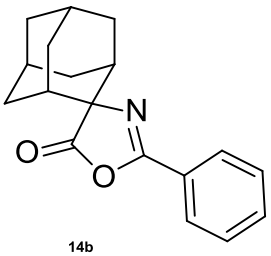
white solid (8.7 g, 39.4 mmol, yield 95 %, mp 138 °C); LC-MS: retention time 4.32 min, m/z [M+H] ^{decarboxylated} = 192.09, m/z [M-H]^{acid} = 236.07. FT-IR (neat, cm^{-1}) *v*: 2907 (s), 1789 (m), 1693 (s), 1448 (m), 1232 (s), 1174 (s), 1046 (s), 971 (s), 900 (s). ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm = 2.51 (d, 2H, *J* = 12 Hz), 2.35 (d, 2H, *J* = 11.2 Hz), 2.19 (s, 3H), 1.94 (s, 2H), 1.71-1.60 (m, 8H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ/ppm = 179.12, 159.27, 72.28, 37.79, 35.04, 33.61, 31.09, 27.48, 26.31, 15.33. HRMS: calculated for $[C_{13}H_{18}NO_2]^+$ 220.1339, found 220.1347.



CCDC860432

The structure was unambiguously confirmed by single X-ray crystallography and deposited at Cambridge University (Crystallographic Data Centre) with the unique reference *CCDC860432*; space group P21/m: a = 6.8926 (4), b = 6.5463 (4), c = 12.267 (2), β = 98.997 (2).

2-Phenyl-4-(adamantane-2'-spiro)-oxazolin-5-one (14b).

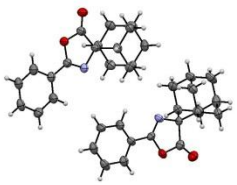


14b

Batch reaction. EXPT A. A solution of 2-phenyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (**13b**) (0.56 g, 2 mmol) in DCM (20 mL) was cooled to -78 °C with acetone/dry ice bath and then ozone was bubbled through the mixture (5 min) until the solution turned light blue in colour. The excess of ozone was removed from the solution by blowing argon and then the reaction was quenched employing supported polymer thiourea. After filtering off the polymer and concentrating the solution, 2-phenyl-4-(adamantane-2'-spiro)-oxazolin-5-one (**14b**) was obtained as a white off solid (0.558 g, 1.98 mmol, yield 99 %); EXPT B. Ozone was

bubbled (20 min) inside a solution of 2-phenyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (**13b**) (0.56 g, 2 mmol) in DCM (20 mL) at room temperature until the solution turned colourless. Afterwards the excess of ozone was removed from the solution blowing argon and then the reaction was quenched employing supported polymer thiourea. After filtering off the polymer and concentrating the solution, the title compound (**14b**) was obtained as a white solid (0.530 g, 1.9 mmol, yield 95 %).

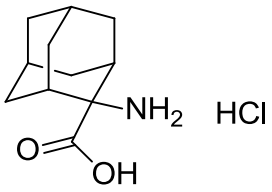
Flow reaction. A solution of 2-phenyl-4-(adamantane-2'-spiro)-5-methylidene oxazoline (**13b**) (10.0 g, 35.8 mmol, flow rate 4.00 mL min^{-1}, pump A) in DCM (350 mL) and a stream of ozone (0.5 bar, flow rate 500 mL min^{-1}) were combined in a T-piece and directly through a 1 mL PTFE tube reactor (180 mm, 2.5 mm i.d., ~15 s residence time). After exiting the tube this flow stream was collected and the excess of ozone was blown out with argon. The solution was then directly delivered to a scavenger cartridge containing QP-TU (3.00 mL min^{-1}, pump B). The collection of the output stream and the concentration of the solution gave the title compound (**14b**) as a white solid (10.09 g, 35.7 mmol, yield 99.8 %, mp 109 °C); LC-MS: retention time 5.64 min, m/z $[M+H]^{decarboxylated}$ = 254.12, m/z $[M-H]^{acid}$= 298.05. FT-IR (neat, cm^{-1}) v: 2893 (s), 1796 (s), 1657 (s), 1453 (m), 1292 (s), 1046 (s), 961 (s), 678 (s); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm = 8.03 (d, 2H, *J* = 6.8 Hz), 7.55-7.51 (m, 1H), 7.49-745 (m, 2H), 2.59 (d, 2H, *J* = 14 Hz), 2.54 (d, 2H, *J* = 14 Hz), 1.99 (s, 2H), 1.87 (s, 2H), 1.82 (s, 2H), 1.70 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ/ppm = 179.04, 157.96, 132.25, 128.68, 127.87, 126.64, 72.79, 37.81, 35.51, 33.67, 31.20, 27.67, 26.39. HRMS: calculated for $[C_{18}H_{20}NO_2]^+$ 282.1494, found 218.1484.



CCDC860431

The structure was unambiguously confirmed by single X-ray crystallography and deposited at Cambridge University (Crystallographic Data Centre) with the unique reference *CCDC860431*; space group P21/m: a = 12.6229 (9), b = 6.9172 (5), c = 17.039 (2), β = 109.380 (3).

2-Aminoadamantane-2-carboxylic acid hydrochloride (8).

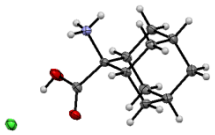


8

Batch reaction. EXPT A. 2-methyl-4-(adamantane-2'-spiro)-oxazolin-5-one (**14a**) (1 g, 4.54 mmol) was dissolved in conc. HCl (10 mL), H2O (50 mL) and acetic acid (230 mL) and heated at reflux (170°C) for 28 h, when the reaction was complete by NMR. The reaction mixture was concentrated in vacuo and the solid triturated with hot Et2O (2 × 100 mL), hot MeCN (2 × 50 mL). The solid was then dissolved in the minimum volume of MeOH (20 mL), added to Et_2O (200 mL) and left to crystallise at 0 ºC overnight. The resulting solid was isolated by filtration to afford the title compound (**8**) as a white crystalline solid (1.00 g, yield 96 %); EXPT B. 2-Phenyl-4-(adamantane-2'-spiro)-oxazolin-5-one (**14a**) (2 g, 7.12 mmol) was dissolved in conc. HCl (15 mL), H_2O (70 mL) and AcOH (350 mL) and heated at reflux (170 °C) for 80 h, when the reaction was complete by NMR. The reaction mixture was concentrated in vacuo and the solid triturated with hot Et_2O (2 × 100 mL), hot MeCN (2 × 50 mL). The solid was then dissolved in the minimum volume of MeOH (20 mL), added to Et_2O (200 mL) and left to crystallise at 0 ºC overnight. The resulting solid was isolated by filtration to afford the title compound (**8**) as a white crystalline solid (1.14 g, yield 70 %).

Flow reactions. EXPT A. A solution of 2-methyl-4-(adamantane-2'-spiro)-oxazolin-5-one (**14a**) (1.15 g, 5.25 mmol, flow rate 0.40 mL min^{-1}, pump A) in glacial acetic acid (150 mL) and a mixture of glacial acetic acid (90 mL), H_2O (50 mL) and hydrochloric acid (10 mL, flow rate 0.40 mL min^{-1}, pump B) were combined in a T-piece and reacted through a 14 mL PFA reactor coil at

150°C (residence time ~18 min). After exiting the tube this flow stream was collected and the mixture was concentrated in vacuo. The solid obtained was washed with hot diethyl ether (50 mL × 2) and hot acetonitrile (50 mL × 2) to obtain the product, that was crystallised from methanol (1.10 g, 4.8 mmol, yield 94 %); EXPT B. A solution of 2-phenyl-4-(adamantane-2'-spiro)-oxazolin-5-one (**14b**) (1.50 g, 5.25 mmol, flow rate 0.25 mL min-1, pump A) in glacial acetic acid (150 mL) and a mixture of glacial acetic acid (90 mL), H_2O (50 mL) and hydrochloric acid (10 mL) (flow rate 0.25 mL min^{-1}, pump B) were combined in a T-piece and reacted through a 14 mL PFA reactor coil at 150°C (residence time ~28 min). After exiting the tube this flow stream was collected and the mixture was concentrated in vacuo. The solid obtained was washed with hot diethyl ether (50 mL × 2) and hot acetonitrile (50 mL × 2) to obtain the product, that was crystallised from methanol (1.05 g, 4.55 mmol, yield 87 %, mp > 258 °C decomposition); LC-MS: retention time 0.31 min, m/z [M+H]decarboxylated = 149.99, m/z $[M+H]^{deaminated}$ = 179.05, m/z [M+H] = 196.10, m/z [M-H] = 194.06. FT-IR (neat, cm-1) v: 3220 (w), 3168 (w), 2775 (s), 1725 (s), 1587 (s), 1506 (s), 1468 (s); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm = 8.70 (s br, 3H), 2.21 (s, 2H), 2.12 (d, 2H, *J* = 13.2 Hz), 1.92 (d, 2H, *J* = 13.2 Hz), 1.75-1.80 (m, 4H), 1.62-1.66 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ/ppm = 171.59, 63.92, 37.54, 34.40, 32.24, 31.22, 26.28. HRMS: calculated for $[C_{11}H_{18}NO_2]^+$ 196.1338, found 196.1336. Microanalysis calculated (found) for $C_{11}H_{18}NO_2Cl$: C 57.02 (56.90%), H 7.83 (7.97%), N 6.04 (6.06%).

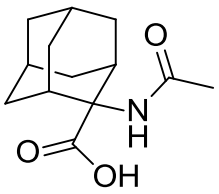


CCDC860428

The structure was unambiguously confirmed by x-ray crystallography and the structure deposited at Cambridge University (Crystallographic Data Centre) with the unique reference

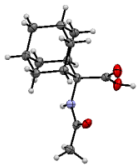
CCDC860428; space group P21/c: a = 6.3862 (2), b = 11.4838 (3), c = 14.9079 (4), β = 95.392 (2).

2-Acetylamido adamantane-2-carboxylic acid (15a).



15a

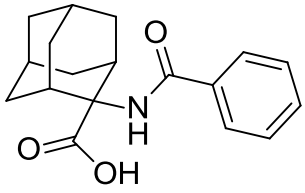
Off-white solid; mp 210-213 °C; LC-MS: retention time 4.06 min, m/z [M-H] = 236.10. FT-IR (neat, cm-1) v: 3386 (m), 2907 (s), 1745 (s), 1632 (m), 1594 (s), 1248 (s), 1208 (s), 849 (m), 721 (m); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm = 12.01 (br. s, 1H), 7.62 (s, 1H), 2.39 (s, 2H), 2.01 (br.s, 4H), 1.82 (s, 3H), 1.73 (s, 2H), 1.62 (br. s, 4H), 1.49 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ/ppm = 174.18, 169.44, 63.10, 38.02, 35.80, 32.50, 32.01, 26.99, 26.71, 23.40. HRMS: calculated for $[C_{13}H_{20}NO_3]$+ 238.1718, found 238.1710.



CCDC874390

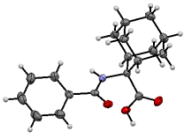
The structure was unambiguously confirmed by x-ray crystallography and the structure deposited at Cambridge University (Crystallographic Data Centre) with the unique reference *CCDC874390*; space group P-1 : a = 6.8572 (2), b = 8.1043 (3), c = 10.8425 (5), α = 90.434(2) °, β = 102.620(2) °, γ = 98.459(3) °.

2-Benzamido adamantane-2-carboxylic acid (15b).



15b

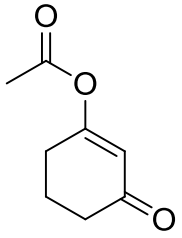
Off-white solid; mp 234-236 °C; LC-MS: retention time 4.28 min., m/z [M+H]+ = 322.14. FT-IR (neat, cm^{-1}) v: 3389.7 (w), 2905.7 (m), 1740.0 (s), 1626.8 (m), 1526.3 (s); ^{1}H-NMR (400 MHz, DMSO-*d6*): δ/ppm = 12.17 (br. s, 1H), 8.10 (s, 1H), 7.79 (d, 2H, J = 7.2 Hz), 7.52 (t, 1H, J = 7.2 Hz), 7.44 (t, 2H, J = 7.2 Hz), 2.63 (s, 2H), 2.12 (m, 4H), 1.80 (s, 2H), 1.65-1.69 (m, 4H), 1.56 (d, 2H, J = 12.7 Hz); ^{13}C-NMR (100 MHz, DMSO-*d6*): δ/ppm = 173.8, 166.4, 135.1, 131.1, 128.1, 127.7, 63.1, 37.5, 33.5, 32.8, 31.5, 26.5, 26.4; HRMS: calculated for $[C_{18}H_{21}NO_3Na]$+ 322.1419, found 322.1421.



CCDC860427

The structure was unambiguously confirmed by x-ray crystallography and the structure deposited at Cambridge University (Crystallographic Data Centre) with the unique reference *CCDC860427*; space group P21/n : a = 14.1635 (4), b = 6.7026 (2), c = 15.8379 (6), β = 99.395 (1).

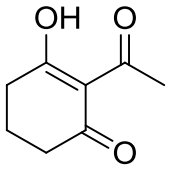
3-Acetyloxy 2-cyclohexen-1-one (22).



22

Batch reaction. To a solution of 1,3-cyclohexandione **16** (2.0 g, 17.8 mmol) and diisopropylamine (4 mL) in anhydrous toluene/dichloromethane (30 mL/5 mL), acetyl chloride (1.4 mL) is added dropwise over 2 minutes. The reaction is stirred for 2 hours at rt. The salts formed are then filtered off to obtain a solution of the 1. For characterisation analyses, a small portion of the solution is extracted with ethyl acetate, washed with water, HCl 2N and brine. The organic fraction, dried and concentrated, affords the product as a yellowish oil in almost quantitative yield (2.45 g, 15.89 mmol, yield 89 %). LC-MS: retention time 1.21 min, m/z [M+H] = 154.48. FT-IR (cm-1, neat): 2954 (w), 1768 (s), 1669 (s), 1640 (m), 1363 (s), 1182 (s) 1115 (s), 1007 (m), 919 (m), 878 (m); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.75 (s, 1H), 2.42 (t, 2H, *J* = 6.9 Hz), 2.30 (t, 2H, *J* = 6.9 Hz), 2.21 (s, 3H), 1.94 (m, 2H, *J* = 6.9 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 199.55, 169.82, 167.30, 117.34, 36.61, 28.22, 21.16, 21.14. HRMS for $[C_8H_{11}O_3]^+$ calculated 155.1110 ; found 155.1111.

2-Acetyl 1,3-cyclohexandione (23).

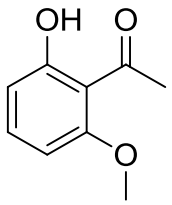


23

Batch reaction. To the solution containing **22** is added PS-DMAP (0.5 g, 3 mmol) and the mixture is microwave irradiated for 3 h. The reaction mixture is then filtered to remove the PS-DMAP and the solution washed with HCl 2N and brine. The organic fraction obtained is dried over

sodium sulfate, filtered and concentrated to obtain **23** as reddish oil. (2.30 g, 14.9 mmol, yield 94 %). LC-MS: retention time 2.62 min, m/z [M+H] = 155.35. FT-IR (cm^{-1}, neat): 2953 (w), 1660 (s), 1550 (s), 1410 (s), 1351 (s) 1188 (s), 1007 (m), 919 (m), 841 (m).^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 15.75 (s, 1H), 2.65 (t, 2H, *J* = 6.9 Hz), 2.61 (s, 3H), 2.48 (t, 2H, *J* = 6.9 Hz), 1.97 (m, 2H, *J* = 6.9 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 203.05, 198.69, 195.38, 108.18, 38.55, 33.21, 28.74, 18.98. HRMS for $[C_8H_{11}O_3]^+$ calculated 155.0708 ; found 155.0705.

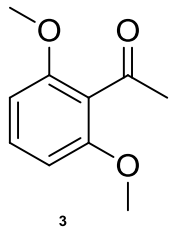
2'-Hydroxy-6'-methoxy-acetophenone (24).



24

Batch reaction. To a solution of **23** (2.0 g, 13 mmol) in methanol (30 mL), iodine (2 eq., 6.8 g) is added and the reaction mixture microwave irradiated for 9 h. The mixture is then passed through calcium carbonate (to remove excess of acid produced during the reaction) and a polymer supported sulfite resin (to eliminate excess of iodine). The organic fraction obtained is concentrated to obtain **24** as yellowish needles. (mp 55-59 °C, 1.75 g, 10.5 mmol, yield 80 %). LC-MS: retention time 4.51 min, m/z [M+H] = 167.37. FT-IR (cm^{-1}, neat): 2944 (w), 1628 (m), 1608 (s), 1483 (s),1367 (m), 1325 (s), 1211 (s), 1180 (m), 1035 (m), 962 (m), 838 (m), 782 (m).^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 13.23 (s, 1H), 7.32 (t, 1H, *J* = 8.9 Hz), 6.55 (d, 1H, *J* = 8.9 Hz), 6.34 (d, 1H, *J* = 8.9 Hz), 3.90 (s, 3H), 2.67 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 205.17 (C), 164.65 (C), 161.55 (C), 136.09 (CH), 111.32 (CH), 101.15 (CH), 55.86 (CH_3), 33.66 (CH_3). HRMS for $[C_9H_{11}O_3]^+$ calculated 167.0708 ; found 167.070.

2',6'-Dimethoxyacetophenone (3).



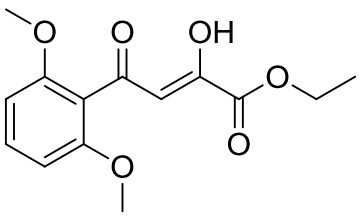
3

Batch reaction. A solution of **24** (1 mmol, 0.166 g) and 1,2-dimethylimidazole (1 mmol, 0.1 g) in dimethyl carbonate (0.8 mL)/methanol (0.8 mL) is microwave irradiated for 1 h at 160°C. The reaction mixture is then passed through a QP-SA scavenger and the solution obtained is concentrated to obtain **3** as a yellowish solid (0.183 g, 0.96 mmol, 96% yield).

Telescoped flow reaction. A solution of 1,3-cyclohexadione (2.0 g, 17.8 mmol) and diisopropylamine (1.1 eq.) (flow rate 0.50 mL min-1, pump A) in toluene (30 mL) and a solution of acetyl chloride (1.4 mL, 19.5 mmol, flow rate 0.50 mL min^{-1}, pump B) in toluene (30 mL) were reacted in a 14 mL CFC PFA at 40 °C after combining at a T-piece (~ 14 min residence time). After exiting the CFC the flow stream was dispensed onto a filter mat to remove any precipitate (eluent collected in a filter flask). The resultant solution was pumped through a monolith containing dimethylaminopyridine (DMAP) catalyst heated to 100 °C (residence time 20 min, flow rate 50 µL min^{-1}). After exiting the CFC the flow stream was collected and the solution concentrated *in vacuo* (2.50 g, 16.7 mmol, yield 94%). A solution of the material obtained in methanol (30 mL) (flow rate 0.20 mL min^{-1}, pump A) and a solution of iodine (8.00 g, 32.00 mmol) in methanol (30 mL) (flow rate 0.20 mL min-1, pump B) were pumped through a CFC PFA at 80 °C after mixing at a T-piece. The solution was then directly delivered to a scavenger cartridge containing calcium carbonate and a polymer supported thiosulfate cartridge.. The resultant solution was delivered (flow rate 0.40 mL min^{-1}, pump A) at a T-piece to meet a solution of 1,2-dimethylimidazole (2.10 g, 13.06 mmol) in dimethyl carbonate (30 mL) (flow rate 0.40 mL min^{-1}, pump B) and reacted into a PFA CFC heated to 150 °C (residence time 14 min). After exiting the CFC the flow stream was directed to a polymer-supported sulfonic acid

(QP-SA) cartridge and then silica. The collection of the output stream and the concentration of the solution gave the title compound (**3**) as a yellowish solid in good yield and purity (mp 67-71 °C, 1.81 g, 10.00 mmol, yield 62%). LC-MS: retention time 4.20 min, m/z [M+H] = 181.37. FT-IR (cm^{-1}, neat): 3000 (w), 2943 (w), 1696 (s), 1590 (s), 1471 (s), 1434 (s), 1358 (s), 1282 (m), 1249 (s), 1107 (s), 781 (s), 759 (m), 735 (s); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.26 (t, 1H, *J* = 8.7 Hz,), 6.55 (d, 2H, *J* = 8.7 Hz), 3.80 (s, 6H), 2.47 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 202.77, 156.76, 130.63, 104.02, 77.26, 55.88, 32.33. HRMS for $[C_{10}H_{13}O_3]^+$ calculated 181.0865; found 181.0857.

Ethyl 4-(2,6-dimethoxyphenyl)-2,4-dioxobutanoate (4).



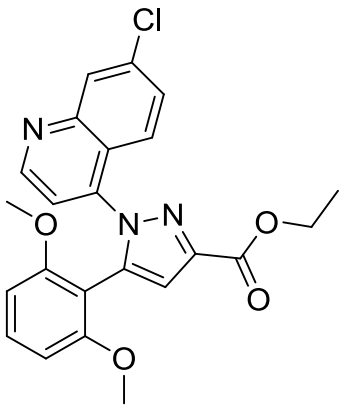
4

Batch reaction. A solution of 2',6'-dimethoxy-acetophenone **3** (0.90 g, 4.90 mmol), diethyl oxalate (1.344 mL, 2 eq.) and a solution of sodium ethoxide (1M, 10mL) in ethanol (8 mL) is microwave irradiated for 25 min at 80°C. Hydrochloric acid (1M, 10 mL) is then added till pH reached 3. The product **4** crystallised overnight as an off-white solid (1.10 g, 3.93 mmol, 80% yield).

Flow reaction. A solution of 2',6'-dimethoxy-acetophenone **3** (1.126 g, 6.18 mmol) and diethyl oxalate (1.664 mL, 2 eq.) in ethanol (36 mL) (flow rate 1.30 mL min^{-1}, pump A) and a solution of sodium ethoxide (1 M) (flow rate 1.30 mL min^{-1}, pump B) were reacted in a 52 mL (10 mm i.d.) PFA reactor at 140 °C after combining at a T-piece (20 minutes residence time). After exiting the reactor, the solution was collected and hydrochloric acid (1M, 10 mL) was added till pH reached 3. The product **4** crystallised overnight as an off-white solid (1.240 g, 4.57 mmol, 74% yield). mp 102-104 ºC; LCMS: retention time = 4.19 min, m/z $[M+Na]^+$= 302.83; FT-IR (neat, cm^{-1}): 1735.66

(m), 1620=5.14 (m), 15486.82 (m), 1475.31 (m), 1257.47 (s); ^{1}H-NMR (400 MHz, $CDCl_3$): 14.33 (br s, 1H), 7.34 (t, 1H, *J* = 8.4 Hz), 6.60 (s, 1H), 6.59 (d, 1H, *J* = 8.4 Hz), 4.35 (q, 2H, *J* = 7.1 Hz), 3.81 (s, 6H), 1.37 (t, 3H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): 195.8, 164.0, 162.6, 158.0, 132.4, 117.2, 106.4, 104.3, 62.5, 56.2, 14.2; HRMS: calculated for $[C_{14}H_{16}O_6Na]^+$ = 303.0845, found 303.0847. Microanalysis: calculated (found) for $C_{14}H_{16}O_6$: C 59.99% (59.99%), H 5.52% (5.75%),

Ethyl 1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carboxylate (5).



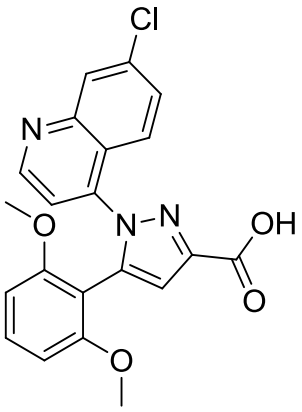
5

Batch reaction. A solution of ethyl 4-(2,6-dimethoxyphenyl)-2,4-dioxobutanoate (**4**) (0.14 g, 0.50 mmol) and 1-(7-chloroquinolin-4-yl)hydrazine (0.12 g, 1.3 eq.) in dimethylformamide (3 mL) was microwave irradiated at 140 °C for 3 h. Sodium hydrogencarbonate (2 g) is added and the mixture stirred until no more evolution of gas is noticed. The mixture is added with water (10 mL) and extracted with dichloromethane (4 x 15 mL). The organic fractions collected are dried over sodium sulfate, filtered and concentrated *in vacuo*. The oil obtained is stirred with hexane (10 mL) overnight to afford a solid which is recrystallized from ethanol/hexane to furnish the product as yellowish crystals (0.18 g, 0.43 mmol, 87% yield).

Flow reaction. A solution of ethyl 4-(2,6-dimethoxyphenyl)-2,4-dioxobutanoate (**4**) (1.12 g, 4.0 mmol) and 1-(7-chloroquinolin-4-yl)hydrazine (0.926 g, 1.2 eq., 4.8 mmol) in dimethylformamide (10 mL) (flow rate 0.50 mL min^{-1}, pump A) and a solution of sulfuric acid

(1.2 mL) in dimethylformamide (10 mL) (flow rate 0.50 mL min-1, pump B) were reacted in a 52 mL (10 mm i.d.) PFA reactor at 140 °C after combining at a T-piece (52 min residence time). After exiting the reactor coil the solution was collected, sodium hydrogencarbonate (5 g) was added and the mixture stirred until no more evolution of gas is noticed. The mixture is added with water (20 mL) and extracted with dichloromethane (4 x 20 mL). The organic fractions collected are dried over sodium sulfate, filtered and concentrated *in vacuo*. The oil obtained is stirred with hexane (20 mL) overnight to afford a solid which is recrystallized by ethanol/hexane to furnish the product as yellowish crystals (1.58 g, 3.58 mmol, 89% yield). mp 147 -149 ºC; LCMS: retention time = 4.58 min, m/z $[M+H]^+$ = 437.93; FT-IR (neat, cm^{-1}): 1719.4 (m), 1590.0 (m), 1476.6 (s), 1434.8 (m), 1232.7 (s); ^{1}H- NMR (400 MHz, DMSO-*d6*): 8.91 (d, 1H, *J* = 4.5 Hz), 8.17 (d, 1H, *J* = 1.8 Hz), 7.72 (dd, 1H, *J* = 9.0, 1.8 Hz), 7.68 (d, 1H, *J* = 9.0 Hz), 7.26 (t, 1H, *J*= 8.4 Hz), 7.23 (d, 1H, *J* = 4.5 Hz), 7.04 (s, 1H), 6.54 (d, 2H, *J* = 8.4 Hz), 4.35 (q, 2H, *J* = 7.1 Hz, H-1'), 3.40 (s, 6H), 1.33 (t, 3H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, DMSO-*d6*): 161.4, 157.5, 151.7, 149.1, 144.5, 143.3, 139.2, 134.8, 132.0, 128.1, 127.7, 125.7, 122.1, 118.3, 111.6, 105.4, 104.0, 60.6, 55.4, 14.2; HRMS: calculated for $[C_{23}H_{21}N_3O_4Cl]^+$ = 438.1221, found 438.1219. Microanalysis: calculated (found) for $C_{23}H_{20}N_3O_4Cl$; C 63.09% (62.80%), H 4.60% (4.62%), N 9.60% (9.53%).

1-(7-Chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carboxylic acid (6).



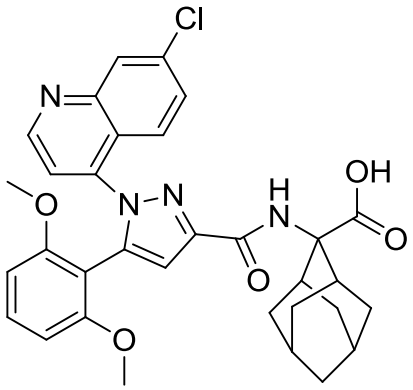
6

Batch reaction. A solution of ethyl 1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carboxylate (**5**) (0.438 g, 1.0 mmol) in tetrahydrofuran (5 mL) was microwave irradiated in the presence of potassium hydroxide aqueous solution (0.5 M, 5 mL) for 2h at 120 °C. The solution obtained was concentrated *in vacuo* and the mixture obtained filtered to eliminate any presence of solid. The solution obtained was then acidified to pH 2-3 with concentrated hydrochloric acid (6 M) in ice water bath. The solid obtained was filtered to afford the product as a yellowish solid (0.370 g, 0.90 mmol, 90% yield).

Flow reaction. A solution of ethyl 1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carboxylate (**6**) (0.876 g, 2.0 mmol) in tetrahydrofuran (10 mL) (flow rate 0.25 mL min-1, pump A) and a solution of potassium hydroxide (0.56 g, 10 mmol) in water (10 mL) (flow rate 0.25 mL min-1, pump B) were reacted in a 14 mL PFA reactor at 120 °C after combining at a T-piece (28 min residence time). The solution obtained is concentrated in vacuo and the mixture obtained filtered to eliminate any presence of solid. The solution obtained is then acidified to pH 2-3 with concentrated hydrochloric acid (6 M) in ice water bath. The solid obtained is filtered to afford the product as a yellowish solid (0.75 g, 1.84 mmol, 92% yield). LCMS: retention time = 4.35 min, m/z $[M+H]^+$ = 410.25; ^{1}H- NMR (400 MHz, DMSO-*d6*): 13.08 (s, 1H), 8.89 (d, 1H, *J* = 4.6 Hz), 8.16 (d, 1H, *J* = 1.9 Hz), 7.73 (d, 1H, *J* = 9.0 Hz), 7.70 (dd, 1H, *J* = 9.0, 1.9 Hz) , 7.26 (t, 1H, *J* = 8.4 Hz), 7.20 (d, 1H, *J* = 4.6 Hz), 6.99 (s, 1H), 6.54 (d, 2H, *J* = 8.5 Hz), 3.39

(s, 6H); ^{13}C- NMR (100 MHz, DMSO-*d6*): 162.9, 157.5, 151.6, 149.1, 145.4, 143.3, 138.9, 134.7, 131.8, 128.0, 127.7, 125.9, 122.1, 118.1, 111.7, 105.6, 104.0, 55.3; FT-IR (neat, cm^{-1}): 1687.7 (m), 1575.2 (s), 1479.2 (s), 1456.9 (m), 1434.7 (s); HRMS: calculated for $[C_{12}H_{17}N_3O_4Cl]^+$ 410.0908, found 410.0921. Microanalysis calculated (found) for $C_{12}H_{16}N_3O_4Cl$: C 61.54 (61.30%), H 3.94% (4.00%), N 10.25% (10.17%).

2-[1-(7-Chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carboxamido]adamantane-2-carboxylic acid (2)

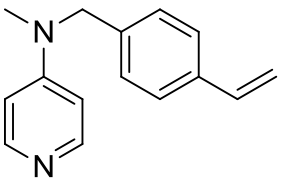


2

Flow reaction. A solution of ethyl 1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carboxylic acid (**7**) (100 mg, 0.23 mmol) in dry DCM (10 mL) (flow rate 0.10 mL min-1, pump A) and solution of 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (Ghosez reagent) (40 µL, 0.30 mmol) in dry DCM (10 mL) (flow rate 0.10 mL min-1, pump B) were combined at a T-piece and heated in a 14 mL PFA coil at 80 °C (70 min residence time). A small portion of the output was analysed to check the acid chloride [LCMS: retention time = 5.00 min, m/z $[M+H]^+$= 428.06; FT-IR: 1758.7 (s), 1633.9 (m), 1593.8 (s), 1475.8 (m), 1444.1 (m); ^{1}H NMR (600 MHz, $CDCl_3$): 9.23 (1H, d, J = 5.7 Hz, H-9), 8.92 (1H, d, J = 1.7 Hz, H-16), 8.34 (1H, d, J = 9.2 Hz, H-13), 7.88 (1H, dd, J = 9.2, 1.7 Hz, H-14), 7.59 (1H, d, J = 5.7 Hz, H-10), 7.37 (1H, t, J =8.5 Hz, H-1), 7.30 (1H, s, H-6), 6.53 (2H, d, J = 8.5 Hz, H-2), 3.52 (1H, s, H-3'); ^{13}C NMR (150 MHz, $CDCl_3$): 161.8, 157.3, 150.6, 149.0, 144.6, 142.0, 141.2, 140.3, 133.1, 131.9, 127.2, 122.6, 121.0, 117.3, 114.3, 104.1, 55.6; HRMS: Calculated for $[C_{21}H_{16}N_3O_3Cl_2]^+$ 428.0569, found 428.0571]. The

solution stream is then delivered to another T-piece where is combined with a solution of amino acid (65 mg, 0.28 mmol) in pyridine (10 mL) which is pumped at a flow rate of 0.2 mL/min was and then heated in a PFA reactor coil (14 mL, residence time = 35 min, 80 ^{o}C). The output solution obtained was extracted with dilute HCl and the organic phase was then dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. LC-MS analysis of this residue indicated that 50% conversion had been obtained. The residue was dry loaded onto silica and flash chromatographed (1-10% gradient of MeOH in DCM). A fraction containing pure meclinertant (**2**) was crystallised from ethanol/hexanes (29 mg, 20% yield). Mp: 219-222 ºC; LCMS: retention time = 5.86 min, m/z $[M+H]^+$ = 643.42; FT-IR (neat, cm^{-1}): 3413.7 (w), 2908.8 (w), 1743.8 (w), 1731.9 (m), 1706.2 (w), 1685.8 (m), 1532.4 (m), 1100.9 (s); ^{1}H- NMR (400 MHz, $CDCl_3$): 8.76 (d, 1H, *J* = 4.6 Hz), 8.13 (d, 1H, *J* = 2.0 Hz), 7.95 (d, 1H, *J* = 9.0 Hz), 7.48 (dd, 1H, *J* = 2.0, 9.0 Hz), 7.21 (t, 1H, *J* = 8.0 Hz), 7.10 (br. s, 1H), 7.07 (s, 1H), 7.04 (d, 1H, *J* = 4.6 Hz), 6.39 (d, 2H, *J* = 8.0 Hz), 3.39 (s, 6H), 2.66 (s, 2H), 2.14 (d, 2H, *J* = 12.9 Hz), 2.05 (d, 2H, *J* = 12.9 Hz), 1.65-1.87 (m, 8H), 1.51 (s, 9H); ^{13}C- NMR (100 MHz, $CDCl_3$): 171.6, 160.6, 158.0, 151.0, 150.2, 148.6, 144.5, 139.4, 136.0, 131.6, 128.5, 128.1, 126.4, 122.9, 117.5, 110.7,106.9, 103.8, 80.9, 64.1, 55.4, 38.1, 34.2, 33.4, 33.0, 28.2, 27.2, 26.9; HRMS: calculated for $[C_{36}H_{40}N_4O_5Cl]^+$ = 643.2687, found 643.2711.

N-(4-vinylbenzyl)-N-methylpyridin-4-amine (26).

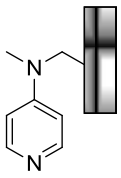


26

To a vigorously stirred solution of N-methyl-4-aminopyridine (9.3 mmol, 1.0 g) in dry THF (20 mL), under complete anhydrous conditions, NaH (1.3 eq, 0.5 g, 60% in mineral oil) is added at 0°C. The suspension is stirred at 0°C for 10 min and then allowed to warm to rt. After 6h, the colourless suspension (n.b.: excess of sodium hydride can be noticed by the solution turning yellowish) is cooled to 0°C and 4-vinyl-benzyl chloride (1.1 mL, 0.8 eq) is added dropwise. After

10 minutes the reaction is warmed gently to rt and stirred for further 26h. The suspension is carefully filtered using filter paper, concentrated in vacuo and the oil obtained dissolved in DCM. The mixture is then washed twice with water, brine and then dried over sodium sulfate. The organic solution is filtered and concentrated to obtain a brown oil which is dissolved in 2 mL of DCM/hexane (1:1) and then passed through basic alumina. The solution is concentrated to obtain the product as a yellowish oil (yield 93%). LC-MS: retention time 3.20 min, m/z [M+H] = 225.17. ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 8.22 (d, *J* = 6.53 Hz, 2H), 7.37 (d, J = 8.13 Hz, 2H), 7.13 (d, *J* = 8.08 Hz, 2H) , 6.70 (q, *J* = 10.89 Hz, *J* = 17.60 Hz, 1H), 6.54 (d, *J* = 6.53 Hz, 2H), 5.73 (d, *J* = 17.57, 1H), 5.24 (d, *J* = 10.93, 1H), 4.56 (s, 2H), 3.07 (s, 3H).^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 154.10, 150.76, 137.63, 136.00, 127.34, 114.02, 107.26, 55.88, 38.33. HRMS for $[C_{10}H_{13}O_3]^+$ calculated 181.0865; found 181.0857.

Dimethylaminopyridine monolith



A stock solution was prepared by dissolving N-(4-vinylbenzyl)-N-methylpyridin-4-amine (**26**) (0.80 g) in 1-dodecanol (4.80 g) with gentle heating (<50°C) then adding divinylbenzene (1.60 g), styrene (0.80 g) and 4,4'-azo*bis*(4-cyanovaleric acid) (0.040 g). A 10 mm i.d./ 4 cm length glass column was filled to 2 cm with this sdtock soliution and both ends of the column were sealed with plugs (Vapourtec). The column was heated at 90 C using the Vapourtec R4 convection heater, resulting in a rigid white monolith. Following polymerisation, the monolith was allowed to cool to room temperature and the end seals were changed for standard tubing connectors. In order to remove the porogen and any residual non-polymeric material, the monolith was heated to 60 °C and washed with THF at 0.25 mL/min for 5 h using the Vapourtec R4/R2+ flow system. Average pressure drop (THF, 0.25 mL/min, 60 C) = 8-10 bar.

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