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Synthesis of new dithiolethione and methanethiosulfonate systems endowed with pharmaceutical interest

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Dedicated to Prof. Jacek Młochowski on the occasion of his 80th anniversary

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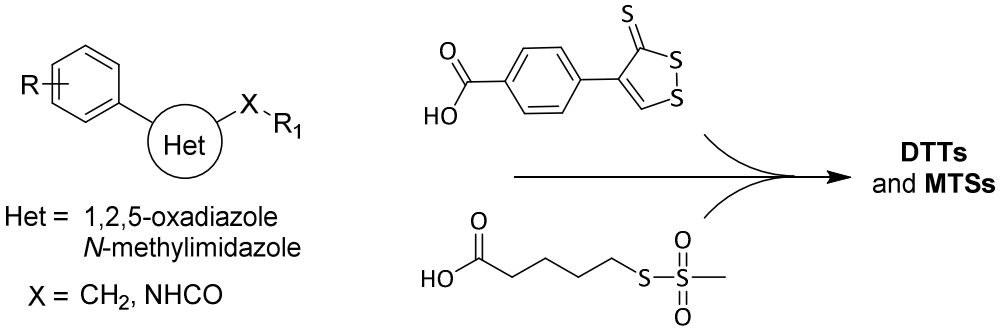
Abstract

Here we report synthetic methodology affording in the most efficient way the rapid preparation of new dithiolethiones (DTTs) and methanethiosulfonates (MTSs). These were evaluated as STAT3 inhibitors since these electrophilic systems could react with thiol groups of STAT3-SH2 domain. The results showed that MTSs strongly interacted with the SH2 domain, whereas the corresponding DTTs possessed lower affinity, independently from the nature of the linked heterocyclic scaffold.



Keywords: 1,2,5-Oxadiazole, *N*-Methylimidazole, STAT3-SH2 domain, Coupling reactions

Introduction

Recent literature[1,2] shows considerable interest in the chemistry and bioactivity of 1,2-dithiole-3-thiones (DTTs) and methanethiosulfonates (MTSs). Both moieties have been reported to exert chemopreventative and anticancer activities.[3-7] Since these electrophilic systems are able to react with thiol groups, and therefore, hypothetically, with cysteines in biologically important peptides and proteins,[8,9] we considered that the introduction of these moieties on a suitable scaffold could lead to new inhibitors able to covalently link the active site of several transcription factors. A similar behavior was observed between a DTT derivative and NFkB (Nuclear Factor Kappa-light-chain-enhancer of activated B cells).[10] As STAT3 (Signal Transducer and Activator of Transcription 3) occupies a noteworthy position in cancer biology,[11] we decided to explore the relevance of these chemical systems against this target. Among the recognized STAT3 direct inhibitors reported in the literature many are sulfur-containing compounds, such as sulfones (Stattic),[12] sulfonates (S31-201)[13] or sulfonamides (SF-1-066 and BP-1-102),[13] but no DTTs or MTSs have been so far considered. As we recently published our results on several oxadiazole derivatives able to interfere with the STAT3 signaling pathway,[14-17] we thought it of interest to link this heterocyclic scaffold to DTT and MTS moieties. In this way, we aimed to promote the targeting of the new compounds towards the SH2 (Src Homology 2) domain.

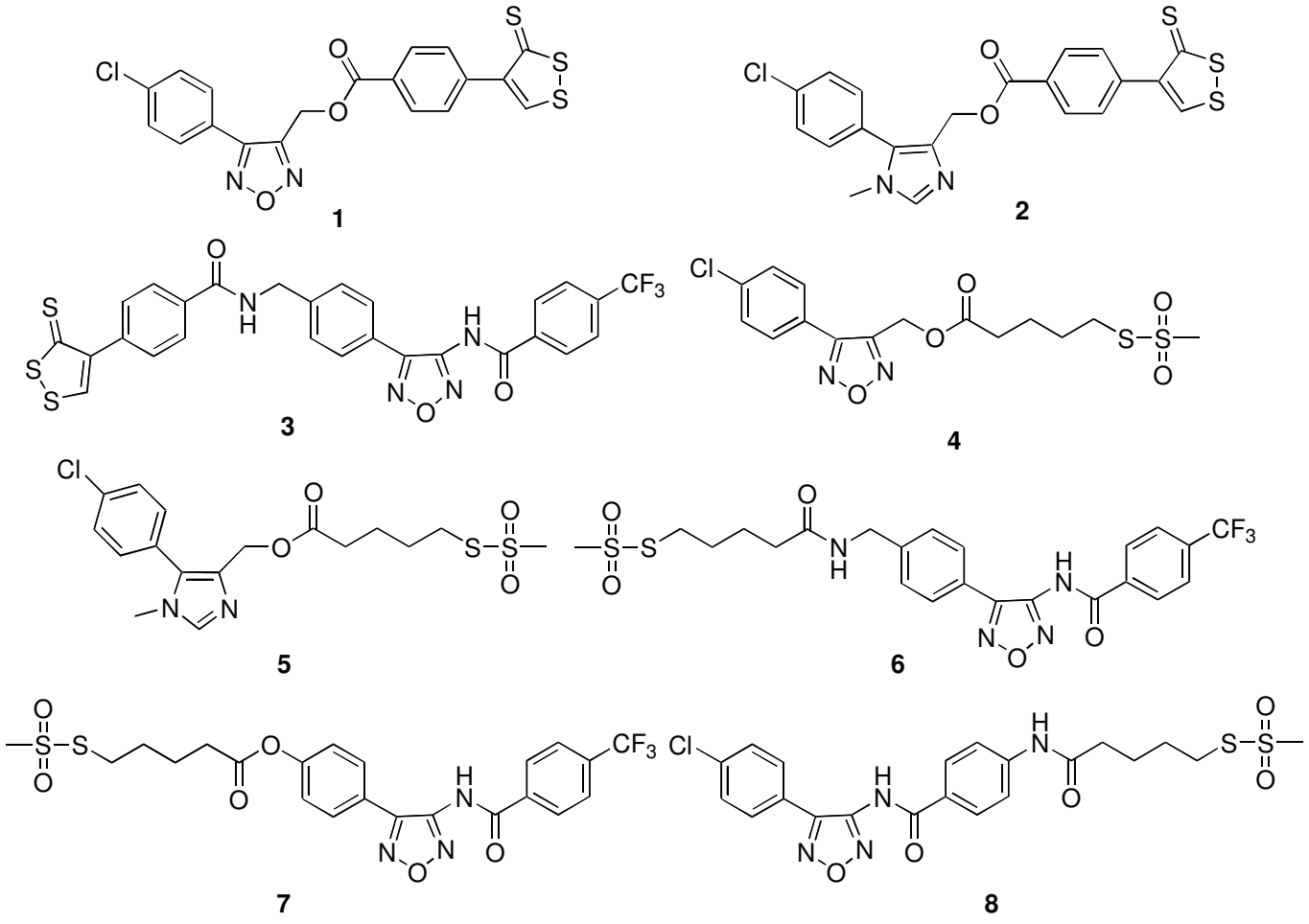


Figure 1. Structures of the new synthesized compounds.

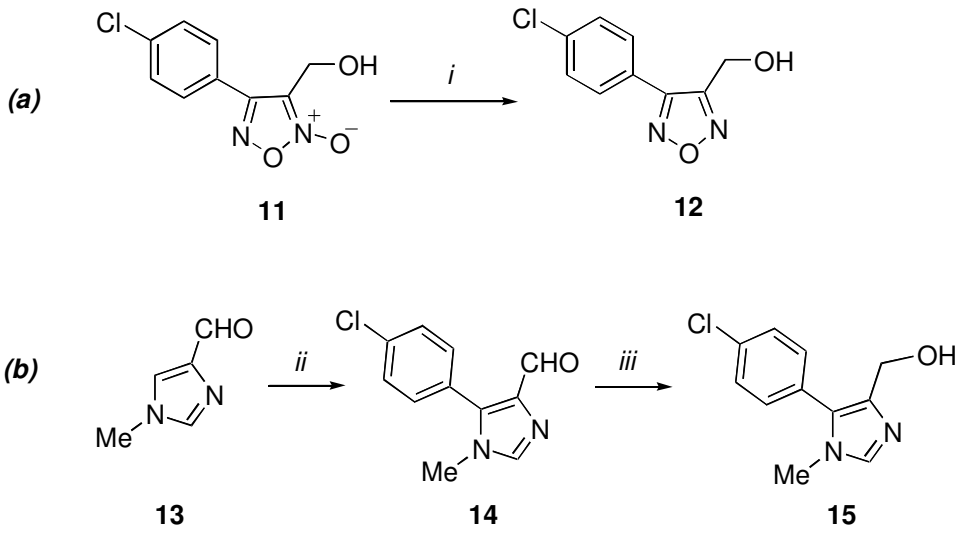
In this paper, we describe synthetic methodology affording in the most efficient way the rapid preparation of new inhibitors having DTT or MTS systems linked to differently substituted 1,2,5-oxadiazole rings through an ester (**1**, **4** and **7**) or an amidic bond (**3**, **6** and **8**) (Figure 1). In addition, we synthesized the bioisosteric analogues of **1** and **4**, by replacing the oxadiazole with a substituted *N*-methylimidazole (**2** and **5**).

Results and Discussion

Chemistry

The key intermediates for the synthesis of the final products (**1**-**8**) were prepared according to the following methods. The synthesis of compounds **9**, 4-(3-thioxo-3*H*-1,2-dithiol-4-yl)benzoic acid, and **10**, 5-(methylsulfonylthio)pentanoic acid, was achieved following literature procedures.[18,19] Intermediates **12**, **15**, **23**, **25** and **27** were prepared as shown in Schemes 1 and 2.

The synthesis of **12**, bearing a suitable 3-hydroxymethyl substituent, was optimized starting from the commercially available *para*-chlorocinnamic acid to give **11**,[20] the *N*-oxide moiety of which was removed by treatment with trimethyl phosphite[21] (Scheme 1a).

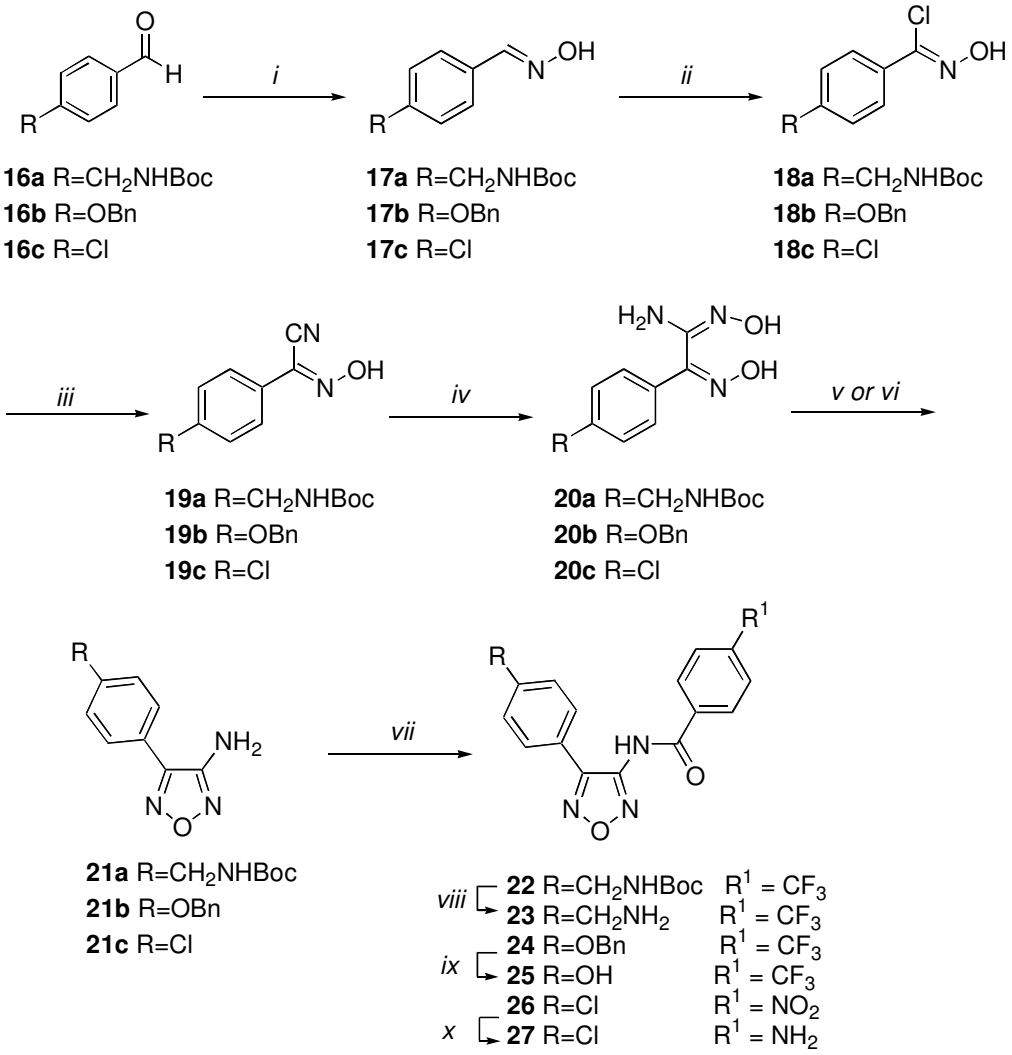


Scheme 1. Reagents and conditions: ***(a)*** *i)* $P(OCH_3)_3$, reflux, 18 h; ***(b)***: *ii)* 4-ClC_6H_4Br, $Pd(OAc)_2$, $P(2\text{-furyl})_3$, K_2CO_3, DMF, 110 °C, 48 h; *iii)* $NaBH_4$, MeOH, rt, 4 h.

Compound **15** was prepared from 1*H*-imidazole-4-carbaldehyde, protected as *N*-methyl derivative by iodomethane[22] (**13**), then transformed into **14** through a selective palladium-catalyzed C-H activation in position 5.[23] The subsequent reduction step afforded the desired product **15** in good yield (Scheme 1b).

The intermediates **23**, **25** and **27** were synthesized from the appropriate benzaldehyde with some variations on the multi-step procedure previously reported[16] (Scheme 2). Since prolonged (12 hours) treatment in refluxing aqueous NaOH of **20a** caused an approximately 50% removal of the Boc (*tert*-butyloxycarbonyl) group, an additional treatment with *tert*-butyloxycarbonyl anhydride (Boc_2O) was required to give **21a**. The amides **22** and **24** were obtained by coupling reactions of intermediates **21a**,**b** with 4-(trifluoromethyl)benzoyl chloride, respectively, while the reaction of compound **21c** with 4-nitrobenzoyl chloride gave **26**.

Deprotection of **22** in acidic conditions led to **23**; compound **24** was debenzylated by a catalytic hydrogenation over Pd/C to **25**; compound **26** was reduced in the presence of tin(II) chloride to **27**.

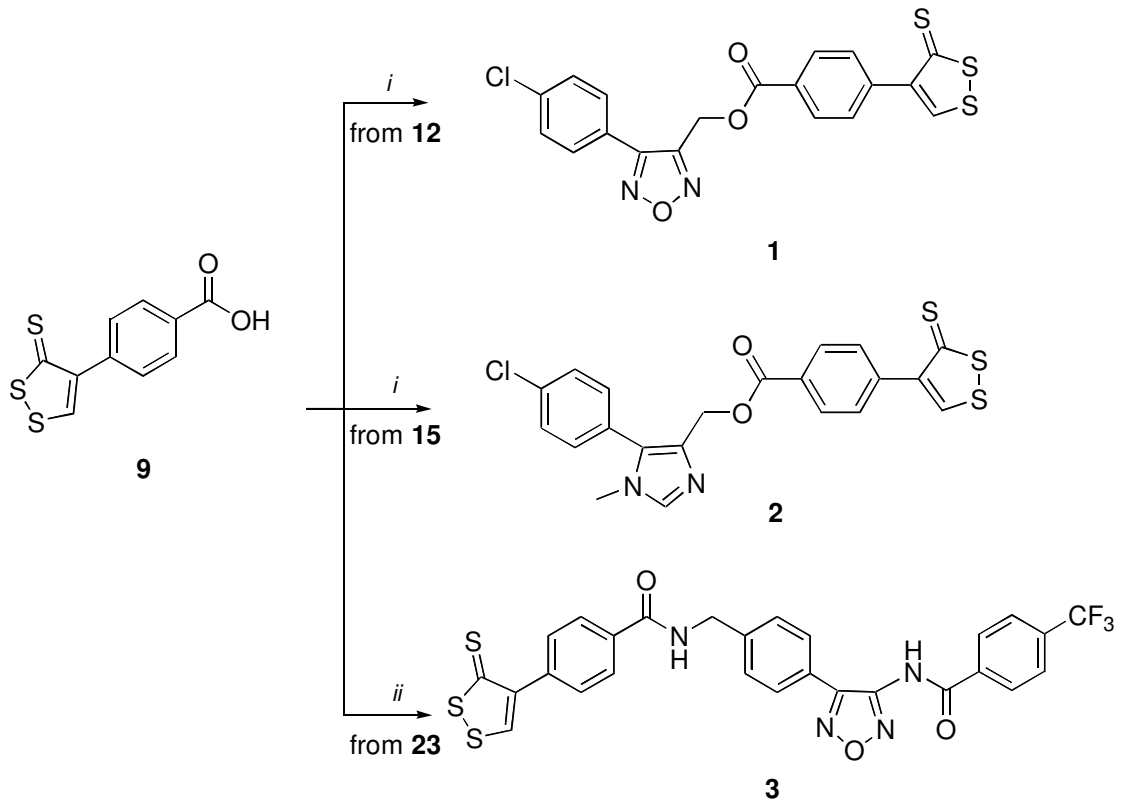


Scheme 2. Reagents and conditions: *i)* $NH_2OH{\cdot}HCl$, $NaHCO_3$, MeOH, reflux, 2 h; *ii)* NCS, DMF, rt, 12 h; *iii)* KCN, Et_2O/H_2O (2:1), rt, 5 h; *iv)* $NH_2OH{\cdot}HCl$, $NaHCO_3$ MeOH, reflux, 12 h; *v)* 2N NaOH, reflux, 12 h; *vi)* a. 2N NaOH, reflux, 12 h; b. Boc_2O, DCM, NEt_3, rt, 4 h; *vii)* appropriately substituted benzoyl chloride, 60% NaH, dry DMF, 60 °C, 12 h; *viii*) HCl, dry 1,4-dioxane, rt, 10 min; *ix*) H_2, Pd/C, EtOAc/MeOH (9:1), 24 h; *x*) $SnCl_2$, EtOAc, reflux, 4 h.

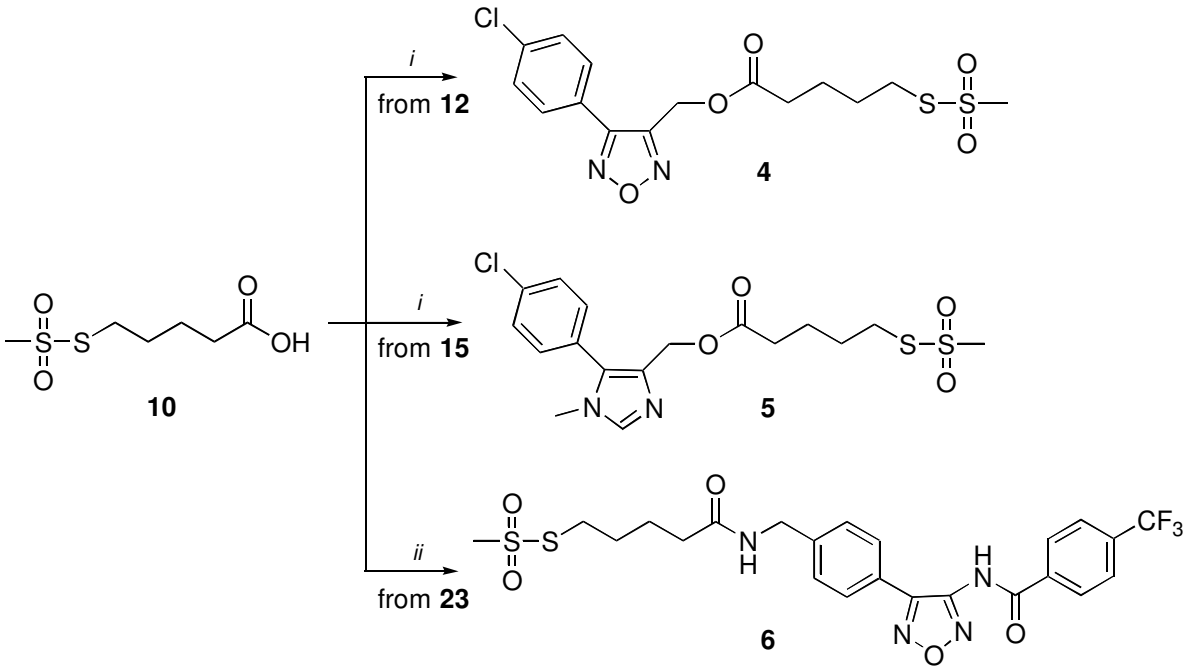
The final products **1**-**8** were prepared by condensation of the suitable intermediate with **9** (Scheme 3) or **10** (Schemes 4, 5), using different coupling reagents.

The DTT products **1**-**3** were obtained in good yields (59-78%) and were easily isolated and purified, thanks to their stability and low solubility.

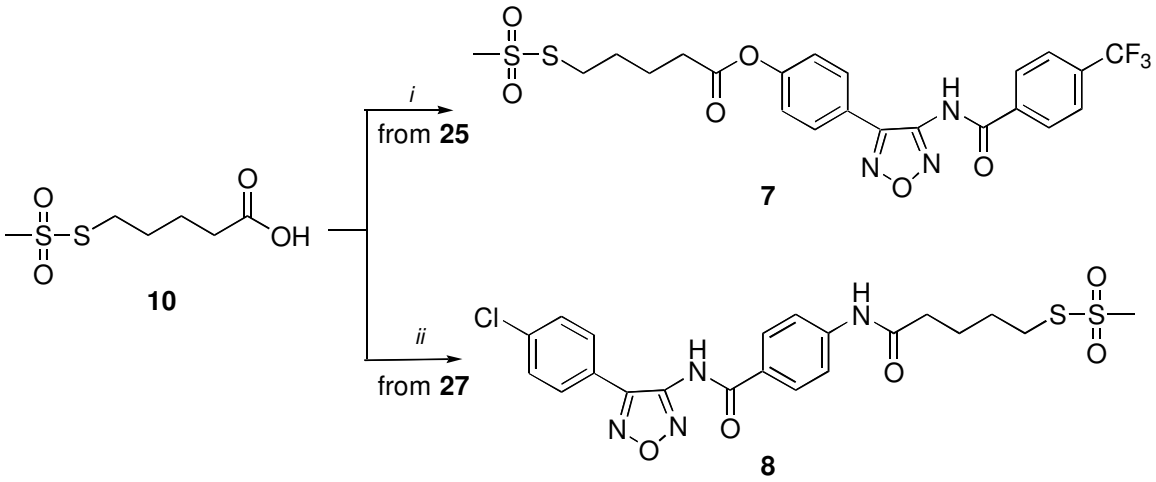
More difficulties during purification were encountered with all MTS derivatives, which were obtained with moderate (28-48%) or low yields (6-7%).



Scheme 3. Reagents and conditions: *i*) EDC*HCl, DMAP, dry DMF, rt, 24 h or 20 h; *ii*) TBTU, NMM, dry DMF, rt, 20 h.



Scheme 4. Reagents and conditions: *i*) DCC, DMAP, dry CH_2Cl_2 or dry DMF, rt, 20 h; *ii*) TBTU, NMM, dry DMF, rt, overnight.



Scheme 5. Reagents and conditions: *i*) DCC, DMAP, dry THF, rt, 4 h; *ii*) EDC*HCl, DMAP, dry DMF, rt, 24 h.

Biology

The effects of the final compounds on STAT3 dimerization were determined by the AlphaScreen-based assay,[24] an *in vitro* competitive binding test used to identify compounds able to directly inhibit the binding of SH2-containing proteins to their correspondent phosphopeptides. To check the selectivity on STAT3, the new products were tested also on the highly homologous (78%) STAT1.

The results showed that MTS derivatives strongly interacted with SH2 domain. Of note, compounds **4** and **7** inhibited STAT3 dimerization with an IC_{50} value of 0.6±0.05 μM, though their selectivity *versus* STAT1 was low (IC_{50} = 5.8±0.3μM and 7.5±0.4μM, respectively). By contrast, the equipotent **6** (IC_{50} = 0.7±0.04 μM *versus* STAT3) was provided with interesting selectivity (IC_{50} > 30 μM *versus* STAT1). The corresponding DTTs (**1**-**3**) possessed lower affinity (58.7, 26.6 and 33.4% of inhibition at 30 μM, respectively), independently from the nature of the linked heterocyclic scaffold.

In addition, their cytotoxic activity (MTT assay)[25,26] was tested on HCT116, a human colon carcinoma cell line which expresses high levels of STAT3.[27] Among the assayed compounds, **6** was the most active (IC_{50} = 84.5±9.8 μM). Since STAT3 inhibition was tested in a cell-free assay, the low correspondence between STAT3 inhibition and cytotoxicity could be related to the physicochemical properties of the compounds, which will require optimization.

Based on these data, the MTS moiety appears worth of further investigation for targeting STAT3-SH2 domain.

Conclusion

In our paper, we describe an easy and direct synthetic approach for the preparation of DTT and MTS derivatives, bearing a 1,2,5-oxadiazole ring, which has been reported as a promising scaffold for STAT3 inhibitors. The bioisosteric replacement of the oxadiazole with an *N*-methylimidazole ring was also considered. Due to their better stability, the DTTs (**1-3**) were obtained in higher yields than the MTSs (**4-8**).

The binding efficiency towards STAT3 and STAT1 SH2 domain was evaluated by means of AlphaScreen-based experiments. In addition, their antiproliferative activity was investigated on HCT116 cancer cell line. The biological results showed that MTSs interacted more strongly with SH2 domain with respect to the

corresponding DTTs. Although we presume that MTS derivatives can react more easily than DTTs with the free thiol group of cysteine residues present in the protein, further studies are required to elucidate the real mechanism of inhibition and the different reactivities of MTSs and DTTs.

In particular, compound **6** showed a higher affinity and selectivity (STAT3 *versus* STAT1), together with a moderate cytotoxic activity. These data suggest that MTS derivatives are promising ligands of the STAT3-SH2 domain. Optimization of their physicochemical properties is still needed to increase their cytotoxicity.

Experimental Section

General. Reagents and solvents were purchased from Sigma-Aldrich and used without further purification. Reactions involving air-sensitive reagents were performed under nitrogen atmosphere and anhydrous solvents were used when necessary. Reactions were monitored by thin layer chromatography analysis on aluminum-backed Silica Gel 60 plates (70-230 mesh, Merck), using an ultraviolet fluorescent lamp at 254 nm and 365 nm. Visualization was aided by appropriate staining reagents. Purification of intermediates and final compounds was performed by flash chromatography using Geduran® Si 60 (40-63 µm, Merck). DMEM (Dulbecco's Modified Eagle Medium), trypsin-EDTA, penicillin, streptomycin, non-essential amino acid solution, fetal calf serum (FCS), disposable culture flasks and petri dishes were purchased from Euroclone S.p.A. (Pero, Milan, Italy).

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD, D_2O, DMSO-d_6 or acetone-d_6 on Bruker DRX Avance 300 MHz or on a Varian 300 MHz Oxford equipped with a non-reverse probe at 25 ^{o}C. Chemical shifts are expressed as δ (ppm) and were referenced to residual solvent proton/carbon peak. Multiplicity is reported as *s* (singlet), *broad s* (broad singlet), *d* (double), *t* (triplet), *q* (quartet), *m* (multiplet), *dd* (double of doublets), *dt* (doublet of triplets). The coupling constants (J-values) are given in Hertz (Hz). All spectroscopic data match the assigned structures. ESI-MS analyses were performed by using a Thermo Finnigan (MA, USA) LCQ Advantage system MS spectrometer with an electronspray ionization source and an 'Ion Trap' mass analyzer. The MS spectra were obtained by direct infusion of a sample solution in methanol under ionization, ESI positive. High-resolution mass spectra (HRMS) were performed by FT-Orbitrap mass spectrometer in positive electrospray ionization (ESI). The melting points were determined on a Buchi Melting Point B540 instrument.

General procedures for the synthesis of dithiolethiones (**1-3**) **and methanethiosulfonates** (**4-8**)

Procedure A. Compound **12, 15** or **27** (0.1 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC*HCl, 0.15 mmol) and 4-dimethylaminopyridine (DMAP, 0.02 mmol) were mixed together in anhydrous *N,N*-dimethylformamide (1 mL) and the relevant sulfurated compound (**9**[18] or **10**[19], 0.11 mmol) was added. The reaction mixture was stirred for 24 h, at rt under an inert atmosphere. The solvent was stripped off and the obtained residue was diluted with EtOAc and washed with a cold solution of 0.5 N HCl and then with a cold solution of 5% $NaHCO_3$, cold water and brine. The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated to dryness to get a residue that was purified by flash chromatography (eluent as indicated for each compound).

Procedure B. The appropriate alcohol (**12**, **15** and **25**, 0.13 mmol), *N*,*N*'-dicyclohexylcarbodiimide (DCC, 0.14 mmol) and DMAP (0.01 mmol) were mixed in anhydrous DMF and 5-(methylsulfonylthio)pentanoic acid (**10**,[19] 0.14 mmol) was added. The reaction mixture was stirred for 4 or 20 h depending on the involved alcohol derivative, at rt under inert atmosphere. After completion, the formed *N*,*N*'-dicyclohexylurea was filtered off and the solvent was evaporated. The obtained residue was than dissolved in EtOAc or CH_2Cl_2 and washed with

a cold solution of 0.5 N HCl, afterward with a cold solution of 5% $NaHCO_3$, then with cold water and brine. The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated to dryness to get a residue that was purified by flash chromatography or preparative silica-TLC (eluent as indicated for each compound).

Procedure C. Compound **23** (0.08 mmol) and the correspondent sulfurated compound (**9**[18] or **10**[19], 0.09 mmol) were mixed together in anhydrous *N,N*-dimethylformamide (1 mL) under inert atmosphere. After cooling to 0 °C, *N,N,N′,N′*-Tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU, 0.1 mmol) and *N*-methylmorpholine (NMM, 0.08 mmol) were added and the reaction mixture was stirred for 24 h at rt. After evaporation of the solvent, the residue was taken up with CH_2Cl_2 or EtOAc and washed with a cold solution of 0.5 N HCl, a cold solution of 5% $NaHCO_3$, cold water and brine. The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated to dryness. The obtained solid residue was then rinsed and recrystallized with the solvent indicated for each compound.

[4-(4-Chlorophenyl)-1,2,5-oxadiazol-3-yl]methyl 4-(3-thioxo-3*H*-1,2-dithiol-4-yl)benzoate (**1**) was synthesized according to procedure **A** using 4-(3-thioxo-3*H*-1,2-dithiol-4-yl)benzoic acid (**9**) and (4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl)methanol (**12**) in *N,N*-dimethylformamide. The obtained residue was purified by column chromatography (silica gel; CH_2Cl_2; isocratic). The fractions containing the purified product were gathered up and the amorphous solid obtained was crystallized with petroleum ether/CH_2Cl_2 (2:0.5) and rinsed with the same bland to provide the title compound as an orange crystal solid. Yield: 63%. mp 135.2-134.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.47 (1H, s, C<u>H</u>=C), 8.02 (2H, d, $^3J_{HH}$ 8.2 Hz, CH aromatic), 7.72 (2H, d, $^3J_{HH}$ 8.2 Hz, CH aromatic), 7.62 (2H, d, $^3J_{HH}$ 8.2 Hz, CH aromatic), 7.48 (2H, d, $^3J_{HH}$ 8.2 Hz, CH aromatic), 5.65 (2H, s, CH_2) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 214.05, 165.24, 161.11, 153.71, 151.14, 147.29, 139.37, 136.64, 130.93, 130.12, 129.95, 129.82, 128.94, 124.35, 56.65 ppm. HRMS (ESI): *m/z* calcd for $C_{19}H_{12}ClN_2O_3S_3$ $[M+H]^+$: 446.9693; found 446.9691.

[5-(4-Chlorophenyl)-1-methyl-1*H*-imidazol-4-yl]methyl 4-(3-thioxo-3*H*-1,2-dithiol-4-yl)-benzoate (**2**) was synthesized according to procedure **A** using 4-(3-thioxo-3H-1,2-dithiol-4-yl)benzoic acid (**9**) and (5-(4-chlorophenyl)-1-methyl-*1H*-imidazol-4-yl)methanol (**15**). The crude product was purified by flash chromatography (silica gel; CH_2Cl_2/MeOH; in gradient); the product was eluted with 0.8% of MeOH. The obtained amorphous solid was crystallized with EtOAc/Et_2O (0.3:1) and rinsed with the same mixture to reach the final product obtained as an orange crystalline solid. Yield: 59%. mp 149.9-152.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.45 (1H, s, C<u>H</u>=C), 8.07 (2H, d, $^3J_{HH}$ 8.8 Hz, CH aromatic), 7.59-7.55 (3H, m, CH aromatic), 7.43 (2H, d, $^3J_{HH}$ 8.8 Hz, CH aromatic), 7.30 (2H, d, $^3J_{HH}$ 8.5 Hz, CH aromatic), 5.22 (2H, s, CH_2), 3.57 (3H, s, CH_3) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 214.10, 165.76, 165.76, 160.97, 147.48, 138.83, 134.07, 133.89, 132.07, 131.26, 130.12, 129.86, 129.65, 129.51, 128.32, 61.15, 32.84 ppm. HRMS(ESI): *m/z* calcd for $C_{21}H_{16}ClN_2O_2S_3$ $[M+H]^+$: 459.0062; found 459.0055.

[4-(3-Thioxo-3*H*-1,2-dithiol-4-yl)-*N*-(4-{4-[4-(trifluoromethyl)benzamido]-1,2,5-oxadiazol-3-yl}benzyl)benzamide (**3**) was synthesized according to procedure **C** using 4-(3-thioxo-3H-1,2-dithiol-4-yl)benzoic acid (**9**). The crude product was rinsed three times with a solution of Et_2O/CH_2Cl_2 (1:1) and it was isolated as an orange solid. Yield: 78%. mp 119.2-124.5 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ_H 10.58 (1H, broad s, NH exchanged with D_2O), 9.10 (1H, s, C<u>H</u>=C), 8.41 (1H, broad s, NH exchanged with D_2O), 8.24 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 8.00 (2H, d, $^3J_{HH}$ 8.6 Hz, CH aromatic), 7.91 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 7.81 (2H, d, $^3J_{HH}$ 8.6 Hz, CH aromatic), 7.72 (2H, d, $^3J_{HH}$ 8.2 Hz, CH aromatic), 7.52 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 4.68 (2H, d, $^2J_{HH}$ 4.2 Hz, CH_2) ppm. ^{13}C NMR (75 MHz, acetone-d_6): δ_C 214.20, 166.06, 157.71, 151.40, 149.61, 147.73, 142.70, 136.25, 134.61, 129.02, 128.90, 128.03, 127.61, 127.36, 127.08, 125.70, 124.58, 124.21, 119.07, 109.40, 42.68 ppm. HRMS (ESI): *m/z* calcd for $C_{27}H_{18}F_3N_4O_3S_3$ $[M+H]^+$: 599.0493; found 599.0497.

[4-(4-Chlorophenyl)-1,2,5-oxadiazol-3-yl]methyl 5-(methylsulfonylthio)pentanoate (**4**) was synthesized according to procedure **B** using [4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl]methanol (**12**), dissolved in anhydrous CH_2Cl_2. The reaction was stirred for 20 h. The crude product was purified by preparative silica-TLC using CH_2Cl_2/MeOH (96:4) as eluent mixture. The title compound was obtained as a colorless oil. Yield: 35%. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.65 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic), 7.51 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 5.40 (2H, s, CH_2), 3.31 (3H, s, CH_3), 3.14 (2H, t, $^3J_{HH}$ 7.3 Hz, CH_2COO), 2.39 (2H, t, $^3J_{HH}$ 7.3 Hz, SCH_2), 1.79-1.72 (4H, m, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 172.71, 135.20, 133.62, 131.24, 129.19, 126.69, 60.43, 59.52, 50.74, 47.40, 36.03, 33.31, 32.55, 28.91, 23.66 ppm. HRMS (ESI): *m/z* calcd for $C_{15}H_{21}ClN_3O_5S_2$ $[M+NH_4]^+$: 422.06057; found 422.0606.

[5-(4-Chlorophenyl)-1-methyl-1*H*-imidazol-4-yl]methyl 5-[(methylsulfonyl)thio]pentanoate (**5**) was synthesized according to procedure **B** starting from [5-(4-chlorophenyl)-1-methyl-1H-imidazol-4-yl]methanol (**15**), dissolved in anhydrous *N,N*-dimethylformamide. The reaction mixture was stirred for 20 h. The crude product was purified by flash chromatography (silica gel; CH_2Cl_2/MeOH; in gradient); the product was eluted with 0.1% of MeOH. The product was collected as a pale yellow-green oil. Yield: 28%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H 7.45 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 7.30 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic), 5.28 (2H, s, CH_2), 3.58 (3H, s, CH_3), 3.31 (3H, s, SO_2CH_3), 3.14 (2H, t, $^3J_{HH}$ 6.9 Hz, CH_2COO), 2.39 (2H, t, $^3J_{HH}$ 6.9 Hz, SCH_2), 1.79-1.72 (4H, m, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 171.89, 148.95, 137.35, 129.68, 129.49, 123.66, 54.88, 50.70, 35.81, 32.96, 29.69, 28.90, 23.40 ppm. HRMS(ESI): *m/z* calcd for $C_{17}H_{21}N_2O_4S_2ClNa$ $[M+Na]^+$: 439.0523; found 439.0531.

***S*-5-Oxo-5-(4-{4-[4-(trifluoromethyl)benzamido]-1,2,5-oxadiazol-3-yl}benzylamino)pentyl methanesulfonothioate** (**6**) was synthesized according to procedure **C** using 5-(methylsulfonylthio)pentanoic acid (**10**). The crude was rinsed three times with pure ethyl ether to give the final product as white-cream crystals. Yield: 42%. mp 179.4-181.3 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ_H 10.55 (1H, broad s, NH exchanged with D_2O), 8.25 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 7.93 (2H, d, $^3J_{HH}$ 8.1 Hz, CH aromatic), 7.78 (2H,d, $^3J_{HH}$ 8.1 Hz, CH aromatic), 7.61 (1H, broad s, NH exchanged with D_2O), 7.42 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 4.43 (2H, d, $^2J_{HH}$ 6.0 Hz, CH_2), 3.41 (3H, s, CH_3), 3.23 (2H, t, $^3J_{HH}$ 6.9 Hz, CH_2COO), 2.30 (2H, t, $^3J_{HH}$ 6.9 Hz, CH_2S), 1.84-1.71 (4H, m, CH_2) ppm. ^{13}C NMR (75 MHz, acetone-d_6): δ_C 171.82, 165.22, 151.42, 149.73, 142.86, 136.27, 134.21, 128.91, 127.94, 127.54, 125.73, 124.17, 49.84, 42.19, 35.60, 34.79, 28.93, 24.24 ppm. HRMS (ES^+): *m/z* calcd for $C_{23}H_{24}F_3N_4O_5S_2$ $[M+H]^+$: 557.1140; found 557.1132.

4-{4-[4-(Trifluoromethyl)benzamido]-1,2,5-oxadiazol-3-yl}phenyl 5-(methylsulfonylthio)pentanoate (**7**) was synthesized according to procedure **B** using *N*-(4-(4-hydroxyphenyl)-1,2,5-oxadiazol-3-yl)-4-(trifluoromethyl) benzamide (**25**) dissolved in anhydrous tetrahydrofuran. The reaction mixture was stirred for 4 h. The product was purified by flash chromatography (silica gel; EtOAc/cyclohexane in ratio 8:2; isocratic). The fractions containing the purified product were gathered up to provide a white solid. Yield: 48%. mp 157.1-158.6 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ_H 10.59 (1H, broad s, NH exchanged with D_2O), 8.25 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 7.95-7.88 (4H, m, CH aromatic), 7.30 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 3.44 (3H, s, CH_3), 3.30 (2H, t, $^3J_{HH}$ 7.2 Hz, CH_2COO), 2.70 (2H, t, $^3J_{HH}$ 7.2 Hz, CH_2S), 2.03-1.82 (4H, m, CH_2) ppm. ^{13}C NMR (75 MHz, acetone-d_6): δ_C 170.99, 165.24, 152.81, 150.96, 149.95, 136.41, 128.91, 128.89, 125.68, 123.15, 122.58, 49.84, 35.45, 32.94, 28.93, 23.35 ppm. HRMS (ESI): *m/z* calcd for $C_{22}H_{21}F_3N_3O_6S_2$ $[M+H]^+$: 544.0824; found 544.0816.

***S*-5-{4-[4-(4-Chlorophenyl)-1,2,5-oxadiazol-3-ylcarbamoyl]phenylamino}-5-oxopentyl methanesulfonothioate** (**8**) was synthesized according to procedure **A** using 4-amino-*N*-[4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl]benzamide (**27**) and 5-(methylsulfonylthio)pentanoic acid (**10**). The crude mixture residue was purified by column chromatography (silica gel; EtOAc/cyclohexane; in gradient); the product was eluted with 40% of EtOAc. The title compound was obtained as a grey-white solid. Yield: 7%. mp 194.8-198.1 °C. ^{1}H NMR (300

MHz, acetone-d_6): δ_H 10.28 (1H, broad s, NH exchanged with D_2O), 9.51 (1H, broad s, NH exchanged with D_2O), 8.00 (2H, d, $^3J_{HH}$ 8.8 Hz, CH aromatic), 7.91-7.80 (4H, m, CH aromatic), 7.55 (2H, d, $^3J_{HH}$ 8.8 Hz, CH aromatic), 3.45 (3H, s, CH_3), 3.29 (2H, t, $^3J_{HH}$ 6.6 Hz, CH_2COO), 2.50 (2H, t, $^3J_{HH}$ 6.6 Hz, CH_2S), 1.97-1.84 (4H, m, CH_2) ppm. HRMS (ESI): *m/z* calcd for $C_{21}H_{22}ClN_4O_5S_2$ $[M+H]^+$: 509.0720; found 509.0712.

(4-(4-Chlorophenyl)-1,2,5-oxadiazol-3-yl)methanol 2-oxide (**11**)**.** This intermediate was synthesized according to the procedure reported in literature.[20] Brown oil. Yield 50%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.79 (2H, d, $^3J_{HH}$ 8.6 Hz, CH aromatic), 7.53 (2H, d, $^3J_{HH}$ 8.6 Hz, CH aromatic), 4.73 (2H, s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 155.81, 137.77, 129.74, 129.06, 124.62, 53.31. MS (ESI) *m/z* calcd for $C_9H_8ClN_2O_3$ $[M+H]^+$: 227.02; found 227.01.

(4-(4-Chlorophenyl)-1,2,5-oxadiazol-3-yl)methanol (**12**)**.**[21] Compound **11** (1.26 mmol) was refluxed with trimethylphosphite (2.62 mL) for 18 h, in a flame-dried flask under nitrogen, monitoring the reaction by TLC (cyclohexane/CH_2Cl_2/EtOAc 5:4.5:0.5). After cooling, 1 N HCl (26.2 mL) was added and the reaction mixture was extracted with Et_2O. The combined organic layers were washed with saturated $NaHCO_3$, and then with brine, dried and evaporated. Flash chromatography (cyclohexane/EtOAc 8:2) was performed to give **12**, as yellow oil. Yield = 15%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.86 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 7.51 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 4.98 (2H, s, CH_2). ^{13}C NMR (75 MHz, acetone-d_6) δ_C 153.42, 153.21, 136.29, 130.19, 129.28, 124.73, 52.89. MS (ESI) *m/z* calcd for $C_9H_7ClN_2O_2Na$ $[M+Na]^+$: 233.01; found 233.6.

1-Methyl-1*H*-imidazole-4-carbaldehyde (**13**)**.** This intermediate was synthesized according to the procedure reported in literature.[22] The final product was collected as a pale-yellow solid. Yield = 65%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.87 (1H, s, CHO), 7.60 (1H, s, CH aromatic), 7.57 (1H, s, CH aromatic), 3.78 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 186.4, 142.9, 139.5, 125.4, 34.2. MS (ES^+): *m/z* calcd for $C_5H_7N_2O$ $[M+H]^+$: 111.06; found 111.1.

5-(4-Chlorophenyl)-1-methyl-1*H*-imidazole-4-carbaldehyde (**14**)**.**[23] 1-bromo-4-chlorobenzene (1.5 mmol), $Pd(OAc)_2$ (0.05 mmol), P(2-furyl)$_3$ (0.1 mmol) and K_2CO_3 (2.0 mmol) were placed in a reaction vessel under a stream of nitrogen. Deaerated **13** (1.0 mmol) and anhydrous *N,N*-dimethylformamide (5 mL) were then added and the resulting mixture was stirred at 110 ^{o}C for 48 h, monitoring the reaction by TLC (CH_2Cl_2/MeOH 95:5). Once the reaction was complete, the mixture was cooled to rt, diluted with EtOAc/CH_2Cl_2 (1:1, 30 mL), and filtered through Celite. The filtrate was concentrated under reduced pressure and purified by flash chromatography with CH_2Cl_2/MeOH 95:5 as the eluent. The product was obtained as a pale yellow solid. Yield: 40%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.80 (1H, s, CHO), 7.62 (1H, s, CH aromatic), 7.50 (2H, d, $^3J_{HH}$ 8.43 Hz, CH aromatic), 7.36 (2H, d, $^3J_{HH}$ 8.43 Hz, CH aromatic), 3.59 (3H, s, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 185.36, 149.25, 137.87, 131.12, 129.54, 129.33, 128.06, 125.98, 33.78 ppm. MS (ESI): *m/z* calcd for $C_{11}H_9ClN_2NaO$ $[M+Na]^+$: 243.03; found 243.1.

[5-(4-Chlorophenyl)-1-methyl-1*H*-imidazol-4-yl]methanol (**15**)**.** To a solution of **14** (0.5 mmol) in MeOH (5 mL) at 0° C, sodium borohydride (0.25 mmol) was added. The reaction was warmed to rt and monitored using TLC analysis (eluent CH_2Cl_2/MeOH 95:5) until the reaction was complete. The mixture was quenched by adding water (5 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The desired product was obtained as a pale-yellow solid after crystallization in CH_2Cl_2/*n*-hexane. Yield: 45%. ^{1}H NMR (300 MHz, CD_3OD) δ_H 7.68 (1H, s, CH aromatic), 7.49 (4H, m, CH aromatic), 4.39 (2H, s, CH_2), 3.59 (3H, s, CH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD) δ_C 138.23, 138.04, 134.52, 131.53, 130.21, 128.75, 127.80, 56.46, 31.61 ppm. MS (ES^+): *m/z* calcd for $C_{11}H_{12}ClN_2O$ $[M+H]^+$: 223.06; found 223.1.

General procedure for the synthesis of benzaldehyde oximes (**17a-c**)**.** $NH_2OH{\cdot}HCl$ (7.2 mmol) and $NaHCO_3$ (7.2 mmol) in water (9 mL) were added to a solution of the proper aldehyde **16a-c** (6.0 mmol) in MeOH (18 mL). The mixture was refluxed under stirring for 2 h and the solvent was evaporated under *vacuum*. The

aqueous phase was extracted with EtOAc (3 x 10 mL), the organic solvent dried over Na_2SO_4 and evaporated under reduced pressure to obtain the oximes (**17a-b**), which were used in the next reaction without further purification.

***tert*-Butyl-4-[(hydroxyimino)methyl]benzylcarbamate** (**17a**) was synthesized starting from **16a** (prepared according to literature procedure[28]). White solid. Yield 81%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.11 (s, 1H, CH), 7.51-7.24 (m, 4H, CH aromatic), 5.27 (broad s, 1H, NH exchanged with D_2O), 4.32 (s, 2H, CH_2), 1.47 (s, 9H, $C(CH_3)_3$) ppm.

4-Phenoxybenzaldehyde oxime (**17b**) was synthesized starting from **16b** and obtained as white solid. Yield: 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.18 (1H, s, CH), 7.55 (2H, d, $^3J_{HH}$ 9.7, CH aromatic), 7.25-7.45 (5H, m, CH aromatic), 7.00 (2H, d, $^3J_{HH}$ 9.7, CH aromatic), 5.12 (2H, s, CH_2) ppm.

4-Chlorobenzaldehyde oxime (**17c**) was synthesized starting from **16c** and obtained as white solid. Yield: 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.04 (1H, s, CH), 7.43 (2H, d, $^3J_{HH}$ 8.5, CH aromatic), 7.29 (2H, d, $^3J_{HH}$ 8.5, CH aromatic), 6.67 (broad s, 1H, NOH exchanged with D_2O) ppm.

General procedure for the synthesis of benzoyl chloride oximes (**18a-c**)**.** The suitable benzaldehyde oxime (**17a-c**, 15 mmol) was dissolved in *N,N*-dimethylformamide (6 mL) and a first portion of *N*-chlorosuccinimide (NCS, 3 mmol) was added. The reaction mixture was cooled at 0°C and a second amount of NCS (12 mmol) was introduced. The solution was stirred for 12h at rt. After addition of water (10 mL) to the reaction mixture, the solvent was evaporated under *vacuum*. The aqueous solution was extracted by EtOAc (3 x 10 mL). The organic phases were collected, dried over Na_2SO_4 and evaporated under *vacuum* to give the correspondent crude hydroxylimino derivative (**18a-c**), that was directly used without further purification in the next reaction.

***tert*-Butyl {4-[chloro(hydroxyimino)methyl]benzyl}carbamate** (**18a**) was synthesized starting from **17a**. Pale oil. Quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.55 (1H, broad s, NOH exchanged with D_2O), 7.75 (2H, d, $^3J_{HH}$ 7.8 Hz, CH aromatic), 7.31 (2H, d, $^3J_{HH}$ 7.8 Hz, CH aromatic), 4.93 (1H, broad s, NH, exchanged with D_2O), 4.35 (2H, d, $^2J_{HH}$ 6.1 Hz, CH_2), 1.47 (9H, s, $C(CH_3)_3$) ppm.

4-Benzyloxybenzoyl chloride oxime (**18b**) was synthesized starting from **17b** and obtained as white solid. Quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.22 (1H, broad s, NOH exchanged with D_2O), 7.78 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 7.46-7.29 (5H, m, CH aromatic), 6.98 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 5.10 (2H, s, CH_2) ppm.

4-Chlorobenzoyl chloride oxime (**18c**) was synthesized starting from **17c** and obtained as pale oil. Yield 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.21 (1H, broad s, NOH exchanged with D_2O), 7.74 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 7.32 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic) ppm.

General procedure for the synthesis of 2-(hydroxyimino)acetonitriles (**19a-c**)**.** The required chloride oxime (**18a-c**, 4.8 mmol) was dissolved in Et_2O (20 mL) and cooled to 0°C. A solution of KCN (9.6 mmol) dissolved in water (10 mL) was added and the reaction mixture was stirred at rt for 5 h. Subsequently the organic layer was separated and the aqueous phase was extracted by EtOAc (3 x 10 mL). The organic solvent was dried over Na_2SO_4 and evaporated under reduced pressure to give the crude 2-(hydroxyimino)acetonitriles (**19a-c**).

***tert*-Butyl {4-[cyano(hydroxyimino)methyl]benzyl)}carbamate** (**19a**) was synthesized starting from **18a** and it was directly used without further purification in the next reaction. Yellow solid. Yield 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 10.15 (1H, broad s, NOH exchanged with D_2O), 7.70–7.28 (4H, m, CH aromatic), 5.04 (1H, bs, NH exchanged with D_2O), 4.37 (2H, s, CH_2), 1.47 (9H, s, $C(CH_3)_3$) ppm.

4-Benzyloxyphenyl-2-(hydroxyimino)acetonitrile (**19b**) was synthesized starting from **18b**. The crude was purified by flash chromatography (eluting with cyclohexane/EtOAc 9:1) and a white solid was obtained. Yield: 61%. ^{1}H-NMR (300 MHz, acetone-d_6) δ_H 12.36 (1H, broad s, NOH exchanged with D_2O), 7.73 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 7.27-7.55 (5H, m, CH aromatic), 7.15 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 5.20 (2H, s, CH_2) ppm.

4-Chlorophenyl-2-(hydroxyimino)acetonitrile (**19c**) was synthesized starting from **18c**. The crude was purified by flash chromatography (eluting with cyclohexane/EtOAc 9:1) and a yellow solid was obtained. Yield: 87%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.59 (1H, broad s, NOH exchanged with D_2O), 7.78 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic), 7.42 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic) ppm.

General procedure for the synthesis of acetamidines (**20a-c**)**.** To a solution of the appropriate intermediate (**19a-c**, 4.7 mmol) in MeOH (13 mL), $NH_2OH{\cdot}HCl$ (7.1 mmol) and $NaHCO_3$ (7.1 mmol) in water (6 mL) were added. The mixture was refluxed under stirring for 12 h and the organic solvent was removed *under vacuum*. The aqueous phase was extracted by EtOAc (3 x 10 mL) and the collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to give the crude acetamidine intermediates (**20a-c**), directly used without further purification in the next reaction.

***tert*-Butyl {4-[2-amino-1,2-bis(hydroxyimino)ethyl]benzyl}carbamate** (**20a**) was synthesized starting from **19a**. White solid. Yield 91%. ^{1}H NMR (300 MHz, CD_3OD) δ_H 7.62 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 7.26 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 4.23 (2H, s, CH_2), 1.45 (9H, s, $C(CH_3)_3$) ppm.

2-[4-(Benzyloxy)phenyl]-N'-hydroxy-2-(hydroxyimino)acetimidamide (**20b**) was synthesized starting from **19b** and obtained as light yellow solid. Yield: 99%. ^{1}H NMR (300 MHz, acetone-d_6) δ_H 10.80 (1H, broad s, NOH exchanged with D_2O), 8.94 (1H, broad s, NOH exchanged with D_2O), 7.60 (2H, d, $^3J_{HH}$ 9.0 Hz, CH aromatic), 7.45-7.52 (2H, m, CH aromatic), 7.24-7.45 (3H, m, CH aromatic), 7.00 (2H, d, $^3J_{HH}$ 9.0 Hz, CH aromatic), 5.45 (2H, broad s, NH_2 exchanged with D_2O), 5.16 (2H, s, CH_2) ppm.

2-(4-Chlorophenyl)-N'-hydroxy-2-(hydroxyimino)acetimidamide (**20c**) was synthesized starting from **19c** and obtained as white solid. Yield: 95%. ^{1}H NMR (300 MHz, CD_3OD) δ_H 7.64 (2H, d, $^3J_{HH}$ 8.5 Hz, CH aromatic), 7.34 (2H, d, $^3J_{HH}$ 8.5 Hz, CH aromatic) ppm.

General procedure for the synthesis of 4-phenyl-1,2,5-oxadiazol-3-amines (**21a-c**)**.** Each acetamidine derivative (**20a-c**, 4.3 mmol) was dissolved in 2 N NaOH (43 mL) and the solution refluxed under stirring for 12 h. After cooling, the volume of the mixture was reduced to approximately 10 mL and the so formed precipitate was collected by filtration and dried *under vacuum*. The crude product was then purified as indicated for each compound.

***tert*-Butyl [4-(4-amino-1,2,5-oxadiazol-3-yl)benzyl]carbamate** (**21a**)**.** The solid obtained from the NaOH treatment was then dissolved in CH_2Cl_2, cooled in an ice bath, and triethylamine (0.8 mmol) followed by *tert*-butyloxycarbonyl anhydride (0.8 mmol) were added. The mixture was left 4 hours at rt, after that time the solvent was removed under *vacuum*, the residue dissolved in $CH_2Cl_2$and washed with sat. $NaHCO_3$, water and brine. The organic solvent was dried over Na_2SO_4 and evaporated under reduced pressure to give crude oxadiazole (**21a**) which was purified by preparative HPLC (column: SunFire Prep C18 19 x 150 mm, gradient: H_2O + TFA 0.1% - MeOH + TFA 0.1%). Solid. Yield: 38 %. ^{1}H NMR (300 MHz, CD_3OD) δ_H 7.98 (2H, d, $^3J_{HH}$ 8.2 Hz, CH aromatic), 7.38 (2H, d, $^3J_{HH}$ 8.2, CH aromatic), 4.30 (2H, s, CH_2), 1.47 (9H, s, $C(CH_3)_3$) ppm.

4-[4-(Benzyloxy)phenyl]-1,2,5-oxadiazol-3-amine (**21b**) was synthesized starting from **20b** and the crude was purified by flash chromatography (eluting with cyclohexane/EtOAc 7:3) to give a white solid. Yield: 80%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ_H 7.66 (2H, d, $^3J_{HH}$ 8.8 Hz, CH aromatic), 7.29-7.49 (5H, m, CH aromatic), 7.11 (2H, d, $^3J_{HH}$ 8.8 Hz, CH aromatic), 5.14 (2H, s, CH_2), 4.19 (2H, broad s, NH_2 exchanged with D_2O) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 160.67, 154.21, 146.79, 136.49, 129.31, 128.95, 128.47, 127.69, 118.24, 116.01, 70.37 ppm.

4-(4-Chlorophenyl)-1,2,5-oxadiazol-3-amine (**21c**) was synthesized starting from **20c** and the crude was purified by flash chromatography (eluting with cyclohexane/EtOAc 8:2) to afford a white solid. Yield: 38%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.69 (2H, d, $^3J_{HH}$ 8.2 Hz, CH aromatic), 7.52 (2H, d, $^3J_{HH}$ 8.0 Hz, CH aromatic), 4.21 (2H, broad s, NH_2 exchanged with D_2O) ppm. ^{13}C NMR (75 MHz, acetone-d_6): δ_C 155.10, 146.20, 135.77, 129.44, 129.31, 124.95 ppm.

General procedure for the synthesis of *N*-(4-phenyl-1,2,5-oxadiazol-3-yl)benzamides (22, 24, 26). In a two-necked flame dried flask, 60% NaH mineral oil (0.3 mmol) was suspended in dry *N,N*-dimethylformamide (3 mL) under nitrogen. The suspension was cooled in an ice bath and the appropriate key intermediate (**21a-c**, 0.25 mmol) was added. The mixture was stirred for 20 min at 0°C. Then, the suitable commercially available acyl chloride (0.3 mmol) was added dropwise and the mixture was stirred at 60°C for 12 h. The reaction was quenched with water (3 mL) and *N,N*-dimethylformamide was removed *under vacuum*. The residue was extracted with EtOAc (3 × 2 mL); the organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by column chromatography to obtain the desired adduct.

***tert*-Butyl (4-{4-[4-(trifluoromethyl)benzamido]-1,2,5-oxadiazol-3-yl}benzyl)carbamate** (**22**) was synthesized starting from **21a** and 4-(trifluoromethyl)benzoyl chloride. After purification by preparative HPLC (column: SunFire Prep C18 19 x 150 mm, gradient: H_2O + TFA 0.1% - MeOH + TFA 0.1%) amide **22** was obtained as a white solid. Yield: 33 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.66 (1H, broad s, NH exchanged with D_2O), 7.99 (2H, d, $^3J_{HH}$ 8.1 Hz, CH aromatic), 7.77 (2H, d, $^3J_{HH}$ 8.2 Hz, CH aromatic), 7.64 (2H, d, $^3J_{HH}$ 8.1 Hz, CH aromatic), 7.39 (2H, d, $^3J_{HH}$ 8.0 Hz, CH aromatic), 4.99 (1H, t, $^3J_{HH}$ 6.1 Hz, NH exchanged with D_2O), 4.33 (2H, d, $^2J_{HH}$ 6.2 Hz, CH_2), 1.44 (9H, s, $C(CH_3)_3$) ppm.

4-Trifluoromethyl-N-[4-(4-benzyloxyphenyl)-1,2,5-oxadiazol-3-yl]benzamide (**24**) was synthesized starting from **21b** and 4-(trifluoromethyl)benzoyl chloride. The crude was purified by flash chromatography (eluting with cyclohexane/EtOAc 7:3) affording a white-gray solid. Yield: 30%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.11 (1H, broad s, NH exchanged with D_2O), 7.99 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 7.79 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 7.64 (2H, d, $^3J_{HH}$ 9.0 Hz, CH aromatic), 7.35-7.44 (5H, m, CH aromatic), 7.09 (2H, d, $^3J_{HH}$ 9.0 Hz, CH aromatic), 5.12 (2H, s, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 136.37 129.36, 129.00, 128.53, 128.32, 127.73, 126.49, 116.10, 70.37 ppm.

4-Nitro-*N*-[4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl]benzamide (**26**) was synthesized starting from **21c** and 4-nitrobenzoyl chloride. The crude was purified by flash chromatography (eluting from dichloromethane / cyclohexane (98:2) to dichloromethane / EtOAc (7:3)) giving a yellow solid. Yield: 46%. mp 229.9-230.2 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ_H 10.70 (1H, broad s, NH exchanged with D_2O), 8.41 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic), 8.29 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic), 7.87 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic), 7.56 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic) ppm. ^{13}C NMR (75 MHz, acetone-d_6): δ_C 165.00, 150.98, 150.69, 149.92, 138.19, 136.50, 129.79, 129.55, 129.48, 124.87, 123.99 ppm.

***N*-[4-(4-Aminomethylphenyl)-1,2,5-oxadiazol-3-yl]-4-(trifluoromethyl)benzamide hydrochloride** (**23**) Compound **22** (0.22 mmol) was dissolved with 4 M HCl in 1,4-dioxane (1 mL) at 0 °C. The reaction was stirred at rt for 30 minutes. After completion of the reaction, monitored by TLC (CH_2Cl_2/MeOH 85:15), the solvent was stripped off and the formed precipitate was washed with cold Et_2O and dried to afford hydrochloride acid salt of compound **23**.

4-[4-(4-Hydroxyphenyl)-1,2,5-oxadiazol-3-yl]-4-trifluoromethylbenzamide (**25**). A solution of **24** (0.54 mmol) and catalytic amount of 10% palladium on carbon (10% p/p) in EtOAc (9 mL) and MeOH (1 mL) was hydrogenated at rt for 24 hours. The resulting mixture was filtered and extracted with Et_2O (3 x 10 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated *in vacuum*. Under reduced pressure the crude oil was purified by flash chromatography eluting with cyclohexane/EtOAc (7:3) to afford the final compound as white foam. Yield: 91.5%. ^{1}H-NMR (300 MHz, acetone-d_6): δ_H 10.38 (1H, broad s, NH exchanged with D_2O), 8.92 (1H, broad s, OH exchanged with D_2O), 8.26 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic), 7.93 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic), 7.71 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic), 6.95 (2H, d, $^3J_{HH}$ 9 Hz, CH aromatic) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 164.7, 158.5, 147.3, 145.2, 137.5, 134.4, 128.9, 127.8, 125.6, 125.2, 124.1, 116.4 ppm.

4-Amino-*N*-[4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl]benzamide (**27**). To a solution of **26** (0.062 mmol) in EtOAc (4 mL), tin(II) chloride (0.310 mmol) was added and the mixture was refluxed for 4 h. The reaction was then quenched by addition of a saturated aqueous solution of $NaHCO_3$ until pH = 7-8 to allow the precipitation of tin salts. The resulting mixture was filtered and washed several times with EtOAc. The aqueous phase was extracted with EtOAc (3 × 10 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was purified on silica gel column chromatography (eluent: cyclohexane/EtOAc 6:4) to give **27** as yellow solid. Yield 70 %. mp 153.5-154 °C. ^{1}H-NMR (300 MHz, acetone-d_6): δ_H 9.82 (1H, broad s, NH exchanged with D_2O), 7.64-7.73 (4H, m, CH aromatic), 7.40 (2H, d, $^3J_{HH}$ 8.7 Hz, CH aromatic), 6.60 (2H, d, $^3J_{HH}$ 8.4 Hz, CH aromatic), 5.35 (2H, broad s, NH_2 exchanged with D_2O) ppm. ^{13}C NMR (75 MHz, acetone-d_6): δ_C 166.09, 153.41, 151.15, 150.88, 136.17, 130.26, 129.36, 129.30, 125.46, 119.84, 113.35 ppm.

Cell culture. The colorectal HCT116 cell lines were cultured in McCoy's media supplemented with penicillin (10,000 U/mL), streptomycin (10 mg/mL), nonessential amino acid and 10% Fetal Calf Serum (FCS). Cells were incubated with newly synthesized compounds dissolved in DMSO. The same volume of solvent was added to control conditions and did not exceed 0.5% v/v.

MTT-assay. The determination of the conversion of MTT (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) to formazan was determined as previously described.[25,26]

AlphaScreen-based Assay. The AlphaScreen-based assay was performed according to literature data.[24]

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