Faculty of Pharmacy

Molecular Design, Synthesis and Biological Evaluation of Heterocyclic Compounds as Potential Targeted Antimicrobial Agents

Thesis

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List of Abbreviations

Abb.	Full term
^{13}C NMR	*Carbon-13 Nuclear Magnetic Resonance*
^{1}H NMR	*Proton Nuclear Magnetic Resonance*
AMR	*Antimicrobial resistance*
BOP	*Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate*
CAMHB	*Cation adjusted Mueller Hinton Broth*
CDC	*Center for disease control and prevention*
CDOCKER	*based docker CHARMm-*
CHARMm	*Chemistry at Harvard Macromolecular Mechanics*
DCC	*N,N′-Dicyclohexylcarbodiimide*
D_2O	*Deuterium oxide*
DCM	*dichloromethane*
DMAP	*4-(Dimethylamino)pyridine*
DMF	*Dimethylformamide*
DMSO	*Dimethyl sulfoxide*
EI-MS	*Electron Ionization Mass Spectrometry*
EtOAc	*Ethyl acetate*
FDA	*Food and Drug Administration*
ΣFIC	*Fractional inhibitory concentration*
GT	*Glycosyl transferase*
HB	*hydrogen bond*
HMM	*High Molecular Mass*
hr	*hours*
HATU	*Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium*
Hz	*Hertz*
IC_{50}	*Half-maximal inhibitory concentration*
LC/MS	*Liquid chromatography–mass spectrometry*
LMM	*Low Molecular Mass*
MS	*Mass spectroscopy*
m/z	*mass-to-charge ratio*
M.P	*Melting point*
M+	*Molecular ion*
MD	*Molecular Dynamics*
MGT	*Monofunctional glycosyl transferase*
MHz	*Mega hertz*
µM	*Micromole*
µl	*Microliter*
MIC	*Minimum inhibitory concentration*
mmol	*Millimole*
MDR	*Multiple drug resistance*
MBC	*Minimum bactericidal concentration*
MRSA	*Methicillin resistance staphylococcus aureus.*

MSSA *Methicillin sensitive staphylococcus aureus*
Mwt.....*Molecular Weight*
NAG.....*N-acetylglucosamine*
NAM.....*N-acetylmuramic acid*
PBP.....*Penicillin binding protein*
PDB.....*Protein data bank*
Ppm.....*Part per million*
QSAR.....*Quantitative structure activity relationship*
R_f.....*Retention factor*
RMSD.....*Root mean square deviation*
rt.....*Room temperature*
S.aureus.....*Staphylococcus aureus*
S.epidermidis.....*Staphylococcus epidermidis*
S.pneumoniae.....*Streptococcus pneumoniae*
SAR.....*Structure activity relationship*
T3P.....*Propanephosphonic acid anhydride*
TBTU.....*2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate*
TEA.....*Triethylamine*
THF.....*Tetrahydrofuran*
TLC.....*Thin layer Chromatography*
TMS.....*Tetramethylsilane*
TP.....*Transpeptidation*
TSA.....*Tryptic Soy Agar*
UDP.....*Uridine diphosphate*

Abstract

The rapid and continuous spread of antimicrobial resistance poses an enormous burden and threat to the global public health, where infections caused by multidrug resistant bacteria are associated with serious morbidity and mortality. The Gram-positive bacterium *Staphylococcus aureus* is a major cause of both nosocomial and community-acquired infections worldwide. The first methicillin-resistant *S. aureus* (MRSA) strain was isolated in the United Kingdom in 1961. This resistance phenotype arose through the acquisition of a gene cassette containing *mecA*, which encoded an altered transpeptidase, penicillin binding protein 2a (PBP2a), that demonstrated low binding affinity to most commercially available β-lactam antibiotics and was capable of cross-linking the peptidoglycan chains of the cell wall when other transpeptidases were inhibited by β-lactams. Since 1990 and up till today, MRSA infections presented a stressing global problem and the development of new antimicrobial agents to combat MRSA infections is of utmost importance. Targeting the key resistance enzyme, penicillin binding protein 2a, with small molecules is a promising therapeutic approach to tackle MRSA infections and was proved successful by the approval of the β-lactam antibiotics, ceftobiprole and ceftaroline.

Our research objectives were to design, synthesize and biologically evaluate new inhibitors targeting MRSA infections via inhibition of the mutant PBP2a. The design process aimed to target PBP2a allosteric site and started by identification of the key interactions between PBP2a enzyme allosteric binding site and a recently reported quinazolinone-based PBP2a inhibitor [(*E*)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4*H*)-yl)benzoic acid], following, rational modification of the lead compound was proposed and three series of derivatives were suggested (1-phenylpyrazole, chromen-4-one, and benzimidazole) and finally molecular modeling studies including field alignment and docking were performed to investigate the predicted binding modes and binding affinities of the designed compounds.

The designed compounds were synthesized, purified and structurally confirmed by different analytical and spectral techniques.

The study involved the synthesis of the following unavailable reported intermediates:

1) 3-Nitroacetophenone **(Ia)**
2) (*E*)-1-(1-(3-Nitrophenyl)ethylidene)-2-phenylhydrazine **(IIa).**
3) 3-(3-Nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **(IIIa)**
4) (*E*)-3-(4-Cyanophenyl)acrylic acid **(VIc).**
5) 2-Acetylphenyl (*E*)-3-phenylacrylate **(VIIIa).**
6) 2-Acetylphenyl (*E*)-3-(4-fluorophenyl)acrylate **(VIIIb).**
7) (*E*)-1-(2-Hydroxyphenyl)-5-phenylpent-4-ene-1,3-dione **(IXa).**
8) (*E*)-5-(4-Fluorophenyl)-1-(2-hydroxyphenyl)pent-4-ene-1,3-dione **(IXb).**
9) (*E*)-2-Styryl-4*H*-chromen-4-one **(Xa).**
10) (*E*)-2-(4-Fluorostyryl)-4*H*-chromen-4-one **(Xb).**
11) (*E*)-4-(2-(4-Oxo-4*H*-chromen-2-yl)vinyl)benzonitrile **(Xc)**
12) (*E*)-3-Bromo-2-styryl-4*H*-chromen-4-one **(XIa).**
13) 2-Acetylphenyl 1-naphthoate **(XIX)**
14) 1-(2-Hydroxyphenyl)-3-(naphthalen-2-yl)propane-1,3-dione **(XX).**
15) 2-(Naphthalen-2-yl)-4*H*-chromen-4-one **(XXI).**
16) *N*-(3-Aminophenyl)methanesulfonamide **(XXIV).**

Also, it comprised the synthesis of the following new intermediates:

1) (*E*)-3-(1-(2-Phenylhydrazono)ethyl)benzonitrile **(IIb).**
2) 3-(4-Formyl-1-phenyl-1*H*-pyrazol-3-yl)benzonitrile **(IIIb).**
3) (*E*)-3-(3-Nitrophenyl)-1-phenyl-4-styryl-1*H*-pyrazole **(IVa)**
4) (*E*)-4-(4-Fluorostyryl)-3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole **(IVb)**
5) 2-Acetylphenyl (*E*)-3-(4-cyanophenyl)acrylate **(VIIIc).**
6) (*E*)-4-(5-(2-Hydroxyphenyl)-3,5-dioxopent-1-en-1-yl)benzonitrile **(IXc).**
7) (*E*)-3-Bromo-2-(4-fluorostyryl)-4*H*-chromen-4-one **(XIb).**
8) (*E*)-4-(2-(3-Bromo-4-oxo-4*H*-chromen-2-yl)vinyl)benzonitrile **(XIc).**
9) (*E*)-4-(2-(3-(3-Aminophenyl)-4-oxo-4*H*-chromen-2-yl)vinyl)benzonitrile **(XIIj).**
10) (*E*)-3-(3-Aminophenyl)-2-styryl-4*H*-chromen-4-one **(XIIIa).**
11) 3-Bromo-2-(naphthalen-2-yl)-4*H*-chromen-4-one **(XXII).**
12) *N*-(3-((2-Nitrophenyl)amino)phenyl)methanesulfonamide **(XXV).**

Furthermore, the study involved the synthesis and characterization of the following new final compounds:

1) (*E*)-4-(2-(3-(3-Nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)vinyl)benzonitrile **(IVc).**
2) (*E*)-3-(4-(4-Methylstyryl)-1-phenyl-1*H*-pyrazol-3-yl)benzonitrile **(IVd).**
3) (*E*)-*N*-(3-(1-Phenyl-4-styryl-1*H*-pyrazol-3-yl)phenyl)methanesulfonamide **(Va).**
4) (*E*)-*N*-(3-(4-(4-Fluorostyryl)-1-phenyl-1*H*-pyrazol-3-yl)phenyl)methanesulfonamide **(Vb).**
5) (*E*)-*N*-(3-(4-(4-Cyanostyryl)-1-phenyl-1*H*-pyrazol-3-yl)phenyl)methanesulfonamide **(Vc).**
6) (*E*)-3-(4-Hydroxyphenyl)-2-styryl-4*H*-chromen-4-one **(XIIa).**
7) (*E*)-3-(4-Oxo-2-styryl-4*H*-chromen-3-yl)benzonitrile **(XIIb).**
8) (*E*)-3-(3-Nitrophenyl)-2-styryl-4*H*-chromen-4-one **(XIIc).**
9) (*E*)-3-(4-(Hydroxymethyl)phenyl)-2-styryl-4*H*-chromen-4-one **(XIId).**
10) (*E*)-3-(4-Hydroxy-3-methoxyphenyl)-2-styryl-4*H*-chromen-4-one **(XIIe).**
11) (*E*)-2-(4-Fluorostyryl)-3-(pyridin-3-yl)-4*H*-chromen-4-one **(XIIf).**
12) (*E*)-3-(2-(4-Fluorostyryl)-4-oxo-4*H*-chromen-3-yl)benzonitrile **(XIIg).**
13) (*E*)-2-(4-Fluorostyryl)-3-(3-methoxyphenyl)-4*H*-chromen-4-one **(XIIIh).**
14) (*E*)-2-(4-Fluorostyryl)-3-(3-nitrophenyl)-4*H*-chromen-4-one **(XIIi).**
15) (E)-3-(3-Aminophenyl)-2-(4-fluorostyryl)-4*H*-chromen-4-one **(XIIIb).**
16) (*E*)-*N*-(3-(4-Oxo-2-styryl-4*H*-chromen-3-yl)phenyl)methanesulfonamide **(XIVa).**
17) (*E*)-*N*-(3-(2-(4-Fluorostyryl)-4-oxo-4*H*-chromen-3-yl)phenyl)methanesulfonamide **(XIVb).**
18) (E)-N-(3-(2-(4-Cyanostyryl)-4-oxo-4*H*-chromen-3-yl)phenyl)methanesulfonamide **(XIVc).**
19) (*E*)-*N*-(3-(4-Oxo-2-styryl-4*H*-chromen-3-yl)phenyl)benzenesulfonamide **(XV).**
20) (*E*)-*N*-(3-(4-Oxo-2-styryl-4*H*-chromen-3-yl)phenyl)acetamide **(XVI).**
21) (*E*)-*N*-(3-(4-Oxo-2-styryl-4*H*-chromen-3-yl)phenyl)cyclopropanecarboxamide **(XVII).**
22) 3-(2-(Naphthalen-2-yl)-4-oxo-4*H*-chromen-3-yl)**benzonitrile (XXIII).**
23) (*E*)-*N*-(3-(2-Styryl-1*H*-benzo[*d*]imidazol-1-yl)phenyl)methanesulfonamide **(XXVI).**

24) *N*-(3-(2-(Naphthalen-2-yl)-1*H*-benzo[*d*]imidazol-1-yl)phenyl)methanesulfonamide **(XXVII)**.

Initial antimicrobial screening identified five chromen-4-one derivatives (**XIIIb**, **XIVa-c** and **XV**) with modest to potent antimicrobial activity (MIC values between 0.008-16 µg/mL) against MRSA USA300 strain, while the 1-phenylpyrazole and benzimidazole derivatives failed to demonstrate any antibacterial activity. Further screening of the chromen-4-one derivatives (**XIIIb**, **XIVa-c**) established them as promising bacteriostatic agents against several Gram-positive bacterial strains, with compounds **XIVa, XIVc** demonstrating nano-range activity (MIC values between 0.008-0.5 µg/mL) particularly against methicillin-sensitive, methicillin-resistant and vancomycin-resistant *S. aureus* strains. Compound **XIVa** outperformed vancomycin in passing into the MRSA infected macrophages cells and reducing the intracellular MRSA burden by 0.066-$\log_{10}$-reduction. Additionally, two compounds (**XIVb, XIVc**) exhibited a synergistic activity when combined with piperacillin against MRSA clinical isolate *in vitro* using the checkerboard assay. All of the tested chromen-4-one derivatives manifested great selectivity for bacterial over mammalian cells (Caco-2, Vero, and J774 cells) and demonstrated an excellent safety profile (non-toxic up to 32 µg/mL). Further mechanistic investigations and pharmacokinetic studies will follow to validate the mode of action of the reported chromen-4-one derivatives and to establish them as a novel class of antibacterial agents with promising activity against MRSA strains.

1. INTRODUCTION

1.1. Overview

Antimicrobial resistance (AMR) is a current threat worldwide with an alarming increase in infection-related morbidity and mortality rates.[1] One of the major causes of hospital and community acquired infections is the methicillin-resistant *Staphylococcus aureus* (MRSA).[2,3] According to the Centers for Disease Control and Prevention (CDC) antibiotic resistance threats report in 2013, MRSA was ranked as a serious threat with more than 80,461 infections and 11,285 deaths per year in USA.[4] Standard treatment options as β-lactam antibiotics are out of use, as the microorganism developed several mutations rendering this class almost inactive against several *S. aureus* strains including MRSA.[5,6] Production of a homologous of penicillin binding protein 2 named PBP2a is the major mechanism employed by MRSA to exhibit a broad clinical resistance to the β-lactam antibiotics.[7] The emergence of such resistance is mediated through the acquisition of a gene cassette containing *mec*A, which encodes an altered, low-affinity transpeptidase; PBP2a.[8] Consequently, an urgent need to develop effective antibiotics to meet the emerging threats of MRSA resistance, is greater than ever.

1.2. Evolution of MRSA

S. aureus is a Gram-positive organism, first discovered by Sir Alexander Ogston in 1880. Since its discovery, it has been regarded as a serious threat to human health, capable of causing a wide spectrum of infections, ranging from acute to life threatening infections such as boils, deep tissue abscesses, enterocolitis, bacteriuria, osteomyelitis, pneumonia, carditis, meningitis and septicemia.[9,10] The prognosis for patients with severe staphylococcal infections was extremely poor until the introduction of penicillin into clinical use in the early 1940s. However, few years later, the first penicillin-resistant strain of *S. aureus* was reported, and by 1946 it was estimated that 60% of hospital isolates in the UK were resistant to this antibiotic.[11] Albeit several antibiotics were introduced later as streptomycin, tetracycline, chloramphenicol and erythromycin, tolerant or resistant strains emerged rapidly.[12,13]