ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2024, Vol. 40, No.(5):
Pg. 1250-1260

An Exquisite Design Process and Impacting Synthesis of 2-methyl Nicotinamide Derivatives and their Anti-bacterial Activity Closet to Fab I Inhibitors

S. RAGHUNADHACHARYULU[1,2], N. SRINIVASU[1]*, SIVARANJANI JAMPALA[2] and ARUNDHUTHI. M[2]

[1]Department of Chemistry, School of Applied Sciences & Humanities, Vignan's Foundation for Science, Technology & Research, Deemed University, Vadlamudi, Guntur - 522 213, Andhra Pradesh, India.
[2]Aurigene Pharmaceutical Sciences Pvt Ltd, Hyderabad, Telangana, India.
*Corresponding author E-mail: navulurisrinivasu@gmail.com

http://dx.doi.org/10.13005/ojc/400506

(Received: August 13, 2024; Accepted: October 15, 2024)

ABSTRACT

Synthesis of "5-(2,5-difluoro-4-((4-methylpiperazin-1-yl) methyl) phenyl)-N-(2-methoxybenzyl)-2-ethyl nicotinamide and 2-fluoro-4-(6-fluoro pyridine-2-yl)-6-(4-methyl piperazine-1-yl)" benzaldehyde derivatives has been developed using the 4–bromo–2,5 –difluoro benzaldehyde and 4–bromo–2,6–diflouro benzaldehyde and further this chemical to prepare the various novel derivatives. Synthesized compounds have been characterized using FTIR, ^{1}H-NMR, ^{13}CNMR etc. Such developed molecules are novel, cost-effective, and can be prepared by industrially viable methods. As a result of the fewer reaction steps, the high yield, and the purity of the organic chemical generated, the procedure described is less strenuous. Compared to earlier synthetic approaches, the newly discovered route is thought to be the most efficient and shortest. The established method may make it easier to prepare a variety of important intermediates and active medicinal compounds. The versatility of this work is the same reagent Titanium isopropoxide was used for both reductive aminations and SNAr couplings. The primary goal of this endeavor is to create novel compounds based on Fab I inhibitor analogs and assess their antibacterial efficacy. The produced substances were examined on "*Gram-positive* bacteria (*S.aureus, B.subtilis*)and also on *Gram-negative* bacteria (*E. coli*, *P.aeruginosa*)." Among all the compounds examined, the nicotinamide derivative 9B showed the MIC 32 (g/mL) against *Staphylococcus aureus* and also on *B.subtilis*. The derivatives 9C and 9D also haveanti-bacterial resistance at 64 (g/mL) on *Gram-positive* bacteria. The aldehyde derivatives 13C and 13D had bacterial resistance at MIC 32 (g/mL) against *Staphylococcus aureus* and also on *B.subtilis*. The docking studies of the synthesized molecules were also examined on the 7ap6 enzyme. The synthesized molecules are very well fit into the enzyme and they have better binding energy than the standard molecules triclosan and MUTO56399.

Keywords: Fab inhibitors,Triclosan, *Staphylococcus aureus*, Nicotanamide, Piperazine.

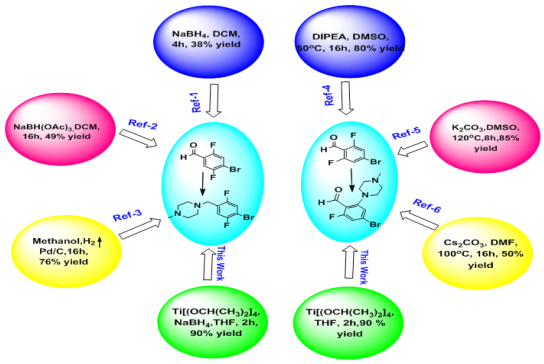


Fig. 1. Various synthetic strategies for the generation of 2,5-difluoro benzaldehyde & 2,6-difluoro benzaldehydes via reductive amination (previous and current)

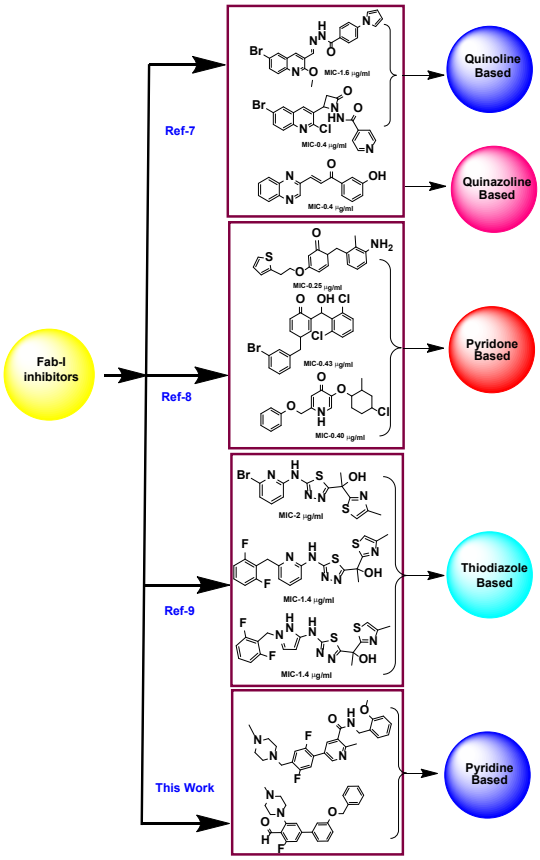


Fig. 2. Schematic representation of various functional based novel molecules showing activity against Fab I

INTRODUCTION

Over the past decade, thousands of infectious diseases[1,2] have been reported around the world, making the development of anti-infective chemotherapeutics more important than ever before. Currently, drug discovery is developing inhibitors of Fab I[3]. The NADH enoyl reductases are manufactured by bacteria using the TYPE-II fatty acid biosynthesis pathway (FAS-II). Bacterial fatty acid biosynthesis (FAS) enzymes are more desirable anti-microbial targets because they generate phospholipid membrane precursors, which are essential for the survival of both *Gram-positive* and *Gram-negative* bacteria. Furthermore, there is an inadequate amount of homology and basic structural differences between the FAS-I system in mammals and the FAS-II method in bacteria. Antibacterial drugs including triclosan, isoniazid, and diazaborines aim their inhibitory efforts at NAD(P)H-dependent enoyl-ACP reductase (ENR), the enzyme responsible for the last stage of the FAS-II elongation cycle.

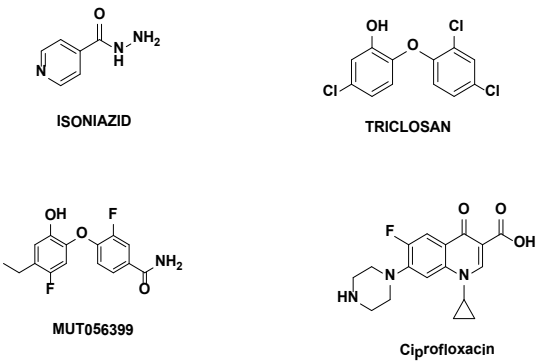


Fig. 3. The well-known FabI inhibitors and anti-infective drugs available in the literature[17]

An established method for inhibiting the biosynthetic process of type II fatty acids produced by bacteria, Fab-I inhibitors are NADH-dependent enoyl reductases (FAS-II). However, it is the underexploited target for drug discovery[18].

On the available literature and the works done in medicinal chemistry and crystallographic studies of Fab K from *Streptococcus pneumoniae*, Kitagawa *et al.,*[19] explored the dual inhibitory properties of phenyl imidazole derivatives of 4-pyridone[20,21]. Fab K showed good activity against *S. pneumoniae*. Additionally, the obtained material did not show any significant cytotoxicity (IC_{50}>69 µM). In the process of obtaining better outcomes, a novel agent is used for treating bacterial infections and the results of the research support the development of the scaffolds.

According to Yong-Mei Zhang *et al.,* vital steps in the production of bacterial membrane lipids should be reviewed. When it comes to producing

cellular membrane lipids, dissociated type II fatty acid synthase is the most energetic component. Controlling the regulatory biochemistry that dictates the speed of cellular lipids, the module of initiation contains the enzymes needed to commence creation of type II fatty acid. According to a 2014 study by Ben C. L. van Schaijk *et al.,* falciparum sporozoites must undergo Type II Fatty Acid Biosynthesis in the midguts of Anopheles mosquitoes. These results demonstrate that the FAS-II pathway is necessary for *P. berghei* and *P. yoelii* parasite development in the liver stage of rodents but is not necessary for oocyst formation in the midguts of Anopheles mosquitoes. Mycobacterium TB resistance mechanisms linked to suppression of type II fatty acid synthase were described by Grzegorzewicz *et al.,* Inhibiting FAS-II dehydratase, an enzyme necessary for mycolic acid production, isoxyl (ISO) and thiacetazone (TAC) kill *Mycobacterium tuberculosis* (Mtb). Many more instant mutations are associated with high-end resilience to ISO and TAC preventing either their active form from being converted or their target from being covalently modified[33-34]. The molecular mechanisms responsible for M.tb spreads strains around are related to mutations in stage II and the non-essential FAS-II dehydratase HadBC, are still unknown[35]. The development of NADH enoyl reductase inhibitors using type-II biosynthetic bacterial fatty acid routes occurs in its infancy[36–38]. Therefore, it is necessary to develop bio-synthetic pathway technologies to discover new key interventions that inhibit a variety of diseases that affect humans.

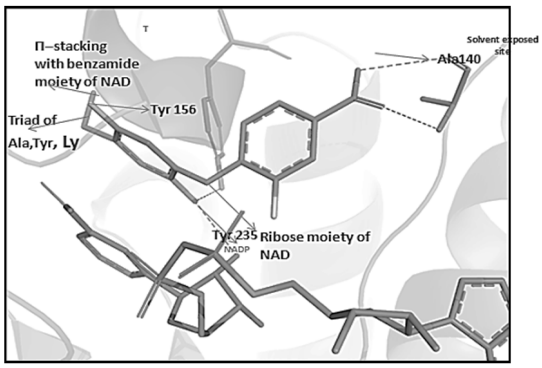


Fig. 4. Binding mode of MUT056399 with enzyme

Based on the previous literature of Fab I inhibitors there should be some important binding centers to the enzyme. The ethereal oxygen was binding with the ribose moiety of NADPH. The LHS halogen atom is bound with Tyr 156. The LHS alkyl chain is bound tothe triad of Ala, Tyr, and Ly as well and π-stacking with the benzamide moiety of NADPH cofactor. The RHS amide part is combined with Ala 140[39-40].

A fluoroquinolone antibiotic called ciprofloxacin is used to treat a variety of infections. Bone and joint problems, infections of the urinary and respiratory systems, skin infections, typhoid fever, and infectious diarrhea are all included in this.The MIC of developed compounds was computed using this as a reference compound.

In this work, the prepared molecules were designed as per the structure of previous Fab I inhibitors and with essential binding sites like piperazine moiety as present in ciprofloxacin[41–42]. It is also noted that One of the most sought-after heterocyclics to develop novel pharmaceutical components with a wide range of applications is piperazine[43–44]. Many molecules emerge with variousbioactivities, like antitumor, antibacterial, anti-inflammatory, antioxidant, and other activities.

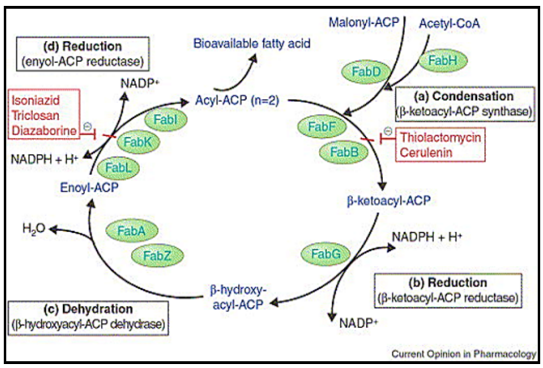
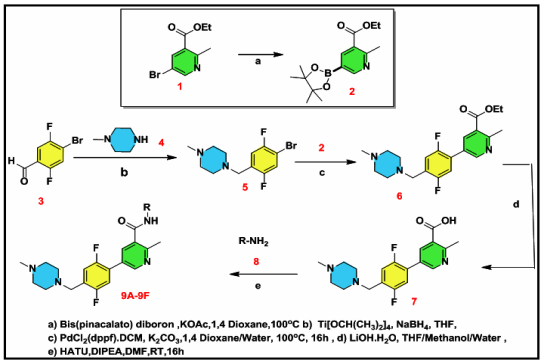


Fig. 5. The biosynthetic pathway of Fab I inhibitors

MATERIALS AND METHODS

All the solvents and the reagents were purchased from Aldrich, BLD Pharma, Rankem India, and Spectrochem. Merck alumina plates coated with silica gel 60 F254 were used for thin-layer chromatography. 5% Methanol/DCM was used as eluent for the spot visibility achieved through UV or iodine vapor exposure. The purification of crude compounds was done on silica gel using a comb flash. Fisher-Johns melting point apparatus was used to determine the melting points in open capillaries.^{1}HNMR and ^{13}C NMR spectra were recorded using JEOL 600 MHz and 100 MHz systems. $CDCl_3$ was used as solvent with TMS as

the internal standard. FT-IR-8400S instrument with KBr pelletswas used to record the FT-IR spectra. HRMS spectra of the samples were recorded on the Waters TQF system.The results of the FT-IR, HRMS, ^{1}HNMR, and ^{13}C NMR spectra were represented and interpreted in the supplementary section.



Scheme. 1

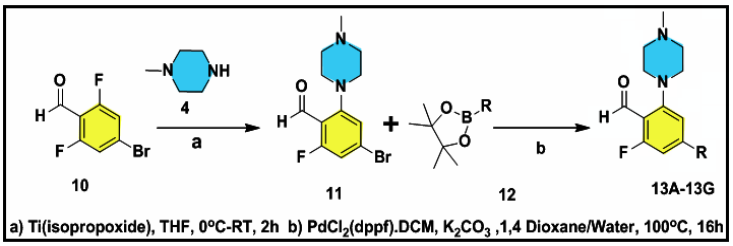
The experimental section of Scheme. 1 was performed with intermediate 5 synthesis by treating n-methyl piperazine with 4-bromo-2,5 -difluoro benzaldehyde via reductive amination in the presence of Titanium isopropoxide. This step was tried with different reducing agents and with different solvents which are listed in Table 1.

Various reducing agents, protic,and aprotic solvents were used for the reductive amination of intermediate **5**. At the beginning, the schematic reaction was conducted by using sodium borohydride as a reducing agent and dichloro methane (acting as aprotic solvent), methanol (protic solvent) under room temperature yielded 39% in 16 hours. But this step was successfully done with $Ti[OCH(CH_3)_2]_4$ in the presence of THF as solvent within 4 hours. And it is very affordable. Then intermediate **6** was successfully prepared by making a Suzuki reaction with bromo intermediate **5** on a reaction with pyridine boronated esters **2**. The final amides 9A-9E have been prepared with different amines in moderate to high yields in the presence of a coupling reagent HATU.

Table 1: Screening of reducing agents for reductive amination for the preparation of intermediate (5) in Scheme 1

Sr. No	Reducing agent	Solvents	Volume	Time(h)	Temprature^{0}C	Yield(%) Intermediate(5)
1	$NaBH_4$	DCM	20.0	16	25-30	39 %
2	$NaHB(OAc)_3$	DCM	20.0	16	25-30	48 %
3	H_2/Pd	Methanol	10.0	16	25-30	30 %
4	$NaCNBH_3$	Methanol	10.0	4	25-30	40 %
5	$Ti\{OCH(CH_3)_2\}_4$,$NaBH_4$	THF	20.0	4	25-30	90 %

Note: Volume* = Weight of input (W) x Solvent ratio (R) (Ex: 1.0 g Compound in 20 mL of Solvent)



Scheme. 2

Initially, reductive amination of intermediate **10** was tried with n-methyl piperazine in the presence of titanium isopropoxide. But surprisingly SNAr coupling happened with the replacement of fluorine. This is the novelty of the present work. According to the literature, titanium isopropoxide would be used as a catalyst for the asymmetric allylation of ketones, the diastereoselective reduction of alpha flour ketones, and the production of acyclic and allylic epoxy alcoholsand also for intra molecular formal [3+2] cyclo addition. So intermediate **11** was obtained through SNAr coupling of n-methyl piperazine to intermediate **10** inthepresence of titanium isopropoxide at room temperature. After that different boranate esters were attached to intermediate **11** via Suzuki coupling to prepare the compounds 13A-13G. This route is very short, affordable, and can be utilized to synthesize various biologically active molecules.

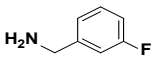
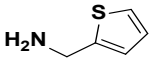
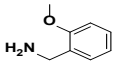
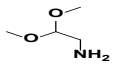
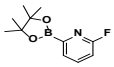
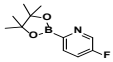
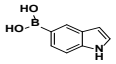
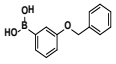
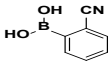
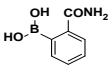
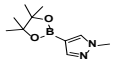
RESULTS AND DISCUSSION

Novel molecules were prepared with novel synthetic routes. The synthetic route proposed was affordable, cost-effective, and well-yielded. The antibacterial activity of the synthesized molecules was established on two types of bacteria.

In vitro antibacterial testing

The synthesized two series of molecules were evaluated on "two *Gram-positive* bacteria (*S. aureus*, *B. subtilis*) and also on two *Gram-negative* bacteria (*E. coli*, *P. aeruginosa*)." The compounds **9A-9F & 13A-13G** were tested in DMSO at 8, 16, 32, 64, 128 & 256 µg/mL concentrations and their MICs were evaluated. Compound 9B has shown the MIC at 32 µg/mL. The compounds 9C and 9D are shown the MIC at 64 µg/mL, against both *Gram-positive* bacteria. At 32 µg/mL, the aldehyde derivatives 13C and 13D demonstrated resistance to the Gram-positive bacteria *S. aureus* and *B. subtilis*.

Table 2: MIC results of synthesized molecules

Compounds (Scheme 1)	Different amines (R)	MIC (µg/mL)			
		S.aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
9A		128	128	256	256
9B		32	32	256	256
9C		64	64	256	256
9D		64	64	256	256
9E		128	128	256	256
9F		128	128	256	256
Compounds (Scheme 2)	Different boranateesters & boronic acids (R)	MIC (µg/mL)			
		S.aureus	*B. subtilis*	*E. coli*,	*P. aeruginosa*
13A		128	128	256	256
13B		128	128	256	256
13C		32	32	256	256
13D		32	32	256	256
13E		256	256	256	256
13F		64	64	256	256
13G		256	256	256	256
Reference Compounds	Triclosan	0.025	1.5	32	1
	MUT056399	0.06	64	32	64

Docking studies

The molecular docking for the compounds with the highest MIC values was corroborated theoretically using molecular docking studies.

The protein used for the docking of molecules is 7ap6. The software used for the molecular docking is AutodockVina 1.5.7. As shown in Table 3, the result shows that the synthesized novel molecules showedhigher binding energy than the standard drug Trichlosan. In the docking picture, the dotted lines indicate the hydrogen bonding donor and acceptors. The various types of interactions were mentioned at the bottom of the picture in the square box. The number of hydrogen bonds that the molecule's protein forms (9B) is 8 with a binding energy of -7.67 kcal/mol, which is more in comparison with the standard molecule Triclosan. Molecule 13 (D) is also the other best molecule with a binding energy of -8.43 kcal/mol and best fitsinto the protein pocket by binding with 10 hydrogen bonds.This is the best molecule among the synthesized molecules. The benzyloxy group is responsible for additional hydrogen bond formation with the protein.

Table 3: Docking studies of the synthesized molecules with protein

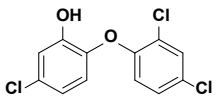
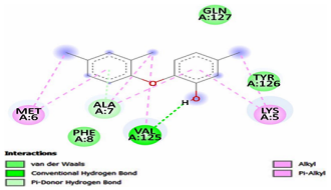
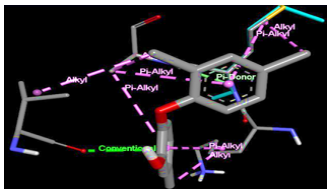
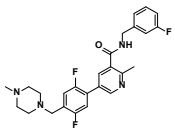
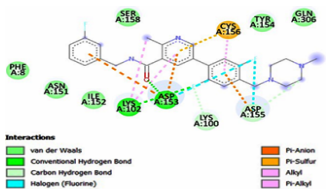
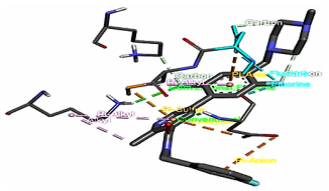
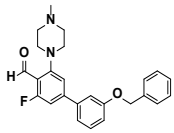
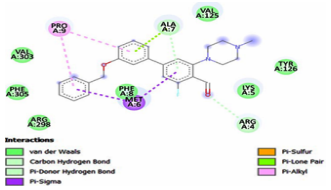
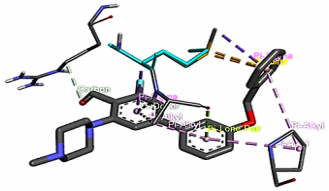
Structure	2D Structure of the molecule with protein	3D Structure of the molecule with protein
Triclosan  Binding Energy = -6.69 kcal/mol		
9(B)  Binding Energy = -7.67 kcal/mol		
13(D)  Binding Energy = -8..44 kcal/mol		

Table 3 illustrates the interaction between compounds 9B & 13Dwith the target enzyme. The Ligand-Protein complex's 2D structure is displayed in the second column, its 2D structure is displayed in the first column, and its 3D structure is displayed in the third column. The binding active residues in the 2D complex are denoted by three-letter codes, whilst the docked compounds are shown as a grey stick color. Yellow lines represent the π-stacking interactions while pink dotted lines represent the Hydrogen Bond (HB) interactions. The 3D representation shows the docked compounds as cyan stick models and the binding active residues as grey stick models. The figure displays interactions with HB on the left side and π-stacking on the right side, shown by the dotted lines, respectively.

General Procedure

Scheme 1. Procedure for the Synthesis of 1-(4-Bromo-2,5-diflourobenzyl)-4-methyl piperazine (5)

The reaction was initiated by mixing 4-bromo-2,5 -difluoro benzaldehyde (5 g, 22,624 mmol, 1eq.), N-methyl piperazine (2.26 g, 22,624 mmol, 1eq.) in THF (100 mL, 20 vol.) to 0°C, which was followed by adding titanium isopropoxide (13.72 mL, 45.248 mmoL). One hour was spent stirring the reaction mixture at room temperature. A further hour of stirring at RT was continued by the addition of $NaBH_4$ (0.85 g, 22.624 mmol,1 eq.). Filtering the reaction blend through a celite bed was the next step. Researchers diluted the celite bed filtrate with “ethyl acetate” and then cleaned it with water, saturated with brine, evaporated over Na_2SO_4, and then condensed at a minimised compression. A light-yellow solid (3.25 g, 90% yield) was obtained

from the crude material using silica gel. LC–MS: m/z 306.16 [M+2].

Procedure for the Synthesis of Ethyl 5-(2,5-diflouro-4-((4-methyl piperazine-1-yl) methyl) phenyl)-phenyl)-2-methyl nicotinate (6)

The compound 1-(4-Bromo-2,5-diflouro benzyl)-4-methyl piperazine (3 g, 9.830 mmol, 1 equivalent) was dissolved in a mixture of 1,4 dioxane/ water (4:1).To this solution, ethyl 2-methyl-5-(4,4,5,5-tetra methyl-1,3,2-dioxaboralan-2-yl) nicotinate (3.43, 11.796 mmol, 1.2 equivalents) and K_2CO_3 (2.71 g,19.66 mmol, 2 equivalents) were added.

The reaction mixture was purged with N_2 gas for 5 min to remove any dissolved gases, and then Pd_2Cl_2(dppf) was added. The amount of dichloromethane adduct used was 1.80 grammes, which corresponds to 0.983 millimoles and is equivalent to 0.1 equivalents. Upon reaching a temperature of 100°C, the mixture was kept at that temperature for duration of 16 hours. TLC oversaw the conclusion of the retreat. A light brown semi-solid, consisting of ethyl "5-(2,5-difluoro-4-((4-methyl piperazine-1-yl) methyl)-phenyl)-2-methyl nicotinate", was produced with a yield of 84% (3.20 g). The resulting product was concentrated under decreased pressure after being diluted in ethyl acetate, cleaned with water and brine solution, and evaporated with Na_2SO_4. Liquid chromatography-mass spectrometry: mass-to-charge ratio of 390.16 with a molecular ion peak at [M+1].

Procedure for the Synthesis of Ethyl 5-(2,5-diflouro-4-((4-methyl piperazine-1-yl) methyl) phenyl)-phenyl)-2-methyl nicotinic acid (7)

A solution of ethyl "5- (2,5-difluoro-4-[(4-methyl piperazine-1-yl)methyl)phenyl)-2-methyl nicotinate" (3.20 g, 8.221 mmol, 1 equivalent) in a mixture of tetrahydrofuran (THF), methanol, and water (20 mL THF, 5 mL methanol, and 5 mL water) was adjusted to room temperature before adding lithium hydroxide monohydrate ($LiOH.H_2O$). The mixture is swirled continuously for over 16 hours. Thin-layer chromatography (TLC) was used to monitor the advancement of the reaction. Citric acid was used as an acidifier to counterbalance the alkalinity of the organic solvent. The system is kept at reduced pressure, and the resulting solids are filtered and dried to produce the compound ethyl "5-(2,5-difluoro-4-((4-methyl piperazine-1-yl) methyl)-phenyl)-2-methyl nicotinic" acid was obtained with a yield of 2.80 grammes, which corresponds to a 94% yield.

^{1}H NMR (400 MHz, DMSO-d_6): 8.76 (s, 1H), 8.28 (s, 1H), 7.51-7.49 (m,1H)7.42-7.38 (m.1H),3.67 (s,2H),3.19 (s,3H),3.06-2.60 (m,8H),2.58 (s,3H). LC–MS: m/z 362.33 [M+1].

General procedure for the synthesis of Ethyl 5-(2,5-diflouro-4-((4-methyl piperazine-1-yl) methyl) phenyl)-phenyl)-2-methyl nicotinamide derivatives (9A-9F)

"A solution of ethyl 5-(2,5-difluoro-4-((4-methyl piperazine-1-yl) methyl)-phenyl)-2-methyl nicotinic acid(7) (0.1 g, 0.28 mmol, 1 eq.) was combined with a solution of DMF (10 mL), along with DIPEA (0.09 mL, 0.552 mmol, 2 eq.) and HATU (0.12 g, 0.331 mmol, 1.2 eq.)." The 2-methoxy benzyl amine (0.037 g, 0.276 mmol, 1 eq.) was added. The reaction mixture was once again agitated for duration of sixteen hours at the ambient temperature. The TLC measuring instrument was used to ascertain the completeness of the reaction. After being diluted with distilled water, the recovered mixtures were subjected to ethyl acetate extraction. The procedure included a freshwater rinse, immersion in a brine solution, drying with Na_2SO_4, and concentration at reduced pressure. The crude chemical was refined using silica gel as the stationary phase and a 5% Methanol/DCM mixture as the luent. This process resulted in the isolation of "(5-(2,5-diflouro-4-(4-methyl piperazin-1-yl) methyl) phenyl) -N-(2 -methoxybenzyl) -2-methyl nicotinamide (9D)" as a light brown solid, with a yield of 0.1 g (75%).

m.p.: 322-324[4c]; FT-IR (KBr): 1673.48 cm^{-1} -C=O stretching, 3125.35 cm^{-1}: -N-H stretching; ^{1}H NMR (400 MHz, DMSO-d_6): 8.80 (s, 1H), 8.79 (s, 1H), 7.58-7.56 (m,1H),7.29-7.26 (m.1H) ,7.27-6.94 (m, 4H), 4.44-4.42 (m,2H), 3.80 (s, 3H), 3.54-3.48 (m, 2H), 2.58 (s, 3H), 2.44–2.30 (m, 8H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.38, 156.20, 148.87, 135.00, 131.62, 128.23, 127.83, 127.11, 126.85, 126.32, 124.47, 120.19, 118.39, 118.13, 116,.92, 110.52, 55.32 54.66, 52.37, 45.73,40.13, 39.92, 39.08, 38.87 & 37.80. HRMS found: ESI m/z 481.21918 $(M+H)^+$, [M.F: $C_{27}H_{30}F_2N_4O_2$, requires 480.23]

Scheme 2

Procedure for the synthesis of 4-bromo-2-flouro-6-(4-methylpiperazine-1-yl) benzaldehyde (11)

During the experiment, titanium isopropoxide (13.72 mL, 45.248 mmol, 2 eq.) was added to the solution of 4-bromo-2, 6-diflouro benzaldehyde (5 g, 22.624 mmol, 1 eq.) and N-methyl piperazine (4) (2.2 g, 22.624mmol, 1eq) in THF. For two hours, the reaction mixture was stirred constantly at room temperature. Monitoring of the reaction was carried out using TLC. Afterward, an ammonium hydroxide solution was utilized to quench the reaction. The celite bed is used to filter the solid. After cleaning in the brine solution, it was dried using Na_2SO_4 and concentrated under reduced pressure. As a yellow liquid, 4-bromo-2-fluoro-6-(4-methylpiperazine-1-yl) benzaldehyde was purified on silica gel in the presence of 50% ethyl acetate/hexane as the eluent to yield 6.20g (90%) crude material.

^{1}H NMR (400 MHz, DMSO-d_6): 10.18 (s, 1H), 6.95-6.94 (m, 1H), 6.92-6.91(m, 1H), 3.15-3.12 (m, 4H), 2.63-2.61 (m.4H), 2.26 (s, 3H). LC–MS: m/z 303.1 [M+2].

General procedure for the synthesis of 4-bromo-2-flouro-6-(4-methylpiperazine-1-yl) benzaldehyde derivatives (13A-13G)

4-Flouro-2-(4-methylpiperazine-1-yl) benzaldehyde (0.3 g, 0.996 mmol, 1 equivalent) was dissolved in a mixture of 1, 4 dioxane and water (4:1). To this solution, 2-Flouro-6 (4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2yl) pyridine (0.22 g, 0.996 mmol, 1.0 equivalent) and K_2CO_3 (0.27 g, 1.993 mmol, 2 equivalents) were added.

After undergoing a degassing process for a duration of 5 min using nitrogen gas, I introduced Pd_2Cl_2(dppf) and DCM (0.08 g, 0.099mmol, 0.1 equivalent). The reaction process was sustained at a temperature of 100 degrees Celsius for duration of 16 hours. Thin-layer chromatography (TLC) was used to observe the progress of the reaction. The combination underwent a water wash, followed by dilution with ethyl acetate. It was then submerged in a "brine solution, dried using Na_2SO_4, and concentrated under reduced pressure." 2-fluoro-4-(6-fluoropyridin-2-yl)The compound-6-(4-methylpiperazine-1-yl) benzaldehyde (13A) was produced with a yield of 0.20 g, which corresponds to a 63% yield."

m.p.: 322-324[4c]; FT-IR (KBr): 1673.48 cm^{-1} -C=O stretching, 3125.35 cm^{-1}: -N-H stretching; ^{1}H NMR (400 MHz, DMSO-d_6):10.28 (s, 1H), 8.44 (s,1H), 8.00-7.98 (m,1H) 7.05-7.00 (m.1H), 6.95-6.90 (m, 2H), 3.21-3.20 (m, 4H), 2.68-2.66 (s, 4H), 2.21 (s,3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.91, 150.62, 144.26, 130.99, 129.84, 129.43, 129.18, 128.16, 127.11, 122.54, 120.84, 117.17 & 116.06; HRMS found: ESI m/z 318.2 $(M+H)^+$, [M.F $C_{17}H_{17}F_2N_3O$ requires 317.13]

CONCLUSION

The prepared molecules are novel with simple structure and prepared in a very simple synthetic way. The methodology adopted for thepreparation was repeatable and the materials used were very much affordable."At 32 (µg/mL), the antibacterial activity of molecules 9B, 13C, and 13D was shown to be the strongest against the *Gram-positive* bacteria *Staphylococcus aureus* and *B. subtilis*."Docking studies of these molecules show that they are the best fit in the enzyme and the binding energies of these molecules are also more than the standard molecule Triclosan. In the future in vivo studies of the molecules were planned on FabI enzymes.

ACKNOWLEDGMENT

The authors are extremely thankful to Aurigene Pharmaceutical Services Pvt Ltd for the execution of this research work. My special thanks to Dr. Amit Murlidhar Jabgunde, Dr. Ravikrishnababu, and APSL analytical team for helping us whenever required.

Supporting Information

The supplemental information file contains the characterization of synthesized compounds, including ^{1}H NMR, ^{13}C NMR spectra, IR spectra, and HRMS data.

Conflict of interest

The authors have no conflict of interest to declare. All co-authors have seen with the contents of manuscript, and there is no financial interest to report. We certify that the submission is original work and it is not under review at any other publication.

REFERENCES

1. Montoya, J.G.; Multani. A.; Diagnosis of infection in immune compromised patients: from microscopy to next generation sequencing and host gene signatures., *Curr. Opin. Infect. Dis.*, **2019**, *32*, 305-322.
2. Bujayr, A.A.; Aljohar, B.A.; Bin Saleh, G.M.; Alanazi, K.H.; Assiri, A.M.; Incidence and epidemiological characteristics of COVID-19 among health care workers in Saudi Arabia: A retrospective cohort study., *J. Infect. Public Health.*, **2021**, *14*, 1174–1178.
3. Payne, D.J.; Warren, P. V.; Holmes, D.J.; Ji, Y; Lonsdale, J.T.; Bacterial fatty-acid biosynthesis: a genomics-driven target for antibacterial drug discovery., *Drug Discov. Today.*, **2001**, *6*, 537–544.
4. Campbell, J.W.; Cronan, J.E.; Bacterial Fatty Acid Biosynthesis: Targets for Antibacterial Drug Discovery., *Annu. Rev. Microbiol.*, **2001**, *55*, 305–332.
5. Heath, R.J.; White, S.W.; Rock, C.O.; Lipid biosynthesis as a target for antibacterial agents., *Prog. Lipid Res.*, **2001**, *40*, 467–497.
6. Parsons, J.B.; Rock, C.O.; Bacterial lipids: Metabolism and membrane homeostasis., *Prog. Lipid Res.*, **2013**, *52*, 249–276.
7. Zheng, L.; Lin, Y.; Lu, S.; Zhang, J. Bogdanov M.; Biogenesis, transport and remodeling of lysophospholipids in *Gram-negative* bacteria, Biochim., *Biophys. Acta-Mol. Cell Biol. Lipids.*, **2017**, *1862*, 1404–1413.
8. Hiltunen, J.K.; Chen, Z.; Haapalainen, A.M.; Wierenga, R.K.;Kastaniotis, A.J.; Mitochondrial fatty acid synthesis–An adopted set of enzymes making a pathway of major importance for the cellular metabolism., *Prog. Lipid Res.*, **2010**, *49*, 27–45.
9. Perez, D.R.; Leibundgut, M.; Wider, G.; Interactions of the Acyl Chain with the Saccharomyces cerevisiae Acyl Carrier Protein., *Biochemistry.*, **2015**, *54*, 2205–2213.
10. Neckles, C.; Eltschkner, S.; Cummings, J.E.; Hirschbeck, M.; Daryaee, F.; Bommineni, Z.; Zhang, G.R.; Spagnuolo, L.; Yu, W.: Davoodi, S.; Slayden, R.A.; Kisker, C.; Tonge, P.J.; Rationalizing the Binding Kinetics for the Inhibition of the Burk holderia pseudomallei FabI1 Enoyl-ACP Reductase., *Biochemistry.*, **2017**, *56*, 1865–1878.
11. Massengo-Tiassé, R.P.; Cronan, J.E. Diversity in enoyl-acyl carrier protein reductases, Cell., *Mol. Life Sci.*, **2009**, *66*, 1507–1517.
12. Heath, R.J.; Rock,, C.O.; Fatty acid biosynthesis as a target for novel antibacterials., *Curr. Opin. Investig. Drugs.*, **2004**, *5*, 146–153.
13. Rana, P.; Ghouse, S.M.; Akunuri, R.; Madhavi, Y. V.; Chopra, S.; Nanduri, S.; FabI (enoyl acyl carrier protein reductase) - A potential broad spectrum therapeutic target and its inhibitors., *Eur. J. Med. Chem.*, **2020**, *208*, 112-757.
14. Lu, H.; Tonge, P.J.; Inhibitors of FabI, an Enzyme Drug Target in the Bacterial Fatty Acid Biosynthesis Pathway., *Acc. Chem. Res.*, **2008**, *41*, 11–20.
15. Tasdemir, D.; Topaloglu, B.; Perozzo, R.; Brun, R.; O'Neill, R.; Carballeira, N.M.; Zhang, X.; Tonge, P.J.; Linden, A.; Rüedi, P.; Marine natural products from the Turkish sponge Agelas oroides that inhibit the enoyl reductases from Plasmodium falciparum, Mycobacterium tuberculosis and *Escherichia coli*, *Bioorg. Med. Chem.*, **2007**, *15*, 6834–6845.
16. Hevener, K.E.; Mehboob, S.; Su, P.C.; Truong, K.; Boci, T.; Deng, J.; Ghassemi, M. ;. Cook, J.L; Johnson, M.E.; Discovery of a Novel and Potent Class of F. tularensis Enoyl-Reductase (FabI) Inhibitors by Molecular Shape and Electrostatic Matching., *J. Med. Chem.*, **2012**, **55**, 268–279.
17. Kang, D.; Song, Y.; Chen, W.; Zhan, P.; Liu, X.; "Old Dogs with New Tricks": exploiting alternative mechanisms of action and new drug design strategies for clinically validated HIV targets., *Mol. Bio Syst.*, **2014**, *10*, 1998–2022.
18. Falconer, S. B.; Brown, E.D.; New screens and targets in antibacterial drug discovery., *Curr. Opin. Microbiol.*, **2009**, *12*, 497–504.
19. Kitagawa, H.; Ozawa, T.; Takahata, S.; Iida, M.; Saito, J.; Yamada, M.; Phenylimidazole Derivatives of 4-Pyridone as Dual Inhibitors of Bacterial Enoyl-Acyl Carrier Protein Reductases FabI and FabK., *J. Med. Chem.*, **2007**, *50*, 4710–4720.

20. Miller, W.H.; Seefeld, M.A.; Newlander, K.A.; Uzinskas, I.N.; Burgess, W.J.; Heerding, D.A.; Yuan, C.C.K.; Head, M.S.; Payne, D.J.; Rittenhouse, S.F.; Moore, T.D.; Pearson, S.C.; Berry, V.; DeWolf, W.E.; Keller, P.M.; Polizzi, B.J.; Qiu, X.; Janson, C.A.; Huffman, W.F.; Discovery of Aminopyridine-Based Inhibitors of Bacterial Enoyl-ACP Reductase (FabI)., *J. Med. Chem.*, **2002**, *45*, 3246–3256.
21. Seefeld, M.A.; Miller, W.H.; Newlander, K.A.; Burgess, W.J.; DeWolf, W.E.; Elkins, M.S.; Head, P.A.; Jakas, D.R.; Janson, C.A.; Keller, P.M.; Manley, P.J.; Moore, T.D.; Payne D.J. Pearson, S.; Polizzi, B.J.; Qiu, X.; Rittenhouse, S.F.; Uzinskas, I.N.; Wallis, N.G.; Huffman, W.F.; Indole Naphthyridinones as Inhibitors of Bacterial Enoyl-ACP Reductases FabI and FabK., *J. Med. Chem.*, **2003**, *46*, 1627–1635.
22. Zhang, Y. M.; Rock, C. O.; Fatty Acid and Phospholipid Biosynthesis in Prokaryotes, in: N.D. Ridgway, R.S. McLeod (Eds.), Biochem. Lipids, Lipoproteins Membr., *Elsevier, Boston.*, **2016**, *6*, 73–112.
23. Zhu, Z.; Hu, Y.; Teixeira, P.G.; Pereira, R.; Chen, Y.; Siewers, V.; Nielsen, J.; Multidimensional engineering of Saccharomyces cerevisiae for efficient synthesis of medium-chain fatty acids., *Nat. Catal. J.*, **2020**, *3*, 64–74.
24. Zhang, Y.M.; Rock, C.O.; Membrane lipid homeostasis in bacteria., *Nat. Rev. Microbio l.*, **2008**, *6*, 222–233.
25. Lasonder, E.; Rijpma, S.R.; van Schaijk, B.C.L.; Hoeijmakers, W.A.M.; Kensche, P.R.; Gresnigt, M.S.; Italiaander, A.; Vos, M.W.; Woestenenk, R.; Bousema, T.; Mair, G.R.; Khan, S.M.; Janse, C.J.; Bártfai, R.; Sauerwein, R.W.; Integrated transcriptomic and proteomic analyses of P. falciparum gametocytes: molecular insight into sex-specific processes and translational repression., *Nucleic Acids Res.*, **2016**, *44*, 6087–6101.
26. Van Schaijk, B.C.L.; Ploemen, I.H.J.; Annoura, T.; Vos, M.W.; Foquet, L.; van Gemert, G.J.; Chevalley-Maurel, S.; van de Vegte-Bolmer, M.; Sajid, M.; Franetich, J.F.; Lorthiois, A.; Leroux-Roels, G.; Meuleman, P.;. Hermsen, C.C; Mazier, D.; Hoffman, C.J. Janse, S.L. ; Khan, S.M.; Sauerwein, R.W. A genetically attenuated malaria vaccine candidate based on P. falciparum b gene-deficient sporozoites., *Elife*. **2014**, *3*, e03582.
27. Vaughan, A.M.; O'Neill, M.T.; Tarun, A.S.; Camargo, N.; Phuong, T.M.; Aly, A.S.I.; Cowman, A.F.; Kappe, S.H.I.; Type II fatty acid synthesis is essential only for malaria parasite late liver stage development, Cell., *Microbiol.*, **2009**, *11*, 506–520.
28. Van Schaijk, B.C.L.; Kumar, T.R.S.; Vos, M.W.; Richman, A.; van Gemert, G.J.; Li, T. ; Eappen, A.G.; Williamson, K.C.; Morahan, B.J.; Fishbaugher, M.; Kennedy, M.; Camargo, N.; Khan, S.M.; Janse, C.J.; Sim, K.L.; Hoffman, S.L.; Kappe, S.H.I.; Sauerwein, R.W.; Fidock, D.A.; Vaughan, A.M.; Type II Fatty Acid Biosynthesis Is Essential for Plasmodium falciparum Sporozoite Development in the Midgut of Anopheles Mosquitoes, Eukaryot., *Cell.*, **2014**, *13*, 550–559.
29. Grzegorzewicz, A. E.; Lelièvre, J.; Esquivias, J.; Angala, B.; Liu, J.; Lee, R.E.; McNeil, M.R.; Jackson, M.; Lack of Specificity of Phenotypic Screens for Inhibitors of the Mycobacterium tuberculosis FAS-II System, Antimicrob., *Agents Chemother.*, **2020**, *65*, 01914-20.
30. Lindner, S.E.; Miller, J.L.; Kappe, S.H.I.; Malaria parasite pre-erythrocytic infection: preparation meets opportunity., *Cell. Microbiol.*, **2012**, *14*, 316–324.
31. Parker, Erica N.; Cain, Brett N.; Behnoush Hajian, Rebecca J.; Ulrich, Emily.; Geddes, J.; Sulyman Barkho.; Hyang Yeon Lee.; John D.; Williams, Malik Raynor.; Diana Caridha.; Angela Zaino, Mrinal Shekhar.; Kristen A.; Muñoz, Kara M.; Rzasa, Emily R.; Temple.; Diana Hunt.; Xiannu Jin.; Chau Vuong.; Kristina Pannone.; Kelly Aya M.; Michael P.; Mulligan.; Katie K.; Lee.; Gee W.; Lau.; Deborah T.; Hung.; Paul.; An Iterative Approach Guides Discovery of the FabI Inhibitor Fabimycin, a Late-Stage Antibiotic Candidate with *In vivo* Efficacy against Drug-Resistant *Gram-negative* Infections., *J. Hergenrother ACS Central Science.*, **2012**, *8*, 1145-1158.
32. Grzegorzewicz, A.E.; Gee, C.; Das, S.; Liu, J.; Belardinelli, J.M.; Jones, V.; McNeil, M.R.; Lee, R.E.; Jackson, M.; Mechanisms of Resistance Associated with the Inhibition of the Dehydration Step of Type II Fatty Acid Synthesis in Mycobacterium tuberculosis., *ACS Infect. Dis.*, **2020**, *6*, 195–204.

33. Unissa, A.N.; Subbian, S.; Hanna, L.E.; Selvakumar, N.; Overview on mechanisms of isoniazid action and resistance in Mycobacterium tuberculosis, Infect., *Genet. Evol*., **2016**, *45*, 474–492.
34. Alahari, A.; Alibaud, L.; Trivelli, X.; Gupta, R.; Lamichhane, G.; Reynolds, R.C.; Bishai, W.R.; Guerardel, Y.; Kremer, L.; Mycolic acid methyltransferase, MmaA4, is necessary for thiacetazone susceptibility in Mycobacterium tuberculosis., *Mol. Microbiol*., **2009**, *71*, 1263–1277.
35. Belardinelli, J.M.; Morbidoni, H.R.; Mutations in the essential FAS II-hydroxyacyl ACP dehydratase complex confer resistance to thiacetazone in Mycobacterium tuberculosis and Mycobacterium kansasii., *Mol. Microbiol*., **2012**, *86*, 568–579.
36. Kleiboeker, B.; Lodhi, I. J.; Peroxisomal regulation of energy homeostasis: Effect on obesity and related metabolic disorders., *Mol. Metab*., **2022**, *65*, 561-577.
37. Heath, R. J.; Jackowski, S.; Rock, C. O.; Fatty acid and phospholipid metabolism in prokaryotes, in: Biochem. Lipids, Lipoproteins Membr. 4th Ed., Elsevier, **2002**, 55–92.
38. Xie, M.; Wang, W.; Zhang, W.; Chen, L.; Lu, X.; Versatility of hydrocarbon production in cyanobacteria., *Appl. Microbial. Biotechnology*., **2017**, *101*, 905–919.
39. Escaich, S.; Prouvensier, L.; Saccomani, M.; The MUT056399 Inhibitor of FabI is a New Anti staphylococcal Compound., *Antimicrobial Agents and Chemotherapy*., **2011**, *55*, 10.
40. Sandra Eltschkner, A long residence time enoyl-reductase inhibitor explores an extended binding region with iso enzyme-dependent tautomer adaptation and differential substrate-binding., *Loop Closure*., **2021**, *7*, 746-758.
41. Bahuguna, A.; Rawat, D.S.; An overview of new anti tubercular drugs, drug candidates, and their targets., *Med. Res. Rev*., **2020**, *40*, 263–292.
42. Kumar, J.; Gill, A.; Shaikh, M.; Singh, A.; Shandilya, A.; Jameel, E.; Sharma, N.; Mrinal, N.; Hoda, N.; Jayaram, B.; Pyrimidine-Triazolopyrimidine and Pyrimidine-Pyridine Hybrids as Potential Acetyl cholinesterase Inhibitors for Alzheimer's Disease., *Chemistry Select*., **2018**, *3*, 736–747.
43. Eissa, K. I.; Kamel, M.M.; Mohamed, L.W.; Kassab, A.E.; Development of new Alzheimer's disease drug candidates using donepezil as a key model., *Arch. Pharm. Weinheim*., **2023**, *356*, 2200398.
44. Mean well, N. A.; Loiseleur, O.; Applications of Isosteres of Piperazine in the Design of Biologically Active Compounds: Part 1., *J. Agric. Food Chem*., **2022**, *70*, 10942–10971.
45. Shaquiquzzaman, M.; Verma, G.; Marella, A.; Akhter, M.; Akhtar, W.; Khan, M.F.; Tasneem, S.; Alam, M.M.; Piperazine scaffold: A remarkable tool in generation of diverse pharmacological agents., *Eur. J. Med. Chem*., **2015**, *102*, 487–529.
46. Zhang, R.H.; Guo, H.Y.; Deng, H.; Li, J.; Quan, Z.S.; Piperazine skeleton in the structural modification of natural products: a review., *J. Enzyme Inhib. Med. Chem*., **2021**, *36*, 1165–1197.