

WESTFÄLISCHE WILHELMS-UNIVERSITÄT

Organisch-Chemisches Institut

Design, Synthesis, and Evaluation of small molecules  
to identify and modulate cellular processes

Inaugural-Dissertation

zur Erlangung des Doktorgrades

der Naturwissenschaften im Fachbereich Chemie und Pharmazie

der Mathematisch-Naturwissenschaftlichen Fakultät

der Westfälischen Wilhelms-Universität Münster

Reshma Vidyadharan

aus

Kochi, Kerala, Indien

2017

# Contents

1	Introduction .....	1
1.1	G-Protein Coupled Receptors .....	2
1.2	Chemokines and Chemokine Receptors .....	4
1.2.1	Roles in Diseases .....	5
1.2.2	Chemokine CCL20 and its Receptor CCR6 .....	5
1.3	Transport of macromolecules across membranes .....	9
1.3.1	Clathrin Mediated Endocytosis .....	9
1.3.2	Nuclear Pore Complexes .....	11
1.4	Guanosine triphosphate hydrolysing enzymes (GTPases) .....	12
1.4.1	Ran (Ras-related nuclear protein) GTPases .....	12
1.4.2	Ras-related C3 botulinum toxin substrate 1 (Rac1) .....	12
1.4.3	Actin .....	13
1.5	Matrix Metalloproteinases (MMPs) .....	13
1.5.1	Role of MMPs in diseases .....	17
1.5.2	MMP-2 and MMP-9 Inhibitors .....	18
1.5.3	Cluster Modelling .....	22
1.6	Computational Studies .....	23
1.6.1	Docking Studies .....	23
1.6.2	Homology Modelling .....	25
1.7	Summary of thesis topic .....	27
2	Synthesis and Testing of small molecule ligands for CCR6 .....	29
2.1	Background and Aim .....	29
2.2	Materials and Methods .....	30
2.3	Results and Discussion .....	37
2.4	Summary and Outlook .....	54
3	Investigation of Off-target effects of Pitstop-2 .....	56
3.1	Background and Aim .....	56
3.2	Materials and Methods .....	56
3.3	Results and Discussion .....	63
3.4	Summary and Outlook .....	65
4	Computational studies on MMP-2 and MMP-9 .....	67

4.1	Background and Aim .....	67
4.2	Materials and Methods.....	68
4.3	Results and Discussion .....	69
5	Experimental.....	74
5.1	General Techniques and Apparatus .....	74
5.1.1	Solvents.....	74
5.1.2	Chemicals and Reagents .....	74
5.1.3	Reactions.....	74
5.1.4	Microwave Assisted Synthesis.....	75
5.2	Chromatography .....	75
5.2.1	Thin Layer Chromatography.....	75
5.2.2	Gas Chromatography .....	75
5.3	Characterization .....	76
5.3.1	Melting point.....	76
5.3.2	Nuclear Magnetic Resonance.....	76
5.3.3	High Resolution Mass Spectrometry .....	77
5.4	Synthesis of CCR6 Ligands .....	77
5.4.1	N-(1H-Benzo[d]imidazol-2-yl)-3-methylbenzofuran-2-carboxamide (1) .....	77
5.4.2	N-(1H-Benzo[d]imidazol-2-yl)benzofuran-2-carboxamide (7) .....	78
5.4.3	2-(5-Bromopentyl)isoindoline-1,3-dione (8) .....	78
5.4.4	N-{1-[5-(1,3-Dioxoisindolin-2-yl)pentyl]-1H-benzo[d]imidazol-2-yl}-3-methylbenzofuran-2-carboxamide (9).....	79
5.4.5	N-[1-(5-Aminopentyl)-1H-benzo[d]imidazol-2-yl]-3-methylbenzofuran-2-carboxamide (10) .....	80
5.4.6	N-[1-(5-Hydroxypentyl)-1H-benzo[d]imidazol-2-yl]-3-methylbenzofuran-2-carboxamide (11) .....	81
5.4.7	3-Methyl-N-(1-vinyl-1H-benzo[d]imidazol-2-yl)benzofuran-2-carboxamide (12).....	81
5.4.8	3-Methyl-N-[1-(prop-2-yn-1-yl)-1H-benzo[d]imidazol-2-yl]benzofuran-2-carboxamide (13) .....	82
5.4.9	N-(1-Allyl-1H-benzo[d]imidazol-2-yl)-3-methylbenzofuran-2-carboxamide (14) .....	83
5.4.10	N-[1-(5-{[5-(Dimethylamino)naphthalene]-1-sulfonamido}pentyl)-1H-benzo[d]imidazol-2-yl]-3-methylbenzofuran-2-carboxamide (15).....	84
5.4.11	3-Methyl-N-[1-(5-{5-[(3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanamido}pentyl)-1H-benzo[d]imidazol-2-yl]benzofuran-2-carboxamide (16) .....	85
5.4.12	Methyl 2-(2-acetyl-4-bromophenoxy)acetate (29).....	86

5.4.13	Methyl 5-bromo-3-methylbenzofuran-2-carboxylate (30) .....	86
5.4.14	5-Bromo-3-methylbenzofuran-2-carboxylic acid (31) .....	87
5.4.15	N-(1H-Benzo[d]imidazol-2-yl)-5-bromo-3-methylbenzofuran-2-carboxamide (28)...	88
5.4.16	Methyl 2-(2-acetyl-4-fluorophenoxy)acetate (32).....	89
5.4.17	Methyl 5-fluoro-3-methylbenzofuran-2-carboxylate (33).....	89
5.4.18	5-Fluoro-3-methylbenzofuran-2-carboxylic acid (34).....	90
5.4.19	N-(1H-Benzo[d]imidazol-2-yl)-5-fluoro-3-methylbenzofuran-2-carboxamide (35)...	91
5.4.20	Methyl 2-(2-acetyl-4-methoxyphenoxy)acetate (36).....	91
5.4.21	Methyl 5-methoxy-3-methylbenzofuran-2-carboxylate (37).....	92
5.4.22	5-Methoxy-3-methylbenzofuran-2-carboxylic acid (38).....	93
5.4.23	N-(1H-Benzo[d]imidazol-2-yl)-5-methoxy-3-methylbenzofuran-2-carboxamide (18)	94
5.4.24	Methyl 2-(2-acetyl-4-ethoxyphenoxy)acetate (39).....	94
5.4.25	Methyl 5-ethoxy-3-methylbenzofuran-2-carboxylate (40).....	95
5.4.26	5-Ethoxy-3-methylbenzofuran-2-carboxylic acid (41).....	96
5.4.27	N-(1H-Benzo[d]imidazol-2-yl)-5-ethoxy-3-methylbenzofuran-2-carboxamide (21) ..	96
5.4.28	3-Acetyl-4-hydroxyphenyl acetate (42).....	97
5.4.29	Methyl 2-(4-acetoxy-2-acetylphenoxy)acetate (43) .....	98
5.4.30	Methyl 5-acetoxy-3-methylbenzofuran-2-carboxylate (44) .....	99
5.4.31	5-Hydroxy-3-methylbenzofuran-2-carboxylic acid (45) .....	99
5.4.32	N-(1H-Benzo[d]imidazol-2-yl)-5-hydroxy-3-methylbenzofuran-2-carboxamide (46)	100
5.4.33	N-(1H-Benzo[d]imidazol-2-yl)-5-(2-fluoroethoxy)-3-methylbenzofuran-2-carboxamide (47).....	101
5.4.34	tert-Butyl 2-(5-hydroxy-3-methylbenzofuran-2-carboxamido)-1H-benzo[d]imidazole-1-carboxylate (48) .....	101
5.4.35	tert-Butyl 2-(5-{[5-(1,3-dioxoisindolin-2-yl)pentyl]oxy}-3-methylbenzofuran-2-carboxamido)-1H-benzo[d]imidazole-1-carboxylate (49).....	102
5.4.36	5-[(5-Aminopentyl)oxy]-N-(1H-benzo[d]imidazol-2-yl)-3-methylbenzofuran-2-carboxamide (50).....	103
5.4.37	N-(1H-Benzo[d]imidazol-2-yl)-3-methyl-5-({5-[(3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentyl}oxy)benzofuran-2-carboxamide (51) .....	104
5.5	Synthesis of Pitstop-2 analogues .....	105
5.5.1	General procedure for coupling naphthalene sulphonyl chloride with pseudothiohydantoin.....	105
5.5.2	General procedure for microwave assisted Knoevenagel condensation.....	105

5.5.3	Preparation of Benzoic acid/piperidine catalyst mixture .....	105
5.5.4	Synthesis of N-(4-oxo-4,5-dihydrothiazol-2-yl)naphthalene-1-sulfonamide(52).....	106
5.5.5	(Z)-N-[5-(4-Bromobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl]naphthalene-1-sulfonamide(53) .....	106
5.5.6	5-(Dimethylamino)-N-(4-oxo-4,5-dihydrothiazol-2-yl)naphthalene-1-sulfonamide (54) 107	
5.5.7	(Z)-N-[5-(4-Bromobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl]-5-(dimethylamino)naphthalene-1-sulfonamide (55).....	107
5.5.8	(Z)-5-(Dimethylamino)-N-[5-(4-fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl]naphthalene-1-sulfonamide (56).....	108
5.5.9	(Z)-5-(dimethylamino)-N-[5-(4-methoxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl]naphthalene-1-sulfonamide (57).....	109
6	Appendix.....	110
6.1	Script for running jobs on PALMA cluster.....	110
6.2	Acronyms and Abbreviations.....	111
7	References.....	114