



UNIVERSITY OF PISA

School of Graduate Studies
“Scienza del Farmaco e delle Sostanze Bioattive”

PhD THESIS
2006-2008

**“New P-glycoprotein inhibitors: potential tools to
reduce Multidrug Resistance ”**

Michael Vanni

DIRECTOR OF THE SCHOOL

Prof. Claudia Martini

2008

INDEX

1. Introduction	6
1.1. Multidrug Resistance	6
1.2. Multidrug Resistance to Anticancer Drugs (neoplastic resistance)	8
1.3. Efflux transporters	10
1.4. ATP-binding cassette (ABC)	12
1.4.1. ABC transporter architecture	16
1.4.2. Transport mechanism	20
1.5. Characteristics of the MRP1 and MRP2 Transporters	21
1.6. Breast cancer resistance protein (BCRP)	24
1.6.1. Structure and cellular localization	25
1.6.2. Transport properties	26
1.6.3. Dimer formation	27
1.7. P-glycoprotein (P-gp)	27
1.7.1. P-gp at the level of blood-brain barrier (BBB)	31
1.7.2. P-gp at the level of placenta	34
1.7.3. Potential role of P-gp and MRP1 at the blood-cerebrospinal fluid barrier	37
1.8. Identification of P-gp binding regions and pockets	40
1.9. The transport cycle of P-glycoprotein	45
1.10. Expression of P-Glycoprotein in Human Tumors	49
1.11. Intentional modulation of the function of P-Glycoprotein	51
1.12. Candidate sites for putative P-glycoprotein inhibitors	54
<i>Bibliography</i>	58
2. Multidrug Resistance Reverting Agents and P-gp modulators	68
2.1. Pharmacological control of MDR	68
2.2. Theoretical SAR studies to predict MDR modulating activity	69
2.3. Transported Substrates of P-gp	72
2.4. P-glycoprotein inhibitor development	73
2.4.1. First generation inhibitors	73
2.4.2. Second generation inhibitors	76

2.4.3. <i>Third generation inhibitors</i>	77
2.5. Structure-Activity Relationship among P-glycoprotein Modulators	78
2.5.1. <i>Verapamil-related compounds</i>	78
2.5.2. <i>Nimodipine-related compounds</i>	80
2.5.3. <i>Quinine-related compounds</i>	82
2.5.4. <i>Dipyridamole-related compounds</i>	84
2.5.5. <i>Cyclosporin A-related compounds</i>	86
2.5.6. <i>Taxanes</i>	87
2.5.7. <i>Trifluoperazine-related compounds</i>	88
2.5.8. <i>Propafenone-related compounds</i>	89
2.5.9. <i>Amiodarone-related compounds</i>	90
2.5.10. <i>Flavonoid-related compounds</i>	91
2.5.11. <i>Alkaloids</i>	92
2.5.12. <i>Terpenes</i>	93
2.5.13. <i>Steroid-related compounds</i>	94
2.8.14. <i>Oligonucleotides</i>	95
2.6. Phamacophore Modeling	96
2.7. Third generation P-gp inhibitors	99
2.7.1. <i>Valspodar</i>	99
2.7.2. <i>Zosuquidar</i>	99
2.7.3. <i>Tariquidar</i>	99
2.7.4. <i>Biricodar</i>	99
2.7.5. <i>Elacridar</i>	100
2.7.6. <i>Timcodar</i>	100
2.7.7. <i>Laniquidar</i>	100
<i>Bibliography</i>	103

3. New P-Glycoprotein inhibitors and their screening: Potential tools to reduce Multidrug Resistance **117**

3.1. Introduction	117
3.2. Current State of Art	119
3.3. Aim of the thesis	125
3.4. Substitution of the Arylmethyl portion linked to the PEP (or 3-methoxy PEP) nucleus	127
3.4.1. <i>Synthesis</i>	128
3.4.2. <i>Results and discussion</i>	131
3.5. Effect of methoxylation on B- and/or C- rings	135
3.5.1. <i>Synthesis</i>	137
3.5.1. <i>Results and discussion</i>	141
3.6. Substitution of the ethyl chain with a methylenoxy linker	145

3.6.1. <i>Synthesis</i>	145
3.7. Bioisoster substitution of oxygen atom with a nitrogen one	147
3.7.1. <i>Synthesis</i>	149
3.7.2. <i>Results and discussion</i>	155
3.8. Replacement of amino function with an amido one	158
3.8.1. <i>Synthesis</i>	159
3.9. Replacement of <i>B</i> -ring with basic nucleuses	160
3.9.1. <i>Synthesis</i>	162
3.9.2. <i>Results and discussion</i>	164
3.10. Further piperazine-nucleus	173
3.10.1. <i>Synthesis</i>	174
<i>Bibliography</i>	176
4. Experimental section	177
4.1. General Methods	177
4.1.1. <i>Elemental Analysis</i>	221
4.2. Biological Methods	223
4.2.1. <i>Cell lines</i>	223
4.2.2. <i>Permeability Experiments. Preparation of Caco-2 Monolayer</i>	224
4.2.3. <i>Drug Transport Experiment</i>	224
4.2.4. <i>Cell ATP Availability Assay</i>	225
4.2.5. <i>[³H] Substrate Transport Inhibition</i>	225
4.2.6. <i>[³H] Mithoxantrone Transport Inhibition</i>	226
4.2.7. <i>Calcein-AM Assay in MDCK-P-gp Cells</i>	227
4.2.8. <i>Radioligand Binding Assay at Rat Human Cloned 5-HT_{1A} Receptor</i>	228
4.2.10. <i>Statistical Analysis</i>	228
<i>Bibliography</i>	230

