Asian Pub. Corp.

www.asianpubs.org

ARTICLE

Synthesis and Biological Evaluation of 3,4-Dihydro-2*H*-benzo[*b*][1,4]-oxazine-2-carboxylic Acid Derivatives as Antitubercular Agents

Macchindra S. Tambe[1,✉], Sonali Gadhe[1], Amit Choudhari[2], Dhiman Sarkar[2], Jaiprakash N. Sangshetti[3] and Rajesh B. Patil[4]

Asian Journal of Organic & Medicinal Chemistry

Volume: 5 Year: 2020
Issue: 2 Month: April–June
pp: 138–148
DOI: https://doi.org/10.14233/ajomc.2020.AJOMC-P261

Received: 14 April 2020
Accepted: 13 June 2020
Published: 2 July 2020

ABSTRACT

A series of side chain modified structurally diverse 3,4-dihydro-2*H*-benzo[*b*][1,4]-oxazine-2-carboxylic acid derivatives were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral study. All the newly synthesized compounds were examined for their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Ra$. The synthesized compounds exhibited minimum inhibitory concentration (IC_{50}) ranging from 5.98 to >30 (µg/mL) against Mtb*H*$_{37}$*Ra*. Among the screened compounds, compounds **5a**, **5c**, **5d**, **5f**, **5g**, **5h**, **5I**, **5j** exhibited IC_{50} as 10.42, 11.81, 18.79, 5.98, 19.21, 24.81 and 14.81 µg/mL, respectively. The antibacterial screening study of these compounds was conducted against four different bacteria to asses there selectivity towards MTB. The antibacterial screening of all the synthesized compounds was conducted against four bacterial strains (Gram-negative strains: *E.coli* and *S.aureus*; Gram-positive strains: *P. aeruginosa* and *B.subtilis.* The compounds **5a**, **5b**, **5c**, **5e** and **5j** showed higher antibacterial activity up to **7- 25** µg/mL. Furthermore, molecular docking studies revealed the binding modes of the compounds in the binding site of the good agreement with the *in vitro* antitubercular screening. The compounds **5a**, **5c** and **5f** with free energy of binding lower than -9.0 Kcal/mol binds more favourably at the binding site of panC as compared to other compounds. Specifically, the compound **5f** with free energy of binding -9.6 Kcal/mol is indeed found more active in docking study as well as in the *in vitro* antitubercular screening. These findings open the possibility for potential lead for antituberculosis chemotherapy.

KEYWORDS

Benzooxazines, Antitubercular agents.

Author affiliations:

[1]P.G. Department of Chemistry and Research Centre, Padmashri Vikhe Patil College, Pravaranagar-413713, India
[2]Organic Chemistry Division Combichem-Bioresource Centre, National Chemical Laboratory, Pune-7, India
[3]Y.B.Chavan College of Pharmacy, Aurangabad-431001, India
[4]Sinhgad Technical Education Society's Smt. Kashibai Navale College of Pharmacy, Pune-Saswad Road, Kondhwa (Bk), Pune-411048, India

✉To whom correspondence to be addressed:

Fax: +91 2422 273425
Tel: +91 2422 273426
E-mail: macchu.tambe@gmail.com

Available online at: http://ajomc.asianpubs.org

INTRODUCTION

Benzoxazine are bicyclic heterocycles containing benzene ring fused with oxazine ring. In general, benzooxazines can be synthesized from formaldehyde, phenols and primary amines by using Mannich reaction [1]. Depending on position of oxygen and nitrogen atoms, the oxazines are observed in three forms as 1,2-benzoxazine, 1,3-benzoxazine and 1,4-benzoxazine [2].

Benzoxazines derivatives was considered precursor for the construction of various heterocyclic compounds as significant heterocyclic system for the possessing remarkable biological properties [3] and several benzoxazine derivatives are currently

in the development phase as potential drug candidates. Among the wide diversity of heterocycles that have been explored for developing medicinally essential molecule 1,4-benzoxazines core play an very important group due to their broad range of biological activity. Moreover, benzoxazine is one of the most privileged core found in many biologically active molecules exhibiting anticancer, antibacterial, antifungal activity and scaffolds for the kinase inhibitors [4,5]. The present treatments for the bacterial and fungal infections are inadequate, due to rapidly developing multi drug resistance and more side effects [6]. 2,3-Dihydro-1,4-benzoxazines have proven their great importance as a part of biologically potent and medicinally high significant value and shows the several biological activities, such as anti-inflammatory, anti-ulcer, anti-bacterial activity [6]. This obscurity of the drug has restricted uses of most antimicrobial agents [7].

In the field of medicinal chemistry, 1,4-oxazine core is one of the privileged sub-structures for drug design. It has been represented an important class of heterocycles possessing remarkable utility as most potent therapeutic agents. Some of the potentially active 1,4-oxazine containing synthetic drugs as ofloxacin (**1**) is one of the antimicrobial agent (MIC = 0.25 µg/mL) [8], levofloxacin (**2**) exhibited good antitumor activity MCF-7 breast cancer cell line with IC_{50} = 6.75 µM, ester (**3**) and amide (**4**) analogues posseses remarkable anticancer activity [9]. The derivatives of 1,4-benzoxazine (**5**,**6**) exhibited excellent antifungal activity with MIC = 0.49l and 0.032 µg/mL, respectively [10]. Li *et al.* [5] reported the synthesis of several 1,4-benzoxazines derivatives (**7-10**) exhibiting excellent activity against *M. tuberculosis* H37Rv. Approved drug and several other drugs with similar mechanism are currently under clinical trial as potent inhibitors of human uric acid transporter 1(hURAT1) (**11-13**) [11-15], Hernandez-Olmos *et al.* [16] reported the SAR study of 1,4-oxazines (**14-16**) and found to be most potent P2X4 antagonists (Fig. 1).

In view of the biocompatibility and biological potential of 1,4-benzoxazine ring core, herein reported the synthesis and biological screening of series of some 4-acyl substituted 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylic acid derivatives and screening of their antitubercular and antibacterial activity [17-28].

EXPERIMENTAL

All the reagents used during the study were purchased from Aldrich and Spectro-Chem. Solvents were dried and redistilled before use. Melting points were recorded on Digital Electro thermal Melting point apparatus (VEEGO, VMP-DS) and are uncorrected. Reaction monitoring was conducted using Thin Layer Chromatography (TLC) using pre-coated Silica gel 60 F_{254} plates with layer thickness 0.25 nm purchased from

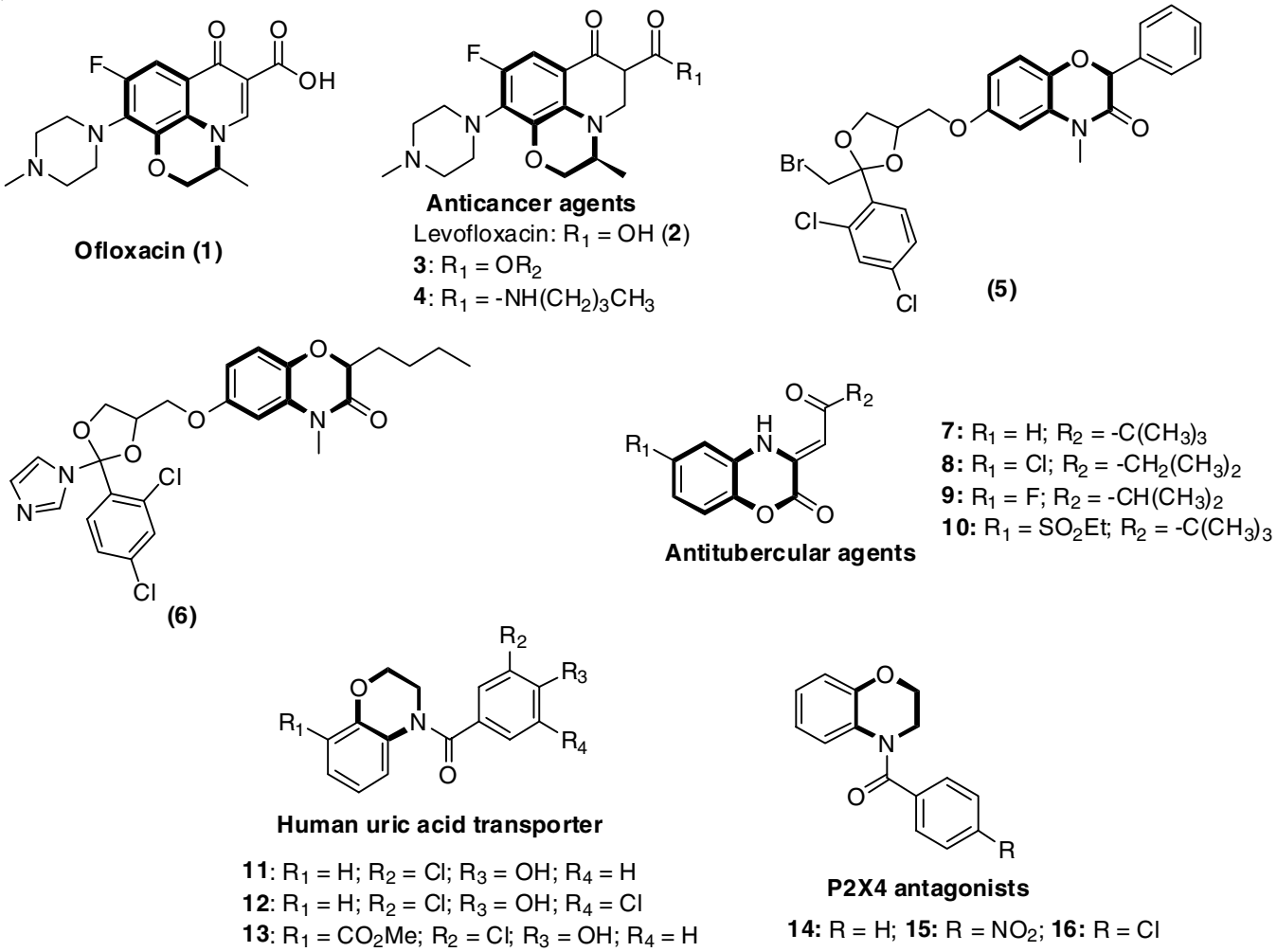


Fig. 1. Potent antibacterial 1,4-benzoxazine derivatives

Merck Ltd. TLC plates were visualized under ultraviolet light at 254 nm wavelength. IR spectra were recorded on KBr discs on Shimazdzu 470 IR spectrophotometer. ^{1}H NMR was recorded on Varian-NMR mercury 300 MHz spectrometer in DMSO-d_6 using TMS as an internal standard. Chemical shifts values (δ) are expressed parts per million (ppm). Mass spectra were recorded on Varian MAT 311 A at 70 eV.

Synthesis of ethyl 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate (3): In oven dried round bottom flask was taken 2-amino phenol (10 g, 0.091 mol) in dry acetonirile, then dried Cs_2CO_3 (38 g, 0.275mol) was added in the reaction flask at room temperature. The reaction mixture was stirred at 90 ºC for 30 min. To this mixture ethyl-2,3-dibromopropionate (16.64 g, 0.109 mol) was added slowly. The reaction mixture was further stirred at 60 ºC for 10 h. After completion of reaction, the reaction mixture was cooled and filtered through celite bed, solvent was evaporated completely then water (50 mL) was added and extracted with ethyl acetate (2 × 100 mL). The organic layer was separated and dried with anhydrous sodium sulphate. The solvent was removed under reduced pressure. The crude product was purified by column chromatography, product was eluted in ethyl acetate:petroleum ether (6-8%), to afford ethyl 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate **(3)** as brown solid (10.12 g). Yield = 53.31%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.22 (t, 3H, -CH_2-CH_3), 3.23 (s, 1H, exchangeable with D_2O, -NH-), 3.54 (dd, *J* = 8.5 Hz, 3.0 Hz, 1H, -CH_2-CH-), 4.24 (q, 2H, -CH_2-CH_3), 4.82 (dd, *J* = 8.5 Hz, 3.0 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 8.5 Hz, 1H, -CH-CH_2-), 6.62 (d, *J* = 7.2 Hz, 1H, Ar-H), 6.84 (dd, *J* = 7.2 Hz, 2H, Ar-H), 6.89 (dd, *J* = 7.2 Hz, 1H, Ar-H); m.f. $C_{11}H_{13}NO_3$ (m.w. 207.23) mass *m/z* (%): 208.17 $(M+H)^+$.

General procedure of synthesis of ethyl 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate derivatives (4): To a mixture of ethyl 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate **(3)** (200 mg, 0.966 mmol) in dry THF (2 mL), pyridine (0.5mL) was added at 25 ºC and stirred for 30 min. To this mixture slowly benzoyl chloride (0.17 mL, 1.44 mmol) solution in THF was added. The reaction mixture was stirred at 65 ºC for 1-2 h. After completion of reaction, reaction mixture was poured in ice cold water and extracted with ethyl acetate. The organic phase was washed by saturated solution of sodium bicarbonate, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The obtained crude product was purified by column chromatography, product was eluted in ethyl acetate:petroleum ether (5-6%) to afford yellow coloured thick oil ethyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate derivatives **(4)** (250 mg), Yield = 70-80%.

Ethyl 4-benzoyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate (4a): ^{1}H NMR (300MHz, DMSO-d_6): δ 1.23 (t, 3H, -CH_2-CH_3), 3.68 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.20 (q, 2H, -CH_2-CH_3), 4.86 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 8.9 Hz, 1H, -CH-CH_2-), 6.58 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.97 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.28-7.46 (m, 5H, Ar-H); m.f. $C_{18}H_{17}NO_4$ (m.w. 311.33) mass *m/z*(%): 312.17 $(M+H)^+$.

Ethyl 4-(4-(*tert*-butyl)benzoyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate (4b): ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.27 (t, 3H, -CH_2-CH_3), 1.29 (s, 9H, Ar-C$(CH_3)_3$), 3.71 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.19 (q, 2H, -CH_2-CH_3), 4.82 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.03 (t, *J* = 8.9 Hz, 1H, -CH-CH_2-), 6.63 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.00 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.32 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.40 (d, *J* = 8.1 Hz, 2H, Ar-H); m.f. $C_{22}H_{25}NO_4$ (m.w. 367.44) mass *m/z* (%): 368.28$(M+H)^+$.

Ethyl 4-(4-(trifluoromethyl)benzoyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate (4c): ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25 (t, 3H, -CH_2-CH_3), 3.62 (dd, *J* = 9.2 Hz, 3.9 Hz, 1H, -CH_2-CH-), 4.20 (q, 2H, -CH_2-CH_3), 4.91 (dd, *J* = 9.2 Hz, 3.9 Hz, 1H, -CH_2-CH-), 5.06 (t, *J* = 9.2 Hz, 1H, -CH-CH_2-), 6.59 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.00 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.56 (d, *J* = 7.8 Hz, 4H, Ar-H); m.f. $C_{19}H_{16}NO_4F_3$ (m.w. 379.33) mass *m/z*(%): 380.44$(M+H)^+$.

Ethyl 4-(4-(methyl)benzoyl)-3,4-dihydro-2*H*-benzo[*b*]-[1,4]oxazine-2-carboxylate (4d): ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.21 (t, 3H, -CH_2-CH_3), 2.35 (s, 3H, Ar-CH_3), 3.69 (dd, *J* = 8.8 Hz, 3.3 Hz, 1H, -CH_2-CH-), 4.19 (q, 2H, -CH_2-CH_3), 4.78 (dd, *J* = 8.8 Hz, 3.3 Hz, 1H, -CH_2-CH-), 5.02 (t, *J* = 8.8 Hz, 1H, -CH-CH_2-), 6.58 (d, *J* = 7.8 Hz, 2H, Ar-H), 6.97 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.11 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.36 (d, *J* = 7.8 Hz, 2H, Ar-H); m.f. $C_{19}H_{19}NO_4$ (m.w. 325.13) mass *m/z* (%): 326.52$(M+H)^+$.

Ethyl 4-pivaloyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate (4e): ^{1}H NMR (300MHz, DMSO-d_6): δ 1.18 (s, 9H, -C$(CH_3)_3$), 1.21 (t, 3H, -CH_2-CH_3), 3.58 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.18 (q, 2H, -CH_2-CH_3), 4.82 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 8.9 Hz, 1H, -CH-CH_2-), 6.48 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.87 (d, *J* = 7.2 Hz, 2H, Ar-H); m.f. $C_{16}H_{21}NO_4$ (m.w. 291.34) mass *m/z* (%): 292.17 $(M+H)^+$.

Ethyl 4-isobutyryl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate (4f): ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.08 (d, 6H, -CH$(CH_3)_2$), 1.23 (t, 3H, -CH_2-CH_3), 3.01 (m, 1H, -CH-$(CH_3)_2$), 3.69 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.17 (q, 2H, -CH_2-CH_3), 4.81 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 8.9 Hz, 1H, -CH-CH_2-), 6.58 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.97 (d, *J* = 7.2 Hz, 2H, Ar-H); m.f. $C_{15}H_{19}NO_4$ (m.w. 277.32) mass *m/z* (%): 250.11$(M+H)^+$.

Ethyl 4-(cyclopropanecarbonyl)-3,4-dihydro-2*H*-benzo-[*b*][1,4]oxazine-2-carboxylate (4g): ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.82 (m, 4H, 2 × -CH_2 cyclopropyl), 1.22 (m, 1H, -CH cyclopropyl),1.23 (t, 3H, -CH_2-CH_3), 3.68 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.20 (q, 2H, -CH_2-CH_3), 4.86 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 8.9 Hz, 1H, -CH-CH_2-), 6.58 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.97 (d, *J* = 7.2 Hz, 2H, Ar-H); m.f. $C_{15}H_{17}NO_4$ (m.w. 275.30) mass *m/z*(%): 276.32 $(M+H)^+$.

Ethyl 4-(4-chlorobenzoyl)-3,4-dihydro-2*H*-benzo[*b*]-[1,4]oxazine-2-carboxylate (4h): ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.23 (t, 3H, -CH_2-CH_3), 3.68 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.20 (q, 2H, -CH_2-CH_3), 4.86 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 8.9 Hz, 1H, -CH-CH_2-), 6.58 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.97 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.40 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.81 (d, *J* = 7.8 Hz, 2H, Ar-H); m.f. $C_{18}H_{16}NO_4Cl$ (m.w. 345.78) mass *m/z*(%): 346.34$(M+H)^+$.

Ethyl 4-(4-fluorobenzoyl)-3,4-dihydro-2*H*-benzo[*b*]-[1,4]oxazine-2-carboxylate (4i): ^{1}H NMR (300 MHz, DMSO-

d$_6$): δ 1.23 (t, 3H, -CH_2-CH_3), 3.68 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.20 (q, 2H, -CH_2-CH_3), 4.86 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 8.9 Hz, 1H, -CH-CH_2-), 6.58 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.97 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.09 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.85 (d, *J* = 7.8 Hz, 2H, Ar-H); m.f. $C_{18}H_{16}NO_4F$ (m.w. 329.32) mass *m/z*(%): 330.23 $(M+H)^+$.

Ethyl 4-(2,4-difluorobenzoyl)-3,4-dihydro-2*H*-benzo-[*b*][1,4]oxazine-2-carboxylic acid (4j): ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.23 (t, 3H, -CH_2-CH_3), 3.68 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.20 (q, 2H, -CH_2-CH_3), 4.86 (dd, *J* = 8.9 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 8.9 Hz, 1H, -CH-CH_2-), 6.58 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.97 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.80 (dd, *J* = 7.8 Hz, 1H, Ar-H), 7.87 (dd, *J* = 7.8 Hz, 1H, Ar-H), 7.92 (d, *J* = 7.8 Hz, 1H, Ar-H); m.f. $C_{18}H_{15}NO_4F_2$ (m.w. 347.31) mass *m/z*(%): 348.17$(M+H)^+$.

General procedure of synthesis of 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylic acid derivatives (5a-j): A mixture of ethyl 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylate derivative (100 mg, 0.325 mmol) in methanol (4 mL), was cooled to 0-5 ºC. To this mixture aq. LiOH solution was added. The reaction mixture was stirred at 25 ºC for 2 h. After completion of reaction, methanol was evaporated at 30 ºC, residue washed with saturated $NaHCO_3$ and ether. Then aqueous layer was acidified by HCl (1N) solution, solid separated was filtered and suck dried to afford series of compounds **5a-j**, Yield: 80-90%.

4-Benzoyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylic acid (5a): ^{1}H NMR: δ 3.67 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.48 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.20 (t, *J* = 9.2 Hz, 1H, -CH-CH_2-), 6.66 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.99 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.38-7.43 (m, 5H, Ar-H), 13.20 (s, 1H, exchangeable with D_2O, -COOH); ^{13}C NMR(100 MHz, DMSO *d*$_6$): δ 40.5, 73.4, 117.2, 120.2, 122.9, 125.6, 125.8, 126.2, 128.3, 130.5, 131.3, 138.2, 146.9, 167.4, 170.8; IR (KBr, ν_{max}, cm^{-1}): 3429 (-OH acid), 3019, 2929 (C-H alkane), 1744 (C=O amide), 1624, 1588 (arom. carbon), 1388 (C-N), 1207 (C-O); m.f. $C_{16}H_{13}NO_4$ (m.w. 283.28) mass *m/z*(%): 284.19 $(M+H)^+$.

4-[4-(*tert*-Butyl)benzoyl)]-3,4-dihydro-2*H*-benzo-[*b*][1,4]oxazine-2-carboxylic acid (5b) : ^{1}H NMR: δ 1.27 (s, 9H, Ar-C(CH_3)$_3$), 3.67 (dd, *J* = 9.2 Hz, 3.6 Hz, 1H, -CH_2-CH-), 4.45 (dd, *J* = 9.2 Hz, 3.6 Hz, 1H, -CH_2-CH-), 5.11 (t, *J* = 9.2 Hz, 1H, -CH-CH_2-), 6.68 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.98 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.00 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.39 (d, *J* = 8.1 Hz, 2H, Ar-H), 13.24 (s, 1H, exchangeable with D_2O, -COOH); IR (KBr, ν_{max}, cm^{-1}): 3417 (-OH acid), 2963, 2869 (C-H alkane), 1748 (C=O amide), 1610, 1587 (arom. carbon), 1386 (C-N), 1235 (C-O); m.f. $C_{20}H_{21}NO_4$ (m.w. 339.39) mass *m/z*(%): 340.55$(M+H)^+$.

4-[4-(Trifluoromethyl)benzoyl]-3,4-dihydro-2*H*-benzo-[*b*][1,4]oxazine-2-carboxylic acid (5c) : ^{1}H NMR: δ 3.66 (dd, *J* = 9.2 Hz, 3.9 Hz, 1H, -CH_2-CH-), 4.45 (dd, *J* = 9.2 Hz, 3.9 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 9.2 Hz, 1H, -CH-CH_2-), 6.70 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.03 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.64 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.80 (d, *J* = 8.4 Hz, 2H, Ar-H), 13.44 (s, 1H, exchangeable with D_2O, -COOH); ^{13}C NMR(100 MHz, DMSO-*d*$_6$): δ 40.5, 73.4, 117.3, 120.0, 122.9, 124.6, 125.6, 126.2, 128.3, 129.5, 130.3, 130.6, 131.0, 139.8, 146.9, 167.4, 170.9; IR (KBr, ν_{max}, cm^{-1}): 3461 (-OH acid), 3063, 2955 (C-H alkane), 1743 (C=O amide), 1645, 1518 (arom. carbon), 1332 (C-N), 1230 (C-O); m.f. $C_{17}H_{12}NO_4F_3$ (m.w. 351.28) mass *m/z* (%): 352.47$(M+H)^+$.

4-[4-(Methyl)benzoyl]-3,4-dihydro-2*H*-benzo[*b*][1,4]-oxazine-2-carboxylic acid (5d) : ^{1}H NMR: δ 2.32 (s, 3H, Ar-CH_3), 3.67 (dd, *J* = 7.8 Hz, 3.4 Hz, 1H, -CH_2-CH-), 4.36 (dd, *J* = 7.8 Hz, 3.4 Hz, 1H, -CH_2-CH-), 4.87 (t, *J* = 7.8 Hz, 1H, -CH-CH_2-), 6.62 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.92 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.18 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.36 (d, *J* = 7.8 Hz, 2H, Ar-H), 13.31 (s, 1H, exchangeable with D_2O, -COOH); IR (KBr, ν_{max}, cm^{-1}): 3408 (-OH acid), 3058, 2945 (C-H alkane), 1750 (C=O amide), 1633, 1587 (arom. carbon), 1332 (C-N), 1230 (C-O); m.f. $C_{17}H_{15}NO_4$ (m.w. 297.31) mass *m/z*(%): 296.29$(M-H)^-$.

4-Pivaloyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylic acid (5e) : ^{1}H NMR: δ 1.19 (s, 9H, -C(CH_3)$_3$), 3.64 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.46 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 9.2 Hz, 1H, -CH-CH_2-), 6.58 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.88 (d, *J* = 7.2 Hz, 2H, Ar-H), 13.24 (s, 1H, exchangeable with D_2O, -COOH); IR (KBr, ν_{max}, cm^{-1}): 3025 (-OH acid), 3043, 2950 (C-H alkane), 1738 (C=O amide), 1614, 1587 (arom. carbon), 1335 (C-N), 1238 (C-O); m.f. $C_{14}H_{17}NO_4$ (m.w. 263.29) mass *m/z*(%): 264.48$(M+H)^+$.

4-Isobutyryl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylic acid (5f): ^{1}H NMR: δ 1.09 (d, 6H, -CH(CH_3)$_2$), 3.02 (m, 1H, -CH(CH_3)$_2$), 3.62 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H, -CH_2-CH-), 4.40 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H, -CH_2-CH-), 5.20 (t, *J* = 9.0 Hz, 1H, -CH-CH_2-), 6.64 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.90 (d, *J* = 7.2 Hz, 2H, Ar-H), 13.20 (s, 1H, exchangeable with D_2O, -COOH); IR (KBr, ν_{max}, cm^{-1}): 3039 (-OH acid), 3066, 2959 (C-H alkane), 1742 (C=O amide), 1622, 1589 (arom. carbon), 1338 (C-N), 1233 (C-O); m.w. $C_{13}H_{15}NO_4$ (m.w. 249.26) mass *m/z*(%): 250.11$(M+H)^+$.

4-(4-Chlorobenzoyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]-oxazine-2-carboxylic acid (5h) : ^{1}H NMR: δ 3.64 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.52 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.22 (t, *J* = 9.2 Hz, 1H, -CH-CH_2-), 6.70 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.78 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.43 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.82 (d, *J* = 7.8 Hz, 2H, Ar-H), 13.24 (s, 1H, exchangeable with D_2O, -COOH); IR (KBr, ν_{max}, cm^{-1}): 3024 (-OH acid), 3078, 2942 (C-H alkane), 1745 (C=O amide), 1634, 1582 (arom. carbon), 1330 (C-N), 1225 (C-O), 752 (C-Cl); m.f. $C_{16}H_{12}NO_4Cl$ (m.w. 317.72) mass *m/z*(%): 318.09$(M+H)^+$.

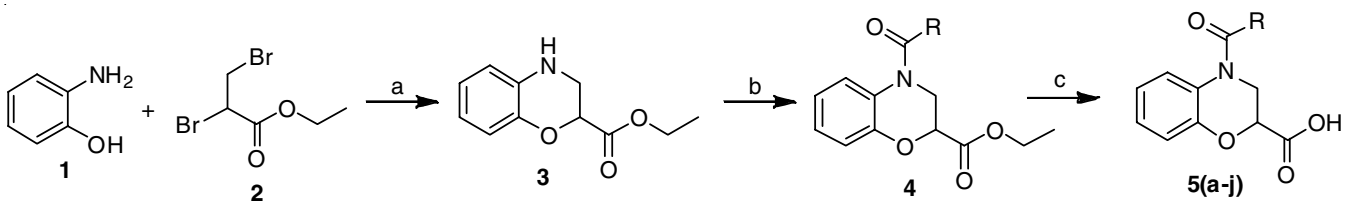
4-(4-Fluorobenzoyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]-oxazine-2-carboxylic acid (5i) : ^{1}H NMR: δ 3.64 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.52 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.22 (t, *J* = 9.2 Hz, 1H, -CH-CH_2-), 6.70 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.78 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.12 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.90 (d, *J* = 7.8 Hz, 2H, Ar-H), 13.24 (s, 1H, exchangeable with D_2O, -COOH); IR (KBr, ν_{max}, cm^{-1}): 3020 (-OH acid), 3060, 2953 (C-H alkane), 1750 (C=O amide), 1644, 1588 (arom. carbon), 1328 (C-N), 1219 (C-O), 814 (C-F); m.f. $C_{16}H_{12}NO_4F$ (m.w. 301.27) mass *m/z*(%): 302.22$(M+H)^+$.

4-(2,4-Difluorobenzoyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]-oxazine-2-carboxylic acid (5j): ^{1}H NMR: δ 3.64 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 4.52 (dd, *J* = 9.2 Hz, 3.1 Hz, 1H, -CH_2-CH-), 5.22 (t, *J* = 9.2 Hz, 1H, -CH-CH_2-), 6.70 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.78 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.82 (dd, *J* = 7.8

Hz, 1H, Ar-H), 7.88 (dd, *J* = 7.8 Hz, 1H, Ar-H), 7.91 (d, *J* = 7.8 Hz, 1H, Ar-H), 13.24 (s, 1H, exchangeable with D_2O, -COOH); IR (KBr, ν_{max}, cm^{-1}): 3064 (-OH acid), 3076, 2969 (C-H alkane), 1740 (C=O amide), 1638, 1592 (arom. carbon), 1339 (C-N), 1233 (C-O), 825 (C-F); m.f. $C_{16}H_{11}NO_4F_2$ (m.w. 319.26) mass *m/z*(%): 320.10$(M+H)^+$.

RESULTS AND DISCUSSION

A series of compounds **5(a-j)** were synthesized as depicted in **Scheme-I**. The required compound **3** was synthesized by cyclization of 2-amino phenol (**1**) and ethyl 2,3-dibromo propionate (**2**) in presence of Cs_2CO_3 in acetonitrile at 90 ºC for 10 h. The cyclized compound **3** on reaction with acid chloride in the presence of pyridine in tetrahydrofuran at 65 ºC to afford compound **4**. The compound **4** which was further hydrolyzed using aqueous LiOH solution in methanol gave 4-aryl/alkyl substituted 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylic acid derivatives **5(a-j)** in a good to excellent yield (80-90 %) (**Scheme-I**, Table-1). The representative compound **5c** showed **IR** absorption at 3461 cm^{-1} due to (-OH acid stretching), 3063, 2955 (C-H alkane), band at 1743 cm^{-1} due to C=O stretching of amide group, 1645, 1518 cm^{-1} indicates presence of aromatic

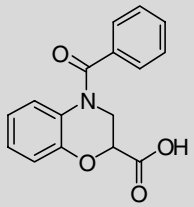
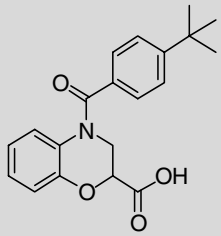
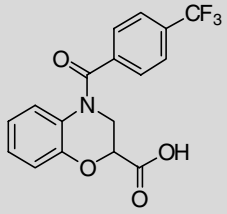
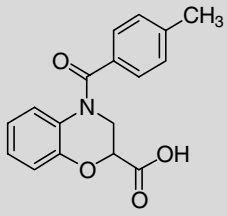


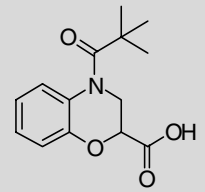
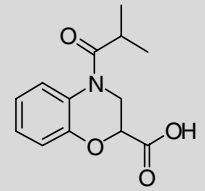
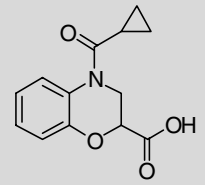
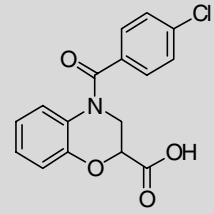
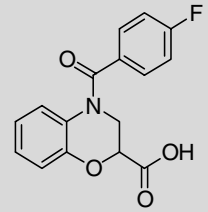
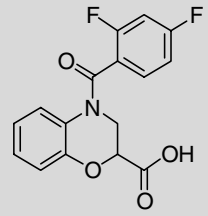
5a: R = C_6H_5
5b: R = 4-*t*-butyl-C_6H_4
5c: R = 4-$CF_3C_6H_4$
5d: R = 4-$CH_3C_6H_4$
5e: R = -$C(CH_3)_3$
5f: R = -$CH(CH_3)_2$
5g: R = Cyclopropyl
5h: R = 4-ClC_6H_4
5i: R = 4-FC_6H_4
5j: R = 2,4-*di*-FC_6H_3

Reagents and conditions:(a) Cs_2CO_3, CH_3CN, 90 °C, 10 h, 53 % (b) Pyridine, R-COCl, THF, 65 °C, 2 h, 70-80 % (c) LiOH, MeOH, H_2O, 0-25 °C, 2 h, 80-90 %

Scheme-I: Synthesis of 4-acyl substituted-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylic acid derivatives

TABLE-1
SYNTHESIS OF 3,4-DIHYDRO-2*H*-BENZO[*b*][1,4]OXAZINE-2-CARBOXYLIC ACID DERIVATIVES (**5a-j**)

Entry	Product (R)	Time (h)	Yield (%)	m.p. (°C)
5a		2.0	85	69-70
5b		2.1	80	121-122
5c		2.5	81	88-89
5d		2.0	87	92-93

5e		2.0	86	84-85
5f		2.0	83	90-91
5g		2.0	86	78-79
5h		2.0	89	110-111
5i		2.5	90	117-118
5j		2.5	89	132-133

[a]Isolated yields of the products after purification. [b]Yields of the products in final step. [c]All synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.

C-C stretching, 1332 cm^{-1} indicates presence of (C-N), 1230 indicates presence of (C-O). The ^{1}H NMR spectrum of compound **5c** (400MHz, DMSO-d_6): δ 3.66 (dd, *J* = 9.2 Hz, 3.9 Hz, 1H, -CH_2-CH-), 4.45 (dd, *J* = 9.2 Hz, 3.9 Hz, 1H, -CH_2-CH-), 5.18 (t, *J* = 9.2 Hz, 1H, -CH-CH_2-), 6.70 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.03 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.64 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.80 (d, *J* = 8.4 Hz, 2H, Ar-H), 13.44 (s, 1H, exchangeable with D_2O, -COOH); ^{13}C NMR (100 MHz, DMSO): δ 40.5, 73.4, 117.3, 120.0, 122.9, 124.6, 125.6, 126.2, 128.3, 129.5, 130.3, 130.6, 131.0, 139.8, 146.9, 167.4, 170.9; m.f. $C_{17}H_{12}NO_4F_3$ (m.w. 351.28) mass *m/z*(%): 352.47(M+H)$^+$.

Anti-tubercular activity: All the synthesized compounds **5a-j** were screened for their *in vitro* antitubercular activity against *M. tuberculosis* H_{37}Ra with concentrations of 30, 10 and 3 μg/mL using XRMA (H37Ra). The percentage inhibition at above concentrations was calculated and tabulated in Table-2. Compounds **5a**, **5c**, **5d**, **5f**, **5g**, **5h** and **5i** exhibited moderate anti-mycobacterial potency (inhibiting >70% of mycobacterial growth at 30 μg/mL with rifampicin as reference drug). In general, the newly synthesized compounds showed moderate selectivity towards *M. tuberculosis* H_{37}Rastrains. The anti-tubercular data obtained showed growth inhibition MTB can be impacted by the introduction of a 4-isobutyryl group (**5f**), MIC = 10.12 μg/mL.

Antibacterial study: The anti-tubercular screening results indicate the fact that the synthesized compounds will exhibits antimicrobial activity. Therefore, antibacterial screening of the synthesized compounds was conducted against four bacteria strains (Gram-negative strains: *E.coli* and *S.aureus*; Gram-positive strains: *P. aeruginosa* and *B. subtilis.* Compounds **5a**, **5b**, **5c**, **5e** and **5j** showed higher antibacterial activity up to 7-25 μg/mL (Table-3).

Molecular docking study: Lipid rich cell wall poses a major challenge in delivery of antimycobacterial agents in the

TABLE-2
ANTITUBERCULAR ACTIVITY OF COMPOUNDS AGAINST AVIRULENT STRAIN OF DORMANT MTBH37Ra

Entry	% Inhibition of *Mycobacterium tuberculosis* H37Ra growth in presence of compounds			IC_{50}	MIC
	30 µg/mL	10 µg/mL	3 µg/mL		
5a	89.64	79.83	77.70	10.42	15.83
5b	22.80	14.54	11.20	> 30	> 30
5c	87.04	88.01	84.17	11.81	17.36
5d	80.50	68.40	22.46	18.79	> 30
5e	18.80	11.58	20.74	> 30	> 30
5f	88.18	86.74	89.92	5.98	10.12
5g	86.09	37.02	15.47	19.21	> 30
5h	70.03	79.13	39.05	24.81	> 30
5i	84.30	70.48	81.30	14.81	28.16
5j	16.29	19.16	13.01	> 30	> 30

[a]IC_{50}/MIC in µg/mL. Antubercular activity of each agent was determined by serial dose dependent dilutions. [b]Standard antitubercular drugs and positive controls. Data were expressed as the means of triplication.

TABLE-3
ANTIBACTERIAL ACTIVITY OF COMPOUNDS AGAINST SELECTED STRAIN

Entry	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilus*
	MIC	MIC	MIC	MIC
5a	22.23	17.23	19.32	>30
5b	9.36	7.65	15.78	12.98
5c	>30	25.39	11.36	4.28
5d	>30	>30	>30	>30
5e	18.88	14.56	23.37	28.53
5f	>30	>30	>30	>30
5g	>30	>30	>30	>30
5h	>30	>30	>30	>30
5i	>30	>30	>30	>30
5j	7.65	5.44	9.89	14.61
Ampicillin	1.46	4.36	1.0	10.32
Kanamycin	1.62	0.49	>30	1.35

[a]IC_{50}/MIC in µg/mL. Antibacterial activity of each agent was determined by serial dose dependent dilutions. [b]Standard antibacterial drugs and positive controls. Data were expressed as the means of triplication.

treatment of *M. tuberculosis*. Large number of genes encoding various enzymes which catalyze the cell wall biosynthesis has been reported [29]. Amongst them the panC gene, which encodes the pantothenate synthetase (PS), remains an attractive target due to its exclusive presence in microorganisms and absence in humans. Pantothenate synthetase catalyzes the production of pantothenate, a key precursor for the biosynthesis of co-enzyme A (CoA) and acyl carrier protein (ACP). The CoA and the ACP play a crucial role in fatty acid biosynthesis, signalling pathways [30]. In recent study, the crystal structure of pantothenate synthetase with the bound ligand (*2S*)-2,3-dihydro-1,4-benzodioxine-2-carboxylic acid was solved, which suggests the inhibitors against this target can become potential agents in the treatment of resistant type of *M. Tuberculosis*. The ligand bound X-ray crystal structure of this complex (PDB:4G5F), reveals that the residues Met40, His47 and Gln164 forms the hydrogen bonds with the oxygen atoms of carboxylate group and oxygen atom of dioxane ring (Fig. 2). These residues are

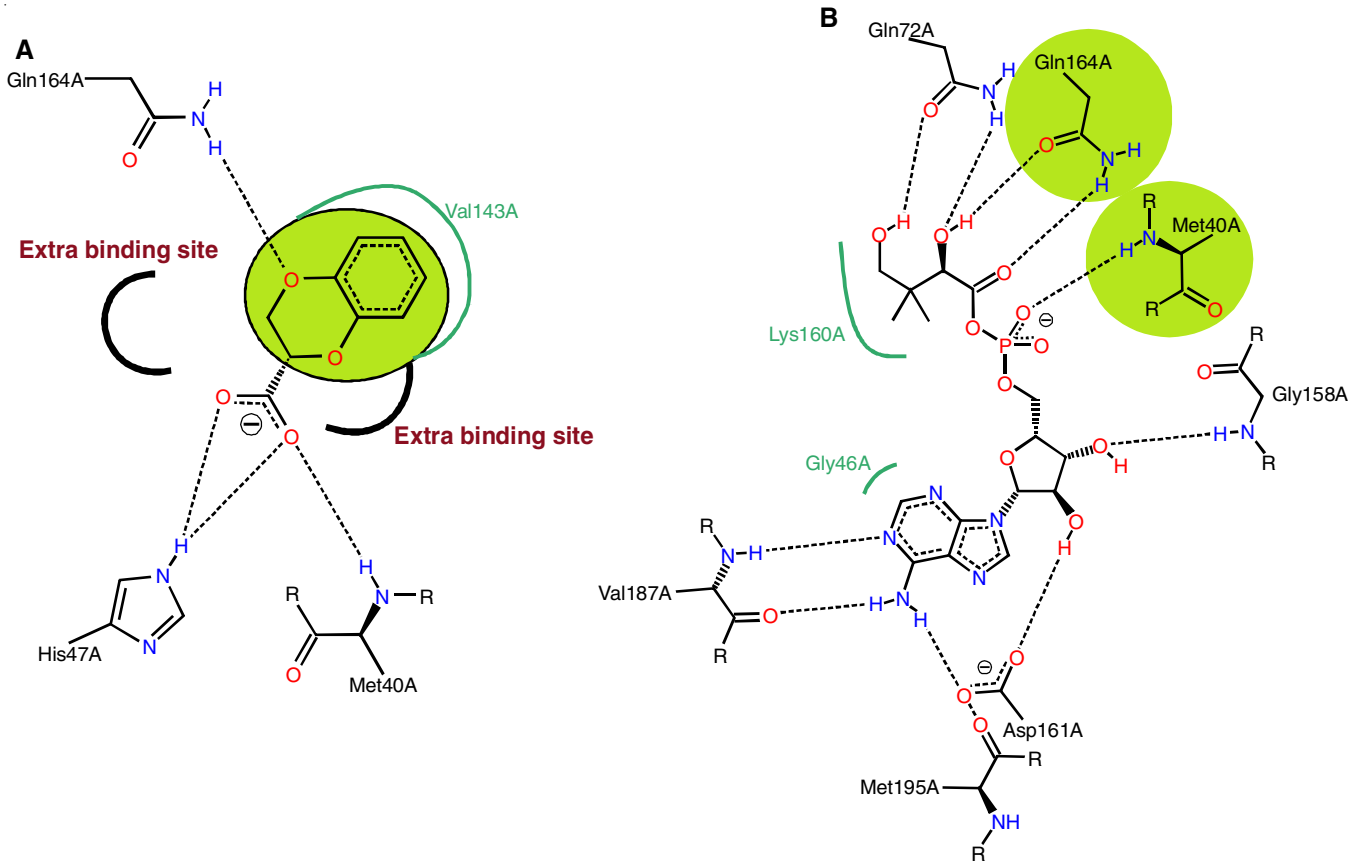


Fig. 2. Key interactions of ligand/substarate at the binding site of panC, (A) interactions with 1,4-benzodioxine-2-carboxylic acid; (B) interactions with pantoate-AMP

also involved in making such hydrogen bonds with the key intermediate pantoate-adenosine monophosphate (AMP) at the binding site of panC. The neighbouring binding cavities holding the residues which can either form hydrophobic or hydrogen bond interactions such as Lys160, Gly46, Gly158, Asp161, Met195 and Gln72 are also important in panC inhibiton [31, 32]. In present work, the synthesized compounds could possibly interact with panC was also investigated through docking studies. It is hypothesized that the additional substituent on benzoxazine-2-carboxylic acid core ring (which closely resembles the co-crystallized ligand benzodioxine-2-carboxylic acid) could access these extra binding pockets and enhance the inhibitory effects.

Molecular docking: The X-ray crystal structure of pantothenate synthetase (panC) in complex with the ligand (*2S*)-2,3-dihydro-1,4-benzodioxine-2-carboxylic acid (PDB ID: 4G5F) was retrieved from the RCSB protein data bank (http://www.rcsb.org/pdb). The molecular docking study was carried out with Autodock vina [33] with rigid docking protocol where the protein structure was kept rigid and ligand structure was kept flexible. The 2D structures of all the compounds were drawn with MarvinSketch 5.6.0.0 which were subsequently transformed into 3D geometry. The geometry of 3D molecules was optimized using UCSF Chimera 1.8 [34] using combination of steepest descent and conjugate gradient geometry search criteria until gradient converses to 0.05 and 0.001, respectively. The crystal structure of panC was also optimized in order to find correct protonation states of key residues, close contacts in the residues and possible clashes of atoms. The crystallized water and other non-standard residues were removed first and the protein was subjected to energy minimization in UCSF Chimera with Amber ff12SB force field. During docking simulation Kollman charges and polar hydrogen were added to protein structure. Grid box of size 16 × 16 × 16 with 1 Å spacing and with X Y Z centres 14.823, 10.671 and 5.488 was chosen to cover the entire binding site of protein. The docking protocol was validated which involves testing the docking programs ability to predict the crystallographic or experimental pose of ligand. The spatial similarity between predicted pose and crystallographic pose is generally measured by RMSD value. The docking protocol is considered to be validated if the RMSD between experimental and predicted pose is below 2 Å [35]. The synthesized compounds were docked using validated docking protocol at the binding site of panC. Discovery studio visualize and PoseView program [36] was utilized for analysis of the docking results. Docking simulation results were analyzed by comparing the binding free energy in Kcal/mol and interactions of ligands with residues at the active site.

Number of recent investigations [29,37,38] in which the *in silico* approaches were explored suggest that these approaches are indeed helpful in design of *M. tuberculosis* panC inhibitors. Thus, in present study we employed one of the structure based design approaches namely molecular docking to investigate the possible modes of binding of compounds under study. The molecular docking studies though reasonably accurate require validation of docking protocol adopted and re-docking experiments. In order to validate the docking protocol the RMSD between crystallographic and predicted pose of co-crystallized ligand was measured which was found to be below 2 Å (Fig. 3).

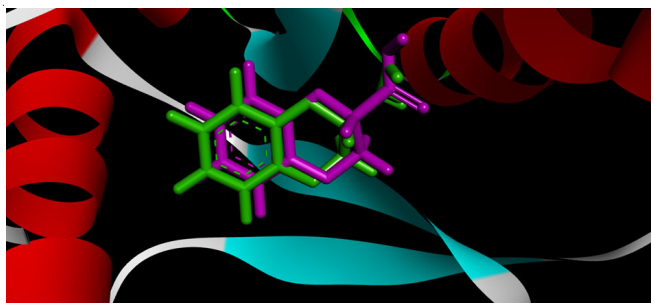


Fig. 3. Docked pose of the ligand in cyan and co-crystallized ligand in green

The residues Met40, His47 and Gln164 are key residues in the formation of hydrogen bonds with the ligand hydrogen bond donors and acceptors. Few other residues are involved in hydrophobic interactions such as Val143, Lys160, Gly46, Gly158, Asp161, Met195 and Gln72. These interactions are possibly responsible for the inhibitory potential of the panC inhibitors and to understand the possible mode of inhibition of panC the molecular docking study was carried out. All the compounds under study and the co-crystallized ligand were successfully docked into the binding site panC. The compounds were found having varying degrees of affinity and varying mode of non-bonded interactions between ligand heteroatoms and nearest residues were observed at the binding site of the ligands. The estimated free energy of binding in Kcal/mol and the key interacting residues are presented in Table-4. Autodock vina program gives the estimates of free energy of binding from the systems intermolecular energy (sum of van der Waal's, hydrogen bond, desolvation and electrostatic energies), total internal energy, torsional free energy and unbound system's energy.

TABLE-4
ESTIMATED BINDING FREE ENERGY AND INTERACTIONS

Entry	EFEB	Key interactions	
		Hydrogen bond interaction	Hydrophobic interaction
L	-6.5	Met40, His47, Gln164	Val143, Pro38
5a	-9.5	Met40, Tyr82	His47, Lys160
5b	-7.6	Lys160, Tyr82	Met40, His44, Asp161
5c	-9.4	Lys160, Gln72	Tyr82, Met40, His44
5d	-8.3	Lys160, Tyr82	His44, Asp161, Met40
5e	-7.8	Ser196, Lys160	His44, Asp161, Met40
5f	-9.6	Ser196, Lys160	Asp161, His44
5g	-8.4	Ser196, Lys160	Met40, His47, Asp161, His44
5h	-8.2	Lys160, Tyr82, Asp161	His44, Met40, Val142
5i	-8.5	Lys160, Tyr82, Asp161	His44, Met40
5j	-7.9	Met40, Tyr82	His44, Lys160, Gln164

L = Co-crystalized ligand; EFEB = Estimated free energy of binding (Kcal/mol)

The docking studies clearly suggest the role of hydrogen bond formation with key residues such as Lys160, Met40, Tyr82, Asp161 and Gln72. The hydrophobic π-π stacking interactions with some residues such as His47, Gln164 and Val142 are also crucial. The estimated free energy of binding in Kcal/mol is in the range -7.6 to -9.6, whereas the free energy of binding for co-crystalized ligand is -6.5 Kcal/mol. This suggests

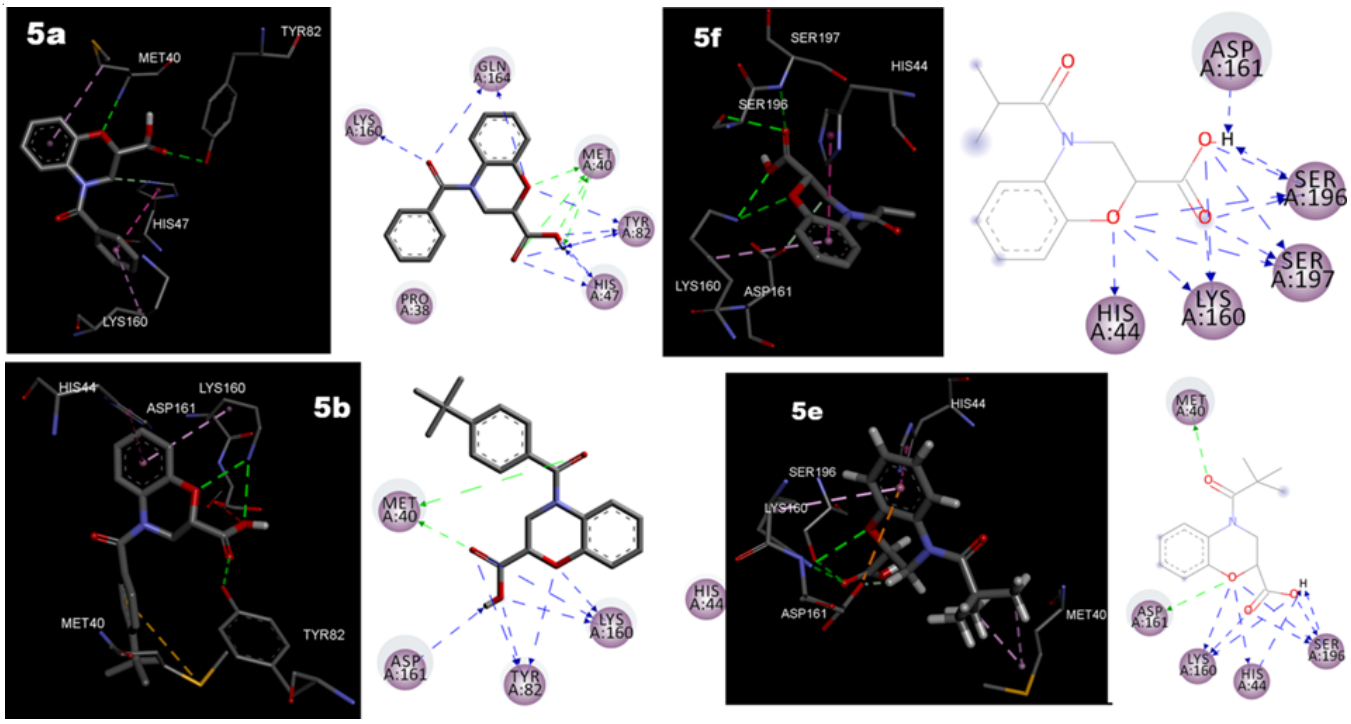


Fig. 4. Interactions observed in docking studies for compounds under study

that the compounds under study have better affinity at the binding site compared to the co-crystallized ligand. The lower binding free energy in the compounds may be attributed to the hydrogen bond formation and hydrophobic interactions. The compounds **5a**, **5c** and **5f** with free energy of binding lower than -9.0 Kcal/mol binds more favourably at the binding site of panC as compared to other compounds. Specifically, the compound **5f** with free energy of binding -9.6 Kcal/mol is indeed found more active in docking study as well as in the *in vitro* antitubercular screening. Fig. 4 shows the interactions produced at the binding site of panC with some more active compounds **5a** and **5f** and less active compounds **5b** and **5e**. In most of the compounds the oxygen of benzoxazine ring act as a hydrogen bond acceptor and the carboxylic acid group also form hydrogen bonds with carboxylate oxygen. The substituent on the nitrogen atom of benzoxazine ring was found involved in either hydrogen bond formation or hydrophobic interaction. The docking results are in good agreement with *in vitro* antitubercular screening. The key interactions observed in docking study are shown in Fig. 3.

Conclusion

In conclusion, a series of 4-aryl/alkyl substituted-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-carboxylic acid derivatives is synthesized and screened of their antitubercular and antibacterial activity. The synthesized compounds were characterized and confirmed by spectral data. The docking results are in good agreement with *in vitro* antitubercular screening. The compounds **5a**, **5c** and **5f** with free energy of binding lower than -9.0 Kcal/mol binds more favourably at the binding site of panC as compared to other compounds. Specifically, compound **5f** with free energy of binding -9.6 Kcal/mol is indeed found more active in docking study as well as in the *in vitro* antitubercular screening. The synthesized compounds were screened for anti-tubercular and antimicrobial studies. Compounds **5a**, **5c** and **5i** were found good anti-tubercular against *Mycobacterium tuberculosis* H37Ra *Mycobacterium bovis* BCG strains but compound **5f** shows a excellent anti-tubercular potential. Compounds **5a**, **5b**, **5c**, **5e** and **5j** showed higher antibacterial activity up to 7-25 µg/mL.

ACKNOWLEDGEMENTS

The authors are thankful to National Chemical Laboratory, Pune, India for biological screening and The Principal, PVP College, Pravaranagar, India for providing necessary the research and laboratory facilities.

REFERENCES

1. L. Zhang, J. Mao, S. Wang, Y. Zheng, X. Liu and Y. Cheng, Benzoxazine Based High Performance Materials with Low Dielectric Constant: A Review, *Curr. Org. Chem.*, **23**, 809 (2019); https://doi.org/10.2174/1385272823666190422130917
2. M. Akhter, S. Habibullah, S.M. Hasan, M.M. Alam, N. Akhter and M. Shaquiquzzaman, Synthesis of Some New 3,4-Dihydro-2*H*-1,3-Benzoxazines Under Microwave Irradiation in Solvent-Free Conditions and their Biological Activity, *Med. Chem. Res.*, **20**, 1147 (2011); https://doi.org/10.1007/s00044-010-9451-x
3. S. Cui, C.R. Arza, P. Froimowicz and H. Ishida, Developing Further Versatility in Benzoxazine Synthesis *via* Hydrolytic Ring-Opening, *Polymers*, **12**, 694 (2020); https://doi.org/10.3390/polym12030694
4. N.A. El-Salam and S. El-Din, 3,4-Dihydro-2*H*-1,3-benzoxazines and their Oxo-Derivatives-Chemistry and Bioactivities, *J. Serb. Chem. Soc.* (2019); https://doi.org/10.2298/JSC1805300015
5. X. Li, N. Liu, H. Zhang, S.E. Knudson, R.A. Slayden and P.J. Tonge, Synthesis and SAR Studies of 1,4-Benzoxazine MenB Inhibitors: Novel Antibacterial Agents against *Mycobacterium tuberculosis*, *Bioorg. Med. Chem. Lett.*, **20**, 6306 (2010); https://doi.org/10.1016/j.bmcl.2010.08.076

6. S. Konda, S. Raparthi, K. Bhaskar, R.J. Munaganti, V. Guguloth, L. Nagarapu and D.M. Akkewar, Synthesis and Antimicrobial Activity of Novel Benzoxazine Sulfonamide Derivatives, *Bioorg. Med. Chem. Lett.*, **25**, 1643 (2015); https://doi.org/10.1016/j.bmcl.2015.01.026
7. D.F. Fidler, Legal Issues Associated with Antimicrobial Drug Resistance, *Emerg. Infect. Dis.*, **4**, 169 (1998); https://doi.org/10.3201/eid0402.980204
8. T. Yamakawa, J. Mitsuyama and K.J. Hayashi, *in vitro* and *in vivo* Antibacterial Activity of T-3912, A Novel Non-Fluorinated Topical Quinolone, *J. Antimicrob. Chem.*, **49**, 455 (2002); https://doi.org/10.1093/jac/49.3.455
9. A. Korolyov, S. Dorbes, J. Azéma, B. Guidetti, M. Danel, D. Lamoral-Theys, T. Gras, J. Dubois, R. Kiss, R. Martino and M. Malet-Martino, Novel Lipophilic 7*H*-Pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic Acid Derivatives as Potential Antitumor Agents: Improved Synthesis and *in vitro* Evaluation, *Bioorg. Med. Chem. Lett.*, **18**, 8537 (2010); https://doi.org/10.1016/j.bmc.2010.10.039
10. R. Fringuelli, N. Giacche, L. Milanese, E. Cenci, A. Macchiarulo, A. Vecchiarelli, G. Costantino and F. Schiaffella, Bulky 1,4-Benzoxazine Derivatives with Antifungal Activity, *Bioorg. Med. Chem.*, **17**, 3838 (2009); https://doi.org/10.1016/j.bmc.2009.04.051
11. J. Peng, Q. Hu, C. Gu, B. Liu, F. Jin, J. Yuan, J. Feng, L. Zhang, J. Lan, Q. Dong and G. Cao, Discovery of Potent and Orally Bioavailable Inhibitors of Human Uric Acid Transporter 1 (hURAT1) and Binding Mode Prediction using Homology Model, *Bioorg. Med. Chem. Lett.*, **26**, 277 (2016); https://doi.org/10.1016/j.bmcl.2015.12.040
12. H. Cho, Y. Iwama, K. Okano and H. Tokuyama, Synthesis of a Human Urate Transporter-1 Inhibitor, an Arginine Vasopressin Antagonist, and a 17β-Hydroxysteroid Dehydrogenase Type-3 Inhibitor, Using Ring-Expansion of Cyclic Ketoximes with DIBALH, *Chem. Pharm. Bull. (Tokyo)*, **62**, 354 (2014); https://doi.org/10.1248/cpb.c13-00961
13. I. Kimiyoshi, New Antihyperuricemic Medicine: Febuxostat, Puricase, *etc*., *Nihon Rinsho* (*J. Clin. Med.*), **66**, 759 (2008) (In Japanese).
14. K. Hirata, N. Ogawa, Y. Shinagawa, T. Kiguchi, T. Inoue, Y. Komeda, I. Yamashita and Y. Kamiya, Method for Producing Nitrogen Containing Fused Ring Compound, WO20071390002 (2017).
15. C. Hidetsura, Y. Iwama, K. Okano and H. Tokuyama, Synthesis of a Human Urate Transporter-1 Inhibitor, an Arginine Vasopressin Antagonist, and a 17β-Hydroxysteroid Dehydrogenase Type-3 Inhibitor, using Ring-Expansion of Cyclic Ketoximes with DIBALH, *Chem. Pharm. Bull*., **62**, 354 (2014); https://doi.org/10.1248/cpb.c13-00961
16. V. Hernandez-Olmos, A. Abdelrahman, A. El-Tayeb, D. Freudendahl, S. Weinhausen and C.E. Müller, N-Substituted Phenoxazine and Acridone Derivatives: Structure-Activity Relationships of Potent P2X4 Receptor Antagonists, *J. Med. Chem.*, **55**, 9576 (2012); https://doi.org/10.1021/jm300845v
17. M.S. Tambe, A. Choudhari, D. Sarkar, J. Sangshetti, R. Patil and S.S. Gholap, Design, Synthesis and Biological Screening of Novel 1,3,4-Oxadiazoles as Antitubercular Agents, *ChemistrySelect*, **3**, 13304 (2018); https://doi.org/10.1002/slct.201802227
18. M.S. Tambe, S.S. Gholap, L. Nawale, D. Sarkar, J. Sangshetti and M. Damale, Design, Synthesis and Pharmacological Evaluation of Fluorinated Azoles as Anti-tubercular Agents, *Arch. Pharm*, **351**, 1700294 (2018); https://doi.org/10.1002/ardp.201700294
19. S.S. Gholap, M.S. Tambe, S. Chakraborty, R. Borkute, A. Choudhri, D. Sarkar, J.N. Sangshetti and R.B. Patil, Synthesis and Biological Screening of Fluorinated Analogues of 4-aminoquinoline Derivatives as Antitubercular Agents, *Asian J. Org. Med. Chem.*, **3**, 190 (2018); https://doi.org/10.14233/ajomc.2018.AJOMC-P158
20. N. Muthukaman, S. Deshmukh, S. Tondlekar, M. Tambe, D. Pisal, N. Sarode, S. Mhatre, S. Chakraborti, D. Shah, V.M. Bhosale, A. Kulkarni, M.Y.A. Mahat, S.B. Jadhav, G.S. Gudi, N. Khairatkar-Joshi and L.A. Gharat, Discovery of 5-(2-chloro-4′-(1H-imidazol-1-yl)-[1,1′-biphenyl]-4-yl)-1*H*-tetrazole as Potent and Orally Efficacious S-Nitrosoglutathione Reductase (GSNOR) Inhibitors for the Potential Treatment of COPD, *Bioorg. Med. Chem. Lett.*, **28**, 3766 (2018); https://doi.org/10.1016/j.bmcl.2018.10.012
21. N. Muthukaman, S. Deshmukh, M. Tambe, D. Pisal, S. Tondlekar, M. Shaikh, N. Sarode, V.G. Kattige, P. Sawant, M. Pisat, V. Karande, S. Honnegowda, A. Kulkarni, D. Behera, S.B. Jadhav, R.R. Sangana, G.S. Gudi, N. Khairatkar-Joshi and L.A. Gharat, Alleviating CYP and hERG Liabilities by Structure Optimization of Dihydrofuran-Fused Tricyclic Benzo[*d*]imidazole Series-Potent, Selective and Orally Efficacious Microsomal Prostaglandin E Synthase-1 (mPGES-1) Inhibitors: Part-2, *Bioorg. Med. Chem. Lett.*, **28**, 1211 (2018); https://doi.org/10.1016/j.bmcl.2018.02.048
22. N. Muthukaman, M. Tambe, S. Deshmukh, D. Pisal, S. Tondlekar, M. Shaikh, N. Sarode, V.G. Kattige, M. Pisat, P. Sawant, S. Honnegowda, V. Karande, A. Kulkarni, D. Behera, S.B. Jadhav, R.R. Sangana, G.S. Gudi, N. Khairatkar-Joshi and L.A. Gharat, Discovery of Furan and Dihydrofuran-Fused Tricyclic Benzo[*d*]imidazole Derivatives as Potent and Orally Efficacious Microsomal Prostaglandin E Synthase-1 (mPGES-1) inhibitors: Part-1, *Bioorg. Med. Chem. Lett.*, **27**, 5131 (2017); https://doi.org/10.1016/j.bmcl.2017.10.062
23. N. Muthukaman, M. Tambe, M. Shaikh, D. Pisal, S. Deshmukh, N. Sarode, S. Tondlekar, L. Narayana, J.M. Gajera, V.G. Kattige, S. Honnegowda, V. Karande, A. Kulkarni, D. Behera, S.B. Jadhav, G.S. Gudi, N. Khairatkar-Joshi and L.A. Gharat, Tricyclic 4,4-Dimethyl-3,4-dihydrochromeno-[3,4-*d*]imidazole Derivatives as Microsomal Prostaglandin E2 Synthase-1 (mPGES-1) Inhibitors: SAR and *in vivo* Efficacy in Hyperalgesia Pain Model, *Bioorg. Med. Chem. Lett.*, **27**, 2594 (2017); https://doi.org/10.1016/j.bmcl.2017.03.068
24. N. Muthukaman, S. Deshmukh, N. Sarode, S. Tondlekar, M. Tambe, D. Pisal, M. Shaikh, V.G. Kattige, S. Honnegowda, V. Karande, A. Kulkarni, S.B. Jadhav, M.Y.A. Mahat, G.S. Gudi, N. Khairatkar-Joshi and L.A. Gharat, Discovery of 2-((2-Chloro-6-fluorophenyl)amino)-*N*-(3-fluoro-5-(trifluoromethyl)phenyl)-1-methyl-7,8-dihydro-1*H*-[1,4]dioxino-[2′,3′:3,4]benzo[1,2-*d*]imidazole-5-carboxamide as Potent, Selective and Efficacious Microsomal Prostaglandin E2 Synthase-1 (mPGES-1) Inhibitor, *Bioorg. Med. Chem. Lett.*, **26**, 5977 (2016); https://doi.org/10.1016/j.bmcl.2016.10.079
25. M.S. Tambe and S.S. Gholap, An Efficient Synthesis of 1,8-Dioxooctahydroxanthenes Promoted by Thiamine hydrochloride (VB_1), *Chem. Biol. Interact.*, **6**, 344 (2016).
26. S.S. Gholap, U.P. Deshmukh and M.S. Tambe, Synthesis and *in vitro* Antimicrobial Screening of 3-Cinnamoyl Coumarin and 3-[3-(1*H*-Indol-2-yl)-3-aryl-propanoyl]-2*H*-chromen-2-ones, *Iranian J. Catal.*, **3**, 171 (2013).
27. S.S. Gholap, V.D. Dhakane, S.N. Shelke, and M.S. Tambe, Green and Sustainable Approach to *bis*-(Indolyl)alkanes, *Bull. Catal. Soc. India,* **11**, 50 (2012).
28. S.S. Gholap, M.S. Tambe, V.L. Malawade and C.H. Gill, Transition Metal Free Michael Addition of Indole or Thiol under Heterogeneous Conditions, *Int. J. Chem. Sci.*, **5**, 2384 (2007).
29. K. Takayama, C. Wang and G.S. Besra, Pathway to Synthesis and Processing of Mycolic Acids in *Mycobacterium tuberculosis*, *Clin. Microbiol. Rev.*, **18**, 81 (2005); https://doi.org/10.1128/CMR.18.1.81-101.2005
30. P.B. Devi, S. Jogula, A.P. Reddy, S. Saxena, J.P. Sridevi, D. Sriram and P. Yogeeswari, Design of Novel *Mycobacterium tuberculosis* Pantothenate Synthetase Inhibitors: Virtual Screening, Synthesis and *in vitro* Biological Activities, *Mol. Inform.*, **34**, 147 (2015); https://doi.org/10.1002/minf.201400120
31. H.L. Silvestre, T.L. Blundell, C. Abell and A. Ciulli, Integrated Biophysical Approach to Fragment Screening and Validation for Fragment Based Lead Discovery, *Proc. Natl. Acad. Sci. USA*, **110**, 12984 (2013); https://doi.org/10.1073/pnas.1304045110
32. S. Wang and D. Eisenberg, Crystal Structures of a Pantothenate Synthetase from *M. tuberculosis* and its Complexes with Substrates and a Reaction Intermediate, *Protein Sci.*, **12**, 1097 (2003); https://doi.org/10.1110/ps.0241803
33. O. Trott and A.J. Olson, AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization and Multithreading, *J. Comput. Chem.*, **31**, 455 (2010); https://doi.org/10.1002/jcc.21334
34. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng and T.E. Ferrin, UCSF Chimera-A visualization System for Exploratory Research and Analysis, *J. Comput. Chem.*, **25**, 1605 (2004); https://doi.org/10.1002/jcc.20084

35. R.D. Kamble, R.J. Meshram, S.V. Hese, R.A. More, S.S. Kamble, R.N. Gacche and B.S. Dawane, Synthesis and *in silico* Investigation of Thiazoles Bearing Pyrazoles Derivatives as Anti-inflammatory Agents, *Comput. Biol. Chem.*, **61**, 86 (2016); https://doi.org/10.1016/j.compbiolchem.2016.01.007
36. K. Stierand and M. Rarey, PoseView-Molecular Interaction Patterns at a Glance, *J. Cheminform.*, **2(S1)**, 50 (2010); https://doi.org/10.1186/1758-2946-2-S1-P50
37. S. Prasanth Kumar, P. Srinivasan, S.K. Patel, R. Kapopara and Y.T. Jasrai, *in silico* Development of Inhibitors against Pantothenate Synthetase of *Mycobacterium tuberculosis*, *J. Adv. Bioinform. Appl. Res.*, **2**, 142 (2011).
38. S. Velaparthi, M. Brunsteiner, R. Uddin, B. Wan, S.G. Franzblau and P.A. Petukhov, 5-*tert*-Butyl-*N*-pyrazol-4-yl-4,5,6,7-tetrahydrobenzo[*d*]-isoxazole-3-carboxamide Derivatives as Novel Potent Inhibitors of *Mycobacterium tuberculosis* Pantothenate Synthetase: Initiating a Quest for New Antitubercular Drugs, *J. Med. Chem.*, **51**, 1999 (2008); https://doi.org/10.1021/jm701372r