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Synthesis and antibacterial activity of 3-benzylamide derivatives as FtsZ inhibitors

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Abstract

The emergence and spread of multidrug-resistant strains of the human pathological bacteria are generating a threat to public health worldwide. In the current study, a series of PC190723 derivatives were synthesized and investigated for their antimicrobial activity. The compounds exhibited good activity against several Gram-positive bacteria as determined by comparison of diameters of the zone of inhibition of test compounds and standard antibiotics. Compound **9** with a fluorine substituted on the phenyl ring showed best antibacterial activity in the series against *M. smegmatis* with the zone ratio of 0.62, and against *S. aureus* with the zone ration of 0.44. The results from this study indicate that based on the unique 3-methoxybenzamide pharmacophore, compound **9** may represent a promising lead candidate against Gram-positive bacteria that are worthy of further investigation

Key Words: antibacterial agents; 3-methoxybenzamide; FtsZ; *Mycobacterium*

tuberculosis; *Staphylococcus aureus*

An increase of multidrug resistance to antibiotics among pathogenic strains of bacteria significantly threatens to public health[1-3]. In particular, the emergence and spread of drug-resistant *staphylococci aureus* is of serious concern[4]. New approaches to combat them are sorely needed. Recent efforts have included finding new ways to modify the structures of existing antibiotics as well as discovering and developing new leads and new molecular targets[5, 6]. Cell division has been of considerable interest to the antibacterial drug discovery as a promising target[1, 2, 7]. The bacterial cell division process encodes essential proteins forming the divisome, which are extremely sensitive to the inhibition. Among those proteins, the essential cytoskeletal cell division protein FtsZ（Filamenting temperature-sensitive mutant Z）is the most important and highly conserved protein in bacteria and archaea cell division machinery[8-10]. During the cell division, FtsZ undergoes GTP dependent polymerization to form a Z-ring, which allows the constriction to give rise to two equal daughter cells. If FtsZ assembly is restrained, the bacterial cell division would eventually fail, thereby resulting in bacterial apoptosis. FtsZ is structurally and functionally homologous to mammalian β-tubulin, which has been successfully exploited for cancer therapy[11]. It suggests that FtsZ may also be amenable to the inhibitor discovery for antibacterial agents. Furthermore, the validation of FtsZ as a novel antibacterial drug target has been confirmed by the work of various research groups, and the recent advances in the development of small molecules that target FtsZ has been the subject of some recently reviews[1, 3, 11-13].

3-Methoxybenzamide (3-MBA) (**Fig. 1**), which has been identified as a small

molecule that stabilizes the polymerization or disturbs the GTPase activity of FtsZ or both of them, was a promising lead for the development of new antibacterial agents[14]. Optimization of this lead with the aim to improving the antibacterial potency and drug like properties, a potent derivative PC190723 was discovered[15, 16]. PC190723 has potent and selective in vitro bactericidal activity against staphylococci, including methicillin- and multi-drug-resistant Staphylococci aureus, and kills staphylococci in vivo[16]. Later modification of PC190723 led to its prodrugs TXY541, TXY436 and TXA709 with superior antibacterial activity and improved drug-like properties[17-20](Fig. 1). Further modifications were also explored by replacing the methyloxy linked thiazolo[5,4-b]pyridine on PC190723 with other heteroaryloxy or aryloxy systems as exemplified by compounds I and II (Fig.1)with enhanced pharmacokinetic and improved in vivo efficacy[21, 22].

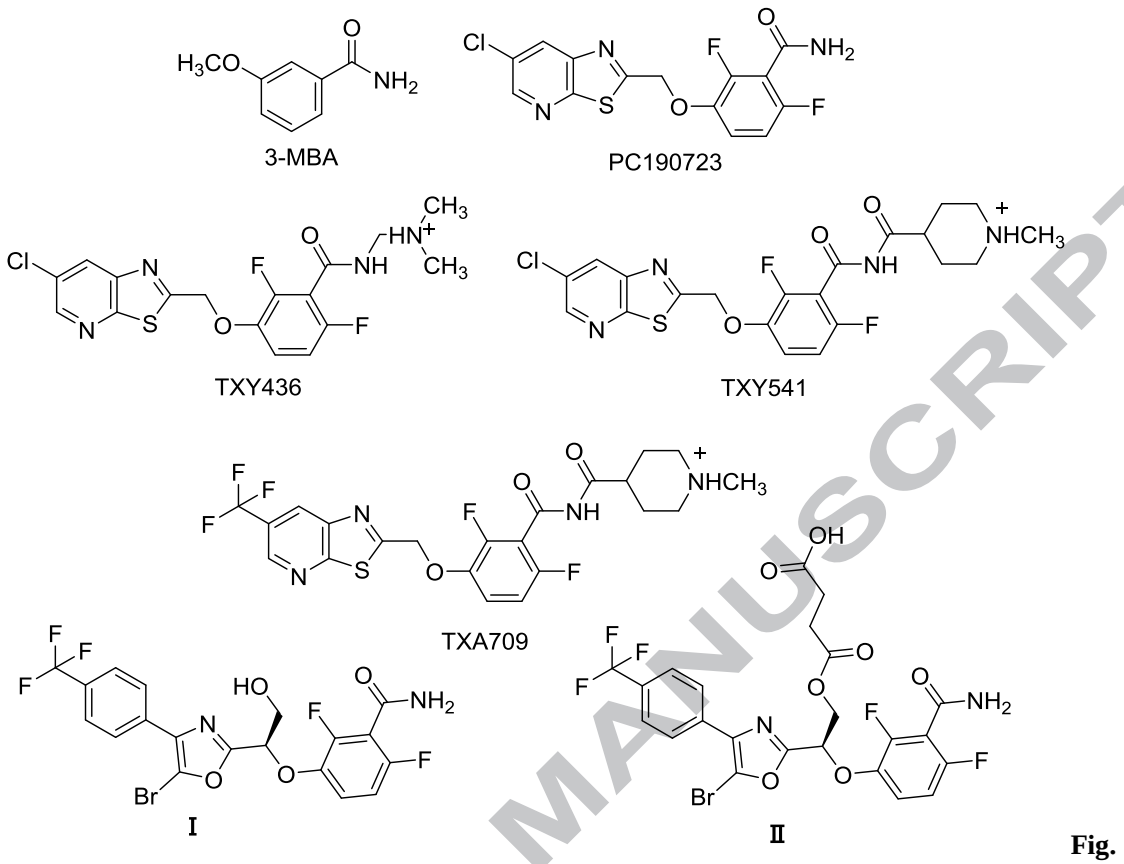


Fig. 1. Chemical Structures of some 3-MBA based FtsZ specific inhibitors

The crystal structure of the *S. aureus* FtsZ-PC190723 complex has demonstrated the mechanism of action of the cell division inhibit and identified the binding site located between the two domains of FtsZ[23]. Several SAR studies demonstrated that benzamide group is critical for the inhibitory activity with little space for the modification, while many reasonably hydrophobic alkyloxy substituents or equivalents linkage on the 3-position of benzamide is helpful for the interaction on the binding site resulting the improvement on the antibacterial activity[15, 21]. On the basis of medicinal chemistry principles and SAR studies of the above findings, a series of new modifications based on PC190723 was considered for the discovery of novel antibacterial agents: a) the amide function as in PC190723 was replaced by the bioisosteric sulphonamide functional group, which is a typical functional group in the

antibacterial sulfa drug used for conditions such as acne and urinary tract infections (Compounds **1** and **2**); b) phenyl functions were introduced at the end of the chain with different oxygen or nitrogen containing linker (Compounds **3**-**22**).

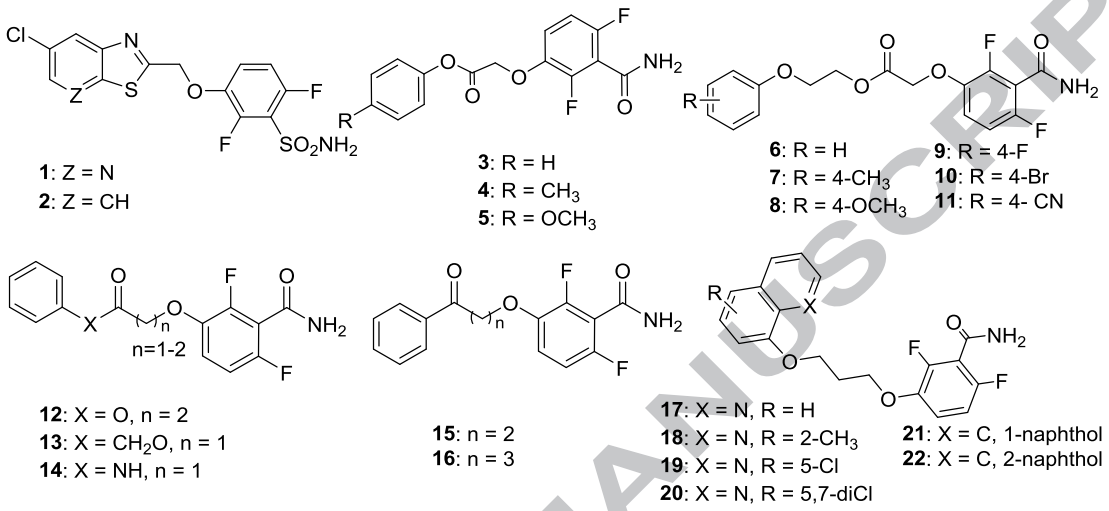


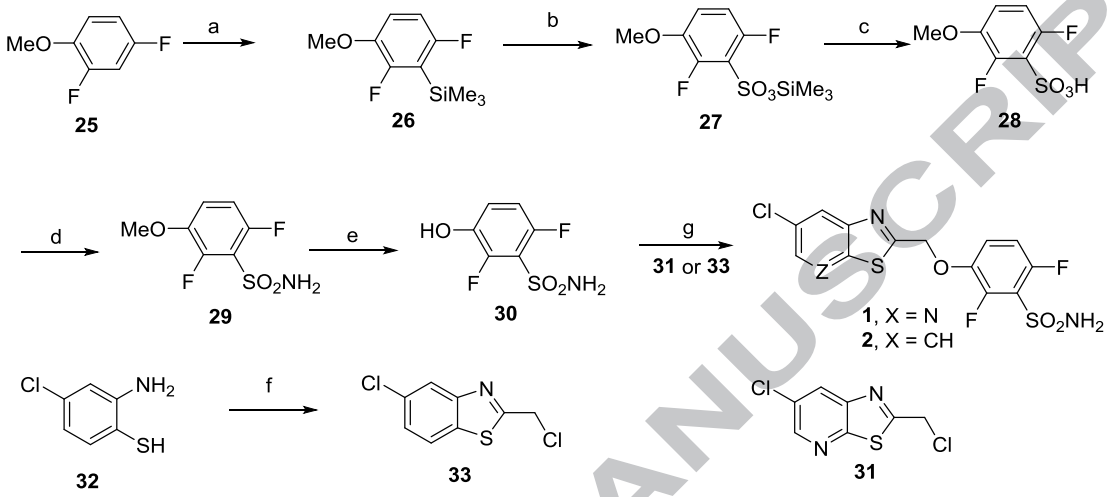
Fig. 2. Structures of 3-MBA and PC190723 analogues

Therefore, in an attempt to design and develop new potential antibacterial agents we report the synthesis and antibacterial activity of compounds **1**-**22**. Our objective was to further explore the effect of the lengths of 3-elongated side chain and various linker group and its bioisosteric version of PC190723 on its antibacterial activity. They were evaluated as antimicrobial agents on the standard bacterial strains using PC190723 as a reference compound to compare their activity with the standard antibacterial drugs.

The synthesis of the bioisosteric version of PC190723 was based on the condensation of 3-hydroxybenzlysuphonamide with 2-halomethyl-thiazolopyridine. This procedure was originally described by Haydon, D. J. et al [24] and further expanded by Sorto, N. A. [25] and our group[26, 27]. Considering these results, our attention was turned to develop a

synthetic approach for the 2,6-difluoro-3-hydroxybenzenesulfonamide (**30**). Since the sulphonamide functional group was on the meta position of the hydroxyl group in the 2,4-difluorophenol, the classical methods involving the direct sulphonation would be ineffective. We resorted to the 3-trimethylsilylated intermediate, which has been successfully used in the functionalisation of the 2 position of 1,3-disubstituted benzenes by para-directing groups[28]. In the synthesis of 3-trimethylsilylated derivative, commercial available 2,4-difluoro-1-methoxybenzene (**25**) was smoothly deprotonated with *sec*-butyllithium at the position flanked by the two fluoro atoms (**Scheme 1**). The deprotonated compound then react with chlorotrimethylsilane to form compound **26** in total of 87.4% yield. The 3-trimethylsilylated compound **26** was then reacted with the freshly prepared trimethylsilyl chlorosulfate to form sulphonic acid **28** after a hydrolysis work-up with sodium bicarbonate in 85.3% yield. The sulphonic acid **28** was further reacted with $PCl_5/POCl_3$ for the chlorination, and subsequently the concentrated ammonia water was added in the system to form the sulphonamide **29**. Treatment of the compound **29** with BBr_3 effected the demethylation to provide the fragment **30** in a high yield. The synthesis of the fragment 6-chloro-2-(chloromethyl)-thiazolo[5,4-b]pyridine (**31**) was completed according to previously described procedures[26, 27]. And the fragment 5-chloro-2-(chloromethyl)benzo[d]thiazole (**33**) was obtained starting from 2-amino-4-chlorobenzenethiol (**32**)[29]. The simultaneously acylation and cyclization between **32** and chloroacetyl chloride under the optimized condition allowed the compound **33** to be efficiently prepared in 51.5 % yield. By using K_2CO_3 as a base in

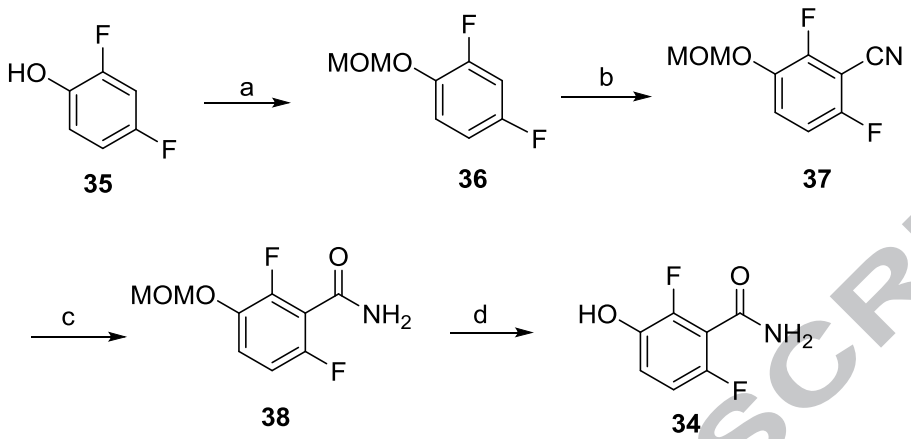
CH_3CN at room temperature, the reaction of the fragment **30** and fragment **31** or **33** catalyzed by the 0.2 eq. of NaI resulted in the formation of **1** or **2** in good yields (**Scheme 1**).



Scheme 1. Reagents and conditions: (a) sec-BuLi, -78 ^{o}C, 1h, Me_3SiCl, -78 ^{o}C, 1h, dry THF; (b) $ClSO_3SiMe_3$, CCl_4, reflux; (c) $NaHCO_3$, H_2O, HCl; (d) $POCl_3$, PCl_5, NH_4OH; (e) BBr_3, CH_2Cl_2; (f) $ClCOCH_2Cl$, Et_3N, toluene; (g) NaI, K_2CO_3, CH_3CN.

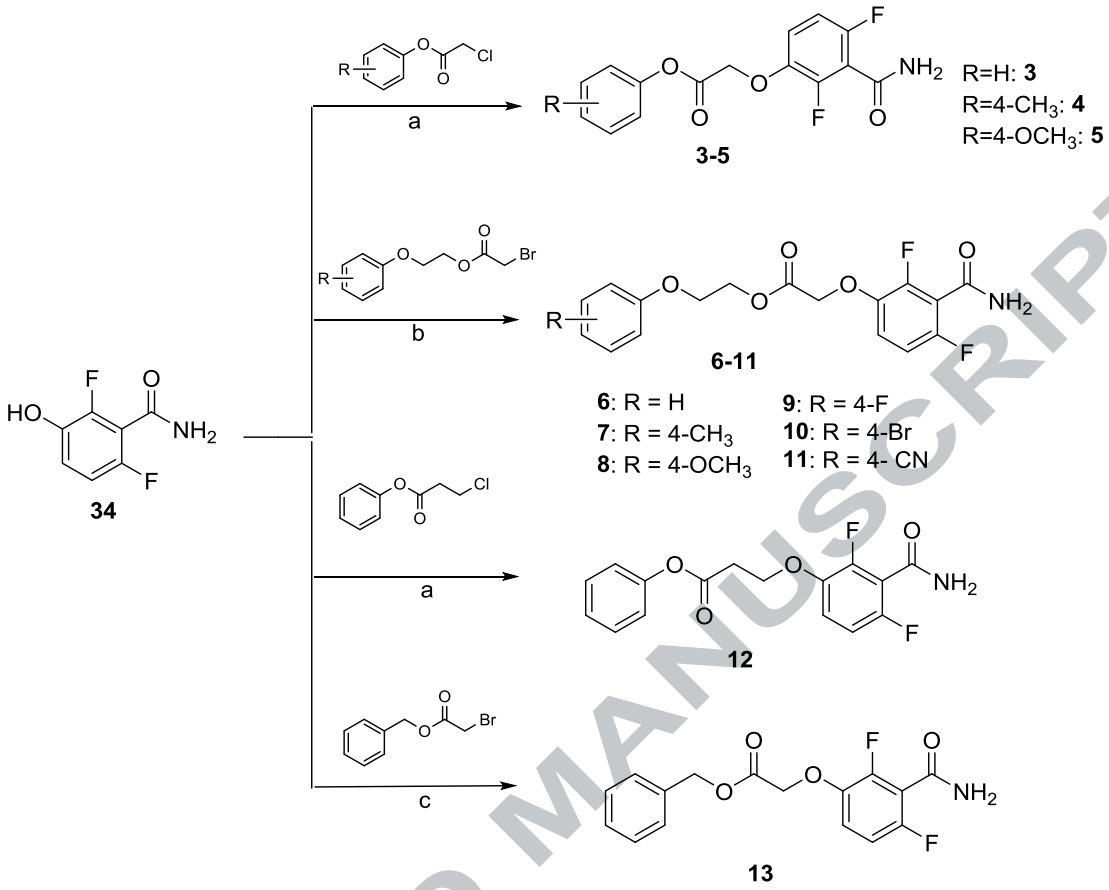
The synthesis of compounds **3-22** was based on the connection from the 2,6-difluoro-3-hydroxybenzamide fragment **34**. As reported in our or others literatures, the 2,6-difluoro-3-hydroxybenzamide (**34**) could be introduced as aldehyde, ester, or carboxylic acid [25, 26, 30]. Since the cyanide functional group was also a common source for the preparation of amide, we began to use the commercially available 2,4-difluoro-phenol (**35**) as the starting material to introduce cyanide functional group for the attempt (**Scheme 2**). Protection of 2,4-difluorophenol with chloromethyl methyl ether followed by regioselective lithiation with s-BuLi, the corresponding aromatic nitrile **37** was then formed by treatment with DMF and $POCl_3$, followed by the reaction with molecular iodine in aq NH_3 [31, 32]. The benzonitrile **37** was converted to amide **38** in aqueous alkaline medium accelerating by hydrogen peroxide. Treatment of compound **38** with methanolic HCl effected deprotection to provide

2,6-difluoro-3-hydroxybenzamide fragment **34** in a high yield.



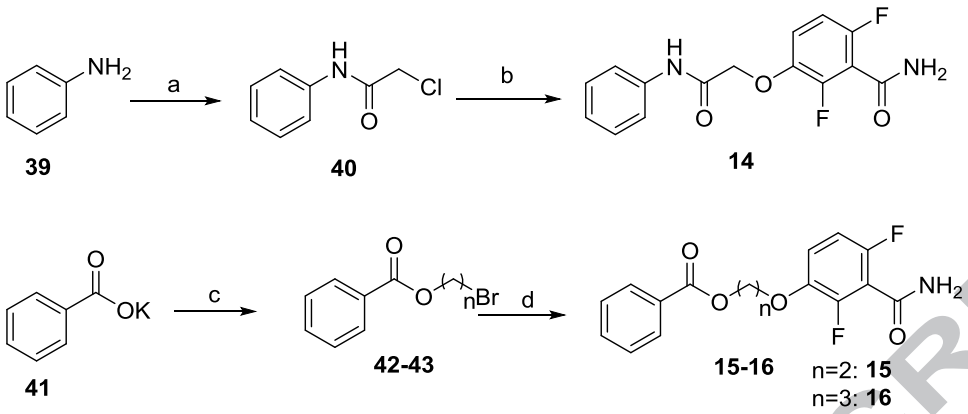
Scheme 2. Reagents and conditions: (a) $ClCH_2OCH_3$, DIPEA, CH_2Cl_2, rt, 2h. (b) ⅰ) *s*-BuLi, anhy THF, -78℃, 2h; ⅱ) DMF, 0℃, 2h; ⅲ) NH_3 (28%), I_2, rt, 3h. (c) NaOH (10%), H_2O_2 (30%), rt, 3h. (d) CH_3OH-6 M HCl, rt, 2h.

Compounds **3-13** were then obtained by the O-alkylation of **34** with prepared substituted phenyl 2-chloroacetate，2-substitutedphenoxyethyl 2-bromoacetate, phenyl 3-chloropropanoate, or benzyl bromoacetate (**Scheme 3**). Substituted phenyl 2-chloroacetates were synthesized from acylation on various phenols with chloroacetyl chloride. The above used intermediates 2-substitutedphenoxyethyl 2-bromoacetates were synthesized from phenols, which were elongated with 2-bromoethanol in aqueous medium to alcoholic derivatives, and then acylated with bromoacetyl bromide to the bromoacetate intermediates. The above used phenyl 3-chloropropanoate was synthesized from the acylation with phenol using 3-chloropropionyl chloride. The followed alkylation with **34** gave compound **12**, while the O-alkylation of **34** with commercial available benzyl bromoacetate formed compound **13** (**Scheme 3**).



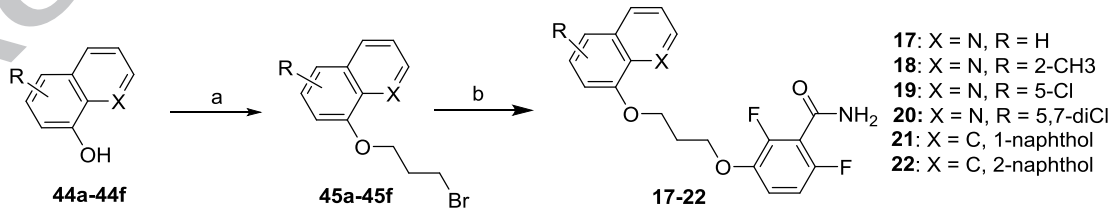
Scheme 3. Reagents and conditions: (a) NaI, K_2CO_3, DMF, rt, 24h. (b) K_2CO_3, rt, 12-24h. (c) K_2CO_3, DMF, rt, 12h

Aniline **39** was acylated with chloroacetyl chloride and was followed by the reaction with 2,6-difluoro-3-hydroxybenzamide (**34**), providing the nitrogen containing compound **14**. Compounds **15** and **16** were prepared starting from potassium benzoate **41**, which was reacted with dibromoalkane (1,2-dibromoethane and 1,3-dibromopropane, respectively) to give mono-bromoalkane **42-43**. The subsequent O-alkylation was achieved through the reaction between the **41** or **42** with of 2,6-difluoro-3-hydroxybenzamide (**34**) to form the title compounds **15** and **16** (**Scheme 4**).



Scheme 4. Reagents and conditions: (a) chloroacetyl chloride, Et_3N, CH_2Cl_2, rt, 1h. (b) **34**, NaI, K_2CO_3, DMF, rt, 12h; (c) 1,2-dibromoethane or 1,3-dibromopropane, CH_3CN, reflux, 12h. (d) **34**, K_2CO_3, DMF, rt, 24h.

The bicyclic aromatic tailing derivatives **17-22** were synthesized from 8-hydroxyquinoline derivatives or 1- or 2-naphthol as outlined in **Scheme 5.** The substituted 8-hydroxyquinoline or 1- or 2-naphthol **44a-44f** were alkylated with 1,3-dibromopropane in the presence of sodium hydroxide aqueous solution and tetrabutylammonium iodide (TBAI) in dichloromethane to afford mono-halogenated intermediates **45a-45f.** Then the target compounds **17-22** were obtained via the alkylation of compound **34** with the corresponding alkyl bromides.



Scheme 5. Reagents and conditions: (a) NaOH, TBAI, CH_2Cl_2, rt, 24h; (b) **34**, K_2CO_3, DMF, rt, 24h.

Compound **1-22** were screened at the loading of 100 μg per disc against a panel of

bacteria (*Staphylococcus aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 9027, *Mycobacterium smegmatis* ATCC 607, *Bacillus subtilis* ATCC 6633, *Klebsiella aerogenes* ATCC 9621, *Escherichia coli* ATCC 25922) and two fungus (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) using paper disc diffusion method[33], and compared with PC190723 tested in the same *in vitro* model. The zone ratios which were used to express the comparative efficiency to the standard drugs are summarized in the Table 1. In general, no inhibitory activity against gram negative bacteria and fungus was observed in the current study. Only the gram positive bacteria *S. aureus*, *B.subtilis*, *M. smegmatis* were sensitive to most of the tested compounds. Compounds demonstrated relatively weaker inhibitory activity against *S. aureus* and *B.subtilis* than positive control cephaloridine with the zone ratio < 1，and none of these compounds showed superiority to PC190728 which demonstrated about equal potency to the cephaloridine (zone ratio = 1.2). These result was consistency with other reports on the PC190723 and 3-MBA analogs, and PC190723 was report to only show the significant efficacy on *Staphylococcus aureus*, including MRSA and MDRSA, but no efficacy on *Escherichia coli*[15, 24].

Table 1. Antimicrobial activity of compounds presented as ratios of zone of inhibition for the test compounds

Compd	Zone Ratio[a,c] (100μg)		
	S. aureus	*B.subtilis*	*M.smegmatis*
1	-[d]	-	-
2	-	-	-
3	0.07	0.19	0.50
4	0.25	0.3	0.07
5	-	0.15	0.19
6	0.18	0.18	0.36
7	0.10	0.25	-
8	-	0.51	0.37

9	0.44	0.18	0.62
10	0.22	0.15	-
11	-	0.11	-
12	0.41	0.3	
13	-	0.18	-
14	0.10	0.10	-
15	-	-	-
16	-	-	-
17	0.07	-	-
18	-	-	-
19	0.25	0.1	-
20	0.18	0.1	0.2
21	-	-	-
22	-	-	-
PC190723	1.2	-	-

[a] $\text{zone ratio} = \frac{\text{inhibition zone of test compound}}{\text{inhibition zone of standard}}$

[b]standard drugs for the calculation: *S.aureus*: cephaloridine disk, *B.subtilis:* Tetracycline, *M.smegmatis*: Streptomycin disk;

[c]The results are averages of three separate experiments

[d] '-'= not active,

In compounds **1** and **2**, the crucial amide functional group in PC190723 was replaced with the bioisosteric sulphonamide functional group, and the rest of the main structure feature was maintained. However, the results show that such a modification is not tolerated. Replacement of thiazolo[5,4-b]pyridine functional with phenyl ring and using an ester linger to connect both ring system derived compounds **3-13.** Compounds **3-5**, which have the ester group directly connected with phenyl ring, showed weak activities on *S. aureus* and *B.subtilis.* Introducing an ethylene linker between the phenyl ring and ester group as in compounds **6-11** only marginally increased the antibacterial activity. Other variations including inserting a methylene either between the ester and phenyl ring or between the ester and alkyloxy group as exampled by compounds **12** and **13,** or replacing the oxygen with amine as in

compound **14**, were deleterious on the antibacterial activity. Ketone analogues showed no activity as evidenced by compounds **15** and **16**. Similarly, replacement with the benzo-condensed bicyclic ring system was unproductive as evidenced by compounds **17-22**. Compounds **17-20** showed slightly activities against a wider spectrum of microorganism, like gram-positive, gram-negative bacterium, even fungus, although the activity was inferior to the standard drugs. Compounds **21-22** showed no efficacy on the screening. It indicates that nitrogen in the ring system may increase polarity and water-solubility of the compounds, and then is beneficial for the antibacterial activity. Among all the tested compounds, compound **9** with a fluorine substituted on the phenyl ring, showed best antibacterial activity in the series against *M. smegmatis* with the zone ratio of 0.62, and against *S. aureus* with the zone ration of 0.44.

A series compounds bearing phenyl or quinolyl side chain of 3-alkyloxy-2,6-difluorobenzamide were designed and synthesised in order to identify new potent bactericidal agents. Most of tested compounds showed activities on Gram-positive bacterium, Compound **9** apparently have better inhibitory activity against *S. aureus* and *M. smegmatis*. Herein these results suggest that based on the unique 3-MBA pharmacophore, compound **9** may represent promising lead candidates against gram positive bacteria that are worthy of further investigation. Halogen atom and polarity of the side chain may improve the antibacterial activity and broad-spectrum properties.

Acknowledgment

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Graphical Abstract

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