Design, Synthesis and Antibacterial Studies of Some New Pyridopyrimidine Derivatives as Biotin Carboxylase Inhibitors

Original Article

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ABSTRACT

Present study reports the development of novel pyridopyrimidine derivatives as biotin carboxylase inhibitors with potent antibacterial activity. These compounds were designed to avoid possibility of resistance development. Accordingly eighteen compounds were synthesised and characterized on the basis of spectral data. These compounds were tested for their antibacterial potential by the enzyme kinetic assay against the biotin carboxylase. The minimum inhibitory concentration (MIC) and single step resistance studies were also performed. Compound 2-((2-Phenylpyrido[2,3-d] pyrimidin-4-yl)amino)phenol (6o) showed promising activity in biotin carboxylase inhibition with low MIC. It showed molecular docking score of -7.96, this compound showed formation of hydrogen bonds with the active site residues and van Der Walls interactions. The MIC of compounds under investigation was in the rage of 2-5µg/mL over most of the strains studied. It also showed the mutant selection windows of around five which is better than the reference compound rifampin. This compound 6o can be studied further and developed into a potential antibacterial lead molecule.

Key Words: Biotin carboxylase, drug resistance, molecular docking, pyridopyrimidine.

Received: 02 March 2019**, Accepted:** 30 June 2019

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ISSN: 1110-0930, xxx 2020 Vol.58, No. 1

INTRODUCTION

In 2014, the World Health Organization (WHO) released an alarming report on microbial resistance to antibiotics especially in bacteria. The WHO report show a comprehensive picture of data obtained from 114 countries.[1] According to it at least seven different common bacteria cause serious diseases like postoperative infections, blood stream infections, hospital acquired infections and gonorrhoea have become drug resistant. Lot of information regarding the spread of drug resistant microorganisms are available still a big gap is observed in tracking antibiotic resistance. United States alone reported over two million deaths due to bacterial infection and 23,000 deaths due to drug resistant bacterial strains.[2] One of the major reasons for rise of drug resistance is lack of new group of antibiotics. The penicillin was discovered during late 1920s, followed by cephalosporin in late 1940s. After a gap of two decades carbapenams and fluoroquinolones were discovered in the 80s. However, since then no such breakthrough discovery has been reported.[3] It is known that about 30-40 targets are generally harnessed for drug discovery process against the bacteria, thus there is an urgent need for smart molecules that target novel targets or multiple targets.[4]

The development of techniques like virtual screening, pharmacophore generation, and fragment based drug discovery, advances in drug delivery has resulted in discovery of newer compounds and validation of newer targets and their proper administration to patients.[5, 6] One of the major metabolic pathways found in bacteria is the fatty acid synthesis pathway and has become a popular target among medicinal chemist. In a recent development, the enzyme biotin carboxylase (BC) was found to be a very attractive target for inhibiting the Acetyl CoA carboxylase mediated reaction. BC plays a vital role in Acetyl CoA carboxylase (ACC) catalysed reactions. Biotin carboxylases catalyse the carboxylation of biotin carboxyl carrier protein (BCCP)-biotin in the presence of bicarbonate to form the BCCP-biotin-CO_2. In the next step, BCCP-biotin-CO_2 transfer the carboxyl group to Acetyl-CoA and forms malonyl-CoA in presence of carboxyltransferase (CT) (Figure 1).[7] The heterocyclic moieties like the pyridopyrimidines such as i to iv were reported to possess significant BC inhibitory activity (Figure 2).[8] Mochalkin *et al*. showed that this class of compounds bind to the ATP binding site of bacterial BC did not bind or inhibit the human BC.[9] Several recent reports on the development of BC inhibitors have validated it as a potential drug target for the development of novel antibacterial compounds.

 DOI: 10.21608/bfpc.2019.10149.1017

Earlier, we have reported the development of various heterocyclic scaffolds like pyrimidines and benzazoles as anti-bacterial and anti-tubercular agents.[10-12] In continuation to our efforts we aim to hit this newly validated target for the development of novel antibacterial agents on the basis of structural analogy and molecular docking studies. The pyridopyrimidine scaffolds provides for a wide range of activity like Bcr-abl inhibitors[13], MexAB-OprM efflux pump inhibitor[14], Adenosine kinase inhibitors[15], PI3K/mTOR dual inihibition[16], Jak1/2 inhibitor[17], cell cycle inhibition[18], antitubercular activity[19, 20], antioxidant activity[21] and antibacterial activity.[9, 22-27] Herein, we report the synthesis, antibacterial evaluation with resistance studies of some novel pyridopyrimidine derivatives and molecular docking analysis of the synthesised compounds.

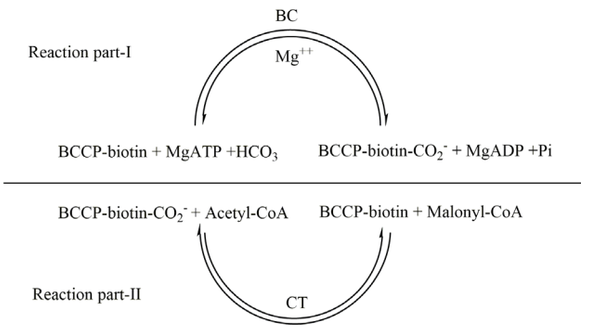


Fig. 1: Reactions catalysed by biotin carboxylase and carboxyltransferase

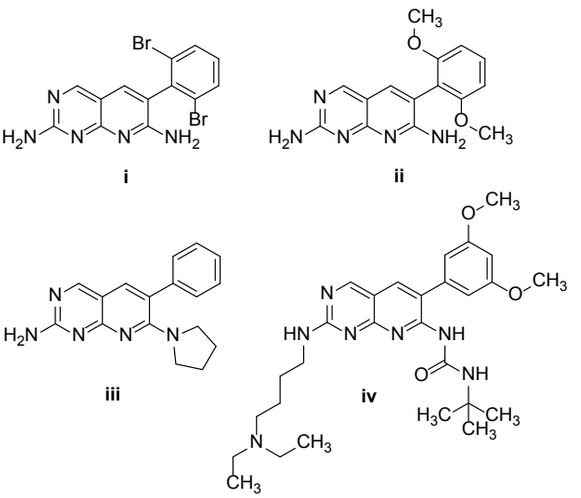


Fig. 2: Pyridopyrimidine inhibitors of biotin carboxylase

2 Experimental

Chemicals, assay kits, reagents and materials were obtained from Sigma Aldrich, Germany, USA and Alfa Aeser, UK. Analytical grade solvents were obtained from the E. Merck, India. Melting points (mp) were detected with open capillaries using Thermonik Precision Melting point cum Boiling point apparatus (C-PMB-2, Mumbai, India) and are uncorrected. Chromatography of the synthesized intermediates and title compounds were performed on silica gel pre-coated plates (Merck: 100–200 mesh). Thin layer chromatography (TLC) was used to monitor the progress and/or completion of the reactions. IR spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Japan). ^{1}H and ^{13}C NMR was obtained using a Bruker Advance-II 400 Spectrometer on 400 MHz using tetramethylsilane (TMS) as internal standard. All chemical shift values were recorded as δ (ppm), coupling constant value J is measured in hertz, the peaks are presented as s (singlet), d (doublet), t (triplet), br s (broad singlet), dd (double doublet), m (multiplet). The purity of compounds was controlled by thin layer chromatography (Merck, silica gel, HF254-361, type 60, 0.25 mm, Darmstadt, Germany). Mass spectra (ESI-MS) were recorded at ESI-MS spectrometer (DPX-400, Bruker, USA). Shimadzu High Performance Liquid Chromatography (HPLC) system with SPD-20A prominence Photodiode Array (PDA) detector and LC-20AB prominence LC solution software were used in this study.

2.2 Materials and Methods

2.1.1. Synthesis of methyl 2-aminonicotinate(2)

2-Aminopyridine 3-carboxyllic acid[1] (13.8g, 0.1 mole) was dissolved in 400 mL of anhydrous methanol, to this solution thionyl chloride (11.8mL, 14.03g, 0.1 mole) was added dropwise for half hour with constant stirring. On complete addition, it was refluxed for 12 hours after which the reaction mixture was cooled to room temperature. The solvent from the mixture was evaporated under vacuum and remaining residue was dissolved in chloroform. The chloroform layer was collected and washed with 5% HCl and 10% $NaHCO_3$ followed by water. This layer was further dried over sodium sulphate and concentrated to give methyl 2-aminonicotinate.

Yield: 71%. mp: 126-129°C (ethanol). IR (KBr) cm^{-1}: 3424.1, 3319.2, 1655.1, 1618.5, 1601.1. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 3.86(s, 3H, CH3), 7.0(d, 1H, J=7.01, Ar), 7.44(dd, 1H, J=7.45, Ar), 8.69(s, 1H, Ar), 12.19(bs, 2H, NH2). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 51.5, 110.0, 111.6, 139.7, 155.4, 158.0, 167.5. HRMS (EI) m/z calcd for $C_7H_8N_2O_2$ 152.1530, found 152.1525. Anal: C (55.26/55.11), H (5.30/5.25), N (18.41/18.37), O (21.03/21.09).

2.1.2. Synthesis of methyl 2-benzamidonicotinate(3)

To a solution of methyl 2-aminonicotinate (7.60g, 0.5 mole) in 50 mL chloroform was added triethylamine (2.5 mL, 0.2 mole). To this solution 3-chlorobenzoyl chloride (3.04 mL, 2.22g, 0.02 mole) in chloroform was added dropwise over a period of one hour with continues stirring. It was further stirred on room temperature for next 20 hours. After that the mixture was filtered to remove any residue formed, the solution was washed with 10% $NaHCO_3$ and 5% HCl. It was further treated with ethyl acetate in presence of hexane to give methyl-2-benzamidonicotinate as solid product.

Yield: 64%. mp: 122-124°C (ethanol). IR (KBr) cm^{-1}: 3320.6, 1900.7, 1845.6, 1742.2, 1715.3, 1651.2, 1455.1. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 3.77 (s, 3H, CH3), 7.7 (d, 2H, J=7.78, Ar), 7.8 (s, 1H, Ar), 8.13 (d, 2H, J=7.12, Ar), 8.3 (d, 1H, J=7.26, Ar), 8.3 (dd, 2H, J=7.38, Ar), 12.73 (bs, 1H, NH). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 51.5, 110.4, 111.2, 127.1, 132.6, 134.7, 137.4, 148.2, 155.5, 167.2, 168.1. HRMS (EI) m/z calcd for $C_{14}H_{12}N_2O_3$ 256.0431, found 256.0459. Anal: C (65.62/65.68), H (4.72/4.60), N (10.62/10.67), O (18.73/18.39).

2.1.3. Synthesis of 2-phenylpyrido[2,3-d]pyrimidin-4(3H)-one(4)

Compound 3 (5.12g, 0.02 mole) was dissolved in 10 mL of methanol and to this a 28% solution of NH_3OH was added. This mixture was then heated for 5 hours and then 40 mL of NaOH was added with vigorous stirring followed by refluxing for 12 hours. It was allowed to cool down to room temperature leading to formation of a precipitate which was further filtered and dried to obtain white solid as 2-phenylpyrido[2,3-d]pyrimidin-4(3H)-one.

Yield: 78%. mp: 168-170°C (ethanol). IR (KBr) cm^{-1}: 3420.1, 3318.2, 1715.3, 1657.4, 1655.1, 1618.5. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 5.8 (bs, 1H, Ar-NH), 6.8 (m, 1H, Ar), 6.8 (m, 5H, Ar), 7.15 (t, 2H, J=7.60, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 115.4, 120.09, 128.2, 129.10, 130.18, 132.40, 136.7, 156.0, 157.5, 159.2, 161.0. HRMS (EI) m/z calcd for $C_{13}H_9N_3O$ 233.0751, found 233.0772. Anal: C (69.95/69.80), H (4.06/4.03), N (18.82/18.67), O (7.17/7.14).

2.1.4. Synthesis of 4-chloro-2-phenylpyrido[2,3-d] pyrimidine(5)

Compound 4 (2.23 g, 0.01 mole) was refluxed with phosphorus oxychloride for five hour during which all the starting material was dissolved and utilised. Excess of phosphorus oxychloride was removed under vacuum and the remaining residue was poured in ice-cold water and chloroform. The chloroform layer was separated and treated with $NaHCO_2$. The chloroform layer was further washed with brine and then purified using silica gel column with chloroform as an eluent to provide 4-chloro-2-phenylpyrido[2,3-d]pyrimidine.

Yield: 67%. mp: 156-158°C (ethanol). IR (KBr) cm^{-1}: 3423.1, 3376.2, 3319.2, 1657.4, 1655.1, 1619.8. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 6.9 (s, 1H, Ar), 7.4 (m, 1H, Ar), 7.49 (m, 2H, Ar), 7.7 (m, 1H, Ar), 7.76 (m, 1H, Ar), 8.01 (t, 2H, J=8.50, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 118.4, 121.09, 128.8, 129.40, 130.13, 132.40, 134.7, 156.0, 157.5, 159.2, 160.10. HRMS (EI) m/z calcd for $C_{13}H_8ClN_3$ 241.0407, found 241.0410. Anal: C (64.61/64.50), H (3.34/3.39), Cl (14.65/14.67), N (17.39/17.30).

2.1.5. General Synthesis of 2-phenyl-N-(substituted phenyl) pyrido[2,3-d] pyrimidin-4-amine (6a-r)

Compound 5 was reacted with various anilines in 4 mL of dry DMF, to this solution 86 microliters of diisopropylamine (DIPA) was added and heated over a period of 3-6 hours. This reaction mixture was cooled to RT and residue was filtered out and purified using column chromatography to yield respective derivatives. Total eighteen derivatives were synthesised, these compounds are enlisted as follows;

2.1.5.1. N-(4-Fluorophenyl)-2-phenylpyrido[2,3-d] pyrimidin-4-amine (6a)

Yield: 59%. mp: 152-154°C (ethanol). IR (KBr) cm^{-1}: 3422.1, 3373.2, 3315.2, 1652.4, 1654.1, 1618.8. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 6.8(m, 1H, NH), 7.3(m, 2H, Ar), 7.36(m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33(t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for C19H13FN4 316.1124, found 316.1126. Anal: C(72.14/72.17), H(4.14/4.12), F(6.01/6.03), N(17.71/17.67).

2.1.5.2. N-(2-Fluorophenyl)-2-phenylpyrido[2,3-d] pyrimidin-4-amine (6b)

Yield: 48%. mp: 150-152°C (ethanol). IR (KBr) cm^{-1}: 3421.1, 3376.4, 3319.5, 1657.6, 1655.3, 1619.1. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.12(m, 2H, Ar), 7.27(m, 2H, Ar), 7.5(m, 2H, Ar), 7.8(m, 2H, Ar), 8.11(s, 2H, Ar), 8.13(d, 2H, Ar), 12.63(bs, 1H, NH). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{13}FN_4$ 316.1124, found 316.1126. Anal: C (72.14/72.17), H (4.14/4.12), F (6.01/6.03), N (17.71/17.67).

2.1.5.3. N-(4-Chlorophenyl)-2-phenylpyrido[2,3-d] pyrimidin-4-amine (6c)

Yield: 64 %. mp: 149-151°C (ethanol). IR (KBr) cm^{-1}: 3420.4, 3375.4, 3317.5, 1656.7, 1656.3, 1618.8. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 6.8(m, 1H, NH), 7.31(m, 2H, Ar), 7.36(m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33(t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{13}ClN_4$ 332.0829, found 332.0835. Anal: C(68.50/68.57), H(3.94/3.90), Cl (10.65), N(16.84/16.88).

2.1.5.4. N-(2-Chlorophenyl)-2-phenylpyrido[2,3-d] pyrimidin-4-amine (6d)

Yield: 60 %. mp: 147-149°C (ethanol). IR (KBr) cm^{-1}: 3423.6, 3376.3, 3319.5, 1657.3, 1655.2, 1619.7. ^1H NMR (DMSO-d6, 400 MHz) δ: 6.8(m, 1H, NH), 7.31(m, 2H, Ar), 7.36 (m, 2H, Ar), 7.4(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33(t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{13}ClN_4$ 332.0829, found 332.0835. Anal: C (68.55/68.57), H (3.90/3.94), Cl (10.65), N (16.84/16.80).

2.1.5.5. N-(4-Bromophenyl)-2-phenylpyrido[2,3-d] pyrimidin-4-amine (6e)

Yield: 51%. mp: 156-158°C (ethanol). IR (KBr) cm^{-1}: 3422.4, 3375.4, 3317.1, 1654.5, 1656.2, 1615.4. ^1H NMR (DMSO-d6, 400 MHz) δ(m, 1H, NH), 7.31(m, 2H, Ar), 7.36 (m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33(t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{13}BrN_4$ 376.0324, found 376.0320. Anal: C (60.49/60.50), H (3.47/3.50), Br (21.18/21.14), N (14.85/14.87).

2.1.5.6. N-(2-Bromophenyl)-2-phenylpyrido[2,3-d] pyrimidin-4-amine (6f)

Yield: 50%. mp: 159-161°C (ethanol). IR (KBr) cm^{-1}: 3422.4, 3375.4, 3317.1, 1654.5, 1656.2, 1615.4. ^1H NMR (DMSO-d6, 400 MHz) δ: 6.8(m, 1H, NH), 7.31(m, 2H, Ar), 7.36(m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33(t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{13}BrN_4$ 376.0324, found 376.0321. Anal: C (60.47/60.49), H (3.48/3.51), Br (21.11/21.15), N (14.82/14.86).

2.1.5.7. 2-Phenyl-N-(4-(trifluoromethyl)phenyl) pyrido[2,3-d]pyrimidin-4-amine (6g)

Yield: 65%. mp: 158-160°C (ethanol). IR (KBr) cm^{-1}: 3423.1, 3378.2, 3317.2, 1659.4, 1654.1, 1620.8. ^1H NMR (DMSO-d6, 400 MHz) δ: 6.8(m, 1H, NH), 7.31(m, 2H, Ar), 7.36(m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33(t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{20}H_{13}F_3N_4$ 366.3472, found 366.3211. Anal: C (65.57/65.60), H (3.58/3.61), F (15.56/15.67), N (15.29/15.40).

2.1.5.8. 2-Phenyl-N-(p-tolyl)pyrido[2,3-d]pyrimidin-4-amine (6h)

Yield: 55 %. mp: 165-167°C (ethanol). IR (KBr) cm^{-1}: 3423.3, 3377.4, 3318.5, 1656.9, 1656.2, 1617.4. ^1H NMR (DMSO-d6, 400 MHz) δ: 2.18(s, 3H, CH3), 6.8(m, 1H, NH), 7.31 (m, 2H, Ar), 7.36(m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33(t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{20}H_{16}N_4$ 312.1375, found 312.1390. Anal: C (76.90/76.85), H (5.16/5.19), N (17.94/17.90).

2.1.5.9. 2-Phenyl-N-(m-tolyl)pyrido[2,3-d]pyrimidin-4-amine (6i)

Yield: 50 %. mp: 164-166°C (ethanol). IR (KBr) cm^{-1}: 3423.1, 3376.2, 3319.2, 1657.4, 1655.1, 1619.8. ^1H NMR (DMSO-d6, 400 MHz) δ: 2.4(s, 3H, CH3), 6.8(m, 1H, NH), 7.11 (t, 1H, J=7.82, Ar), 7.31(m, 2H, Ar), 7.36(m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33(t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{20}H_{16}N_4$ 312.1375, found 312.1399. Anal: C (76.90/76.84), H (5.16/5.18), N (17.94/17.91).

2.1.5.10. 2-Phenyl-N-(o-tolyl)pyrido[2,3-d]pyrimidin-4-amine (6j)

Yield: 58 %. mp: 160-162°C (ethanol). IR (KBr) cm^{-1}: 3423.4, 3377.8, 3318.6, 1656.5, 1656.3, 1618.7. ^1H NMR (DMSO-d6, 400 MHz) δ: 2.12(s, 3H, CH3), 6.8(m, 1H, NH), 7.11 (t, 1H, J=7.82, Ar), 7.31(m, 2H, Ar), 7.36(m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33(t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{20}H_{16}N_4$ 312.1375, found 312.1299. Anal: C (76.90/76.89), H (5.16/5.22), N (17.94/17.87).

2.1.5.11. N-(4-Methoxyphenyl)-2-phenylpyrido[2,3-d]pyrimidin-4-amine (6k)

Yield: 66%. mp: 167-169°C (ethanol). IR (KBr) cm^{-1}: 3425.3, 3378.2, 3319.7, 1656.5, 1656.3, 1618.8. ^1H NMR (DMSO-d6, 400 MHz) δ: 3.81(s, 3H, CH3), 6.8(m, 1H, NH), 7.01 (t, 1H, J=7.82, Ar), 7.21(m, 2H, Ar), 7.36(m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33 (t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{20}H_{16}N_4O$ 328.1324, found 328.0259. Anal: C (73.15/73.34), H (4.91/5.02), N (17.06/17.13), O (4.87/4.18).

2.1.5.12. N-(3-Methoxyphenyl)-2-phenylpyrido[2,3-d]pyrimidin-4-amine (6l)

Yield: 66%. mp: 167-169°C (ethanol). IR (KBr) cm^{-1}: 3423.1, 3376.2, 3319.2, 1657.4, 1655.1, 1619.8. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 2.8(s, 3H, CH3), 6.5(m, 2H, Ar), 6.02(m, 3H, Ar), 7.4(s, 2H, Ar), 7.6(s, 3H, Ar), 2.5(d, 2H, J=7.2, Ar), 9.0(bs, 1H, NH). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{20}H_{16}N_4O$ 328.1324, found 328.0259. Anal: C (73.15/73.34), H (4.91/5.02), N (17.06/17.13), O (4.87/4.18).

2.1.5.13. N-(2-Methoxyphenyl)-2-phenylpyrido[2,3-d]pyrimidin-4-amine (6m)

Yield: 66%. mp: 167-169°C (ethanol). IR (KBr) cm^{-1}: 3423.5, 3375.3, 3318.1, 1656.3, 1656.2, 1618.7. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 3.82(s, 3H, CH3), 6.8(m, 1H, NH), 7.01 (t, 1H, J=7.82, Ar), 7.21(m, 2H, Ar), 7.36(m, 2H, Ar), 7.41(m, 1H, Ar), 7.59(m, 2H, Ar), 8.33 (t, 3H, J=7.76, Ar), 8.59(dd, 1H, J=7.26, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{20}H_{16}N_4O$ 328.1324, found 328.0259. Anal: C (73.15/73.34), H (4.91/5.02), N (17.06/17.13), O (4.87/4.18).

2.1.5.14. 4-((2-Phenylpyrido[2,3-d]pyrimidin-4-yl) amino)phenol (6n)

Yield: 65 %. mp: 130-132°C (ethanol). IR (KBr) cm^{-1}: 3612.1, 3423.6, 3376.3, 3319.3, 1657.6, 1655.5, 1619.7. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 6.7(m, 2H, Ar), 7.0(m, 2H, Ar), 7.21(m, 2H, Ar), 7.85(t, 2H, J=7.8, Ar), 7.9(m, 2H, Ar), 8.30(m, 2H, Ar), 8.91(d, 2H, J=7.75, Ar), 10.89(s, 1H, Ar-OH). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{14}N_4O$ 314.1168, found 314.1100. Anal: C (72.60/72.69), H (4.49/4.56), N (17.85/17.67), O (5.09/5.11).

2.1.5.15. 2-((2-Phenylpyrido[2,3-d]pyrimidin-4-yl)amino)phenol (6o)

Yield: 67 %. mp: 129-131°C (ethanol). IR (KBr) cm^{-1}: 3651.5, 3423.5, 3378.2, 3319.4, 1657.5, 1655.5, 1619.7. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.2(m, 7H, Ar), 7.32(t, 2H, J=7.58, Ar), 7.5(m, 2H, Ar), 8.0(d, 2H, J=7.8, Ar), 12.52(bs, 1H, Ar-OH). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{14}N_4O$ 314.1168, found 314.1110. Anal: C (72.60/72.65), H (4.49/4.57), N (17.85/17.69), O (5.09/5.10).

2.1.5.16. N-(4-Nitrophenyl)-2-phenylpyrido[2,3-d]pyrimidin-4-amine (6p)

Yield: 66%. mp: 172-174°C (ethanol). IR (KBr) cm^{-1}: 3423.1, 3376.2, 3319.7, 1658.4, 1655.1, 1619.1. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 6.89(bs, 1H, NH), 7.44(m, 4H, Ar), 7.50 (m, 3H, Ar), 8.03(d, 2H, J=7.16, Ar), 8.36(t, 2H, J=7.32, Ar), 8.59(t, 1H, J=7.75, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{13}N_5O_2$ 343.1069, found 343.1259. Anal: C (66.47/66.32), H (3.82/3.90), N (20.40/20.67), O (9.32/9.44).

2.1.5.17. N-(3-Nitrophenyl)-2-phenylpyrido[2,3-d]pyrimidin-4-amine (6q)

Yield: 60%. mp: 177-179°C (ethanol). IR (KBr) cm^{-1}: 3425.2, 3377.1, 3318.1, 1657.7, 1655.8, 1619.2. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 6.89(bs, 1H, NH), 7.2(m, 1H, Ar), 7.44(m, 3H, Ar), 7.50(m, 3H, Ar), 8.03(d, 2H, J=7.16, Ar), 8.36(t, 2H, J=7.32, Ar), 8.59(t, 1H, J=7.75, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{13}N_5O_2$ 343.1069, found 343.1123. Anal: C (66.47/66.34), H (3.82/3.95), N (20.40/20.45), O (9.32/9.40).

2.1.5.18. N-(2-Nitrophenyl)-2-phenylpyrido[2,3-d]pyrimidin-4-amine (6r)

Yield: 65%. mp: 172-174 °C (ethanol). IR (KBr) cm^{-1}: 3423.9, 3376.2, 3319.5, 1657.1, 1656.6, 1617.2. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 6.89 (bs, 1H, NH), 7.2 (m, 1H, Ar), 7.44 (m, 3H, Ar), 7.50 (m, 3H, Ar), 8.03 (d, 2H, J=7.16, Ar), 8.36 (t, 2H, J=7.32, Ar), 8.59 (t, 1H, J=7.75, Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 103.6, 114.0, 116.1, 120.7, 124.1, 126.5, 127.1, 129.2, 130.1, 131.5, 136.7, 153.0, 155.1, 157.9, 159.0. HRMS (EI) m/z calcd for $C_{19}H_{13}N_5O_2$ 343.1060, found 343.1089. Anal: C (66.47/66.32), H (3.82/3.94), N (20.40/20.44), O (9.32/9.39).

3.2. Biological Studies

3.2.3. Kinetic Assays

Chemicals and enzymes

The enzyme biotin carboxylase was isolated from Escherichia coli by gene overexpression method as reported elsewhere.[28] The obtained enzyme was purified using the nickel affinity chromatography by histidine-tag attached to N-terminal of the enzyme.[29] Bradford method was used to determine the concentration of enzyme using bovine serum albumin as reference standard.[30] All other reagents, intermediates and solvents were procured from Sigma Aldrich.

Biotin carboxylase assay

The enzyme activity of biotin carboxylase was measured by continuous spectrophotometric method. ADP production was detected by coupling of enzymes pyruvate kinase and lactate dehydrogenase as described earlier.[30] The effect of synthetic compounds on the activity of coupling enzymes was measured at 340 mM. All the experiments were conducted in variable concentrations and by measuring the inhibition of the enzyme activity. The reactions were carried out at a total of 0.5 mL of solution in quartz cuvette with 1 cm path. Before every measurement, the reactions were initiated by the addition of enzyme. All the spectrophotometric experiments were carried out on a Shimadzu UV-vis spectrophotometer with data acquisition software.

Data analysis

The data obtained from the enzyme inhibition assay were analysed by the nonlinear regression method by application of computer program developed by Cleland.[31] The data obtained was fitted in to the equation mentioned below;

$$v = \frac{VA}{Km\left(1 + \frac{I}{Kis}\right) + A}$$

$$v = \frac{VA}{Km\left(1 + \frac{I}{Kis}\right) + A\left(1 + \frac{I}{Kii}\right)}$$

Where,
v = initial velocity
V = maximal velocity
A = substrate concentration
Km = Michalis constant
I = concentration of the inhibitor
Kis and Kii = slopes and intercept inhibition constants.

3.2.4. Determination of MICs.

Minimum Inhibitory concentration MICs were determined by the broth microdilution methods using a cation-adjusted Mueller Hinton (MHII) medium.[32] The strains of microorganism were obtained from the Institute of Microbial Technology (IMTEC), Chandigarh and the ATCC numbers are mentioned accordingly. Agar medium was streaked with test microorganism and incubated overnight at 35°C. The test compounds were prepared over a serial dilution method, by solubilizing compounds in 100% DMSO and sterile water for standard drugs gentamicin and kanamycin to attain a final stock concentration of 10 mg/L. The inoculum of 5 × 105 CFU/mL/0.1 mL in standardized form was incubated with antibiotic for 18−24 h at 35 °C. Visual measures were used to record the MICs and scored accordingly and lowest concentration of antibiotic and the test compounds where observed for the visual inspection for turbidity.

3.2.5. Generation of Spontaneous Resistant Mutant Strains and Resistance Studies.

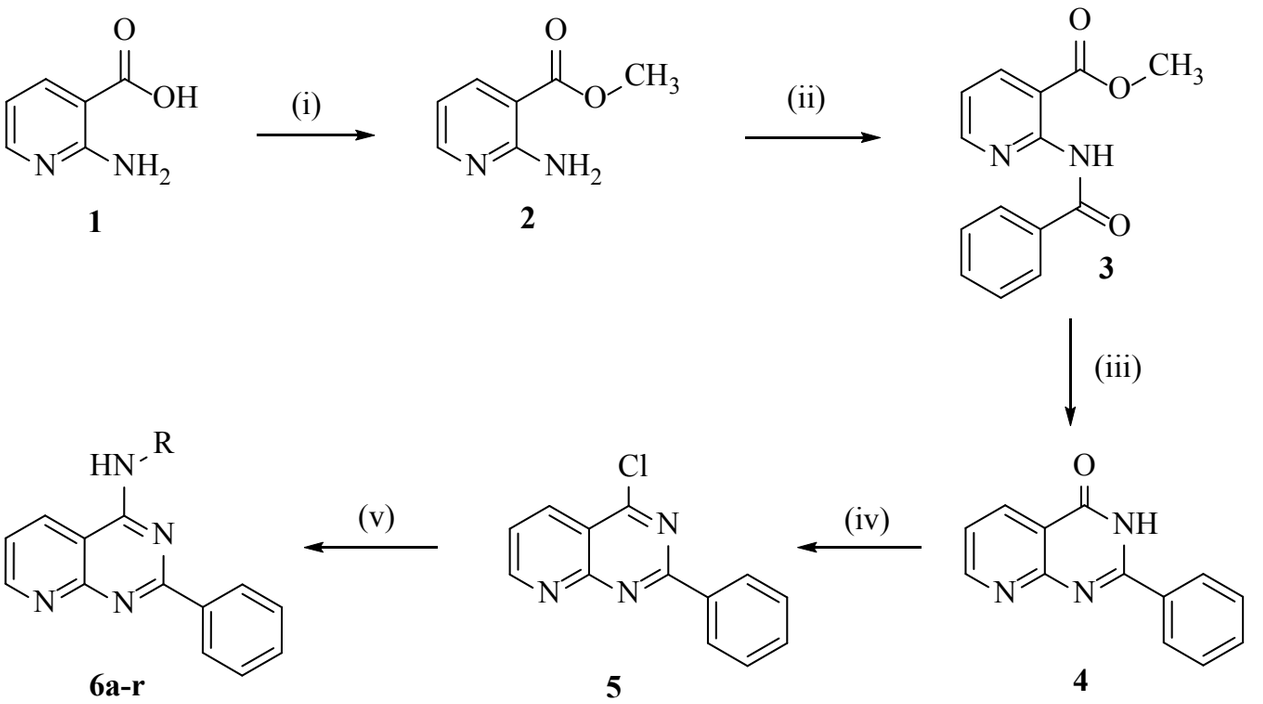
MHII agar was molten and cooled to 55 °C, to this compounds 6d, 6l, 6n, 6o were added in a concentration of two-fold increase starting with initial MIC observed from the previous experiment. Single-step spontaneous resistance selected cultures were obtained as described elsewhere. The microorganisms were allowed to grow overnight to saturation in MHII broth. The microbial cells were concentrated by centrifugation of the medium and a viable number of colony forming units (CFU) were determined by serial dilutions and plating technique. It was taken care that each individual plate had around 4 × 109 CFU/plate. Subsequently the culture plates were monitored for the development of drug-resistant colonies and were observed visually. A detailed study on the development of moiramide resistance and further evaluation was performed as per the earlier reported procedure.[33]

3.2.6. Molecular Docking Studies

All the in silico designing and docking studies were performed on the Schrodinger modelling suite (Version 15.3), the compounds designed were subjected to energy minimisation by Ligprep module of the software.[34] All the protein preparation work was carried out on the protein preparation wizard of the Glide module, the crystal structure obtained from the protein data bank contains the ligands, cofactors, water molecules, they may also be deficient of certain loops, and these anomalies are refined by the protein preparation wizard. The cofactor unless essential are removed the ligand is replaced and the water molecules are also eliminated. The missing loops are filled and the caps are inserted. Thus, when the protein is ready energy minimisation program is run to optimise the protein for molecular docking studies. Glide module was employed for the molecular docking; compounds were docked in the grid designed in the module. The docking was carried out with flexible ligands and the protein with the simulations made on the OPLS5.0 force field. All the results were based on 10 conformations for each molecule in the extra precision (XP) mode. The crystal structure (PDB 2V58)[8] was employed as the receptor with compounds 6a-r as ligands. The results obtained from the simulation were obtained in the form of dock score; these values represent the minimum energies. Interactions between the ligand and residues were presented in the form of H-bond, van der Waals forces and the pi bonds. The results in the form of 3D and 2D representation were obtained for simplified understanding.

RESULTS AND DISCUSSION

Biotin carboxylase acts as an important catalyst in the synthesis of malonyl-CoA which is an important precursor for the synthesis of fatty acids. These are essential for formation of the bacterial cell wall. With the advent of drug resistant bacterial strains and urgent need for drugs with potential for inhibiting drug resistant bacteria was felt. Herein, we are reporting the development of some new pyridopyrimidine derivatives with their potential for inhibiting the drug resistant bacterial strains. Earlier, Chakravarty *et al*., has reported several quinazolines as TGF-β inhibitors.[35] Pyridopyrimidines were designed on the basis of structural analogy to the compounds previously reported with antibacterial potential; these consisted of some common features like the pyridopyrimidine nucleus as shown in (Figure 2).[8, 14, 22-24] Eighteen pyridopyrimidine derivatives were synthesised following a five-step synthetic protocol as shown in scheme 1.



Scheme 1: Synthesis of pyridopyrimidine derivatives. Reagents and conditions: (i) SOCl2, CH3OH, (ii) 3-Chlorobenzoyl chloride, CHCl3, (C2H5)3N, (iii) 2M NH3/CH3OH, 28% NH4OH (iv) POCl3 (v) (C2H5)3N, DMF.

2-Amino pyridine carboxylic acid (1) undergoes Fischer-Speier esterification[36] and provides with methyl 2-aminonicotinate (2). The free primary amine reacts with 3-chlorobenzoyl chloride in the presence of triethyl amine and forms the methyl 2-benzamidonicotinate (3). Compound (3) undergoes cyclisation in the presence of ammonia leading to formation of 2-phenylpyrido[2,3-d]pyrimidin-4(3H)-one (4). On treatment with phosphorus oxychloride, compound (4) forms the 4-chloro-2-phenylpyrido[2,3-d]pyrimidine (5). The chloro group of compound five is substituted with various anilines in basic medium to give eighteen final derivatives. The structural confirmation was made on the basis of IR, NMR and mass spectra.

The spectral details of compounds are mentioned in the experimental section with spectra of selected compounds in the supplementary material available with the online version of this article. Compound (2) shows the characteristic amine absorption spectra around 3424.1 cm^{-1} and the absorption for ester around 1655.1 cm^{-1}. Proton NMR spectra of this compound shows a broad singlet corresponding to the amine at 12.19 ppm and a singlet at 3.86 ppm with three protons of the methyl group. The compound (3) shows absorption around 1455.1 cm^{-1} for the secondary amine group, the proton NMR spectra shows a singlet of the methyl group at 3.77 ppm with three protons, aromatic protons around 7.7 to 8.3 ppm whereas the amide proton is seen at 12.73 ppm. In case of compound (4) the methyl group seen in compound (3) disappears as a result of cyclisation and a single proton of secondary nitrogen is observed at 5.8 ppm as abroad singlet.

The compound (5) shows all the protons in the aromatic region because chlorination of compound (4) leads to disappearance of amide proton. In the series of compounds (6a-r), the characteristic properties of derivatives involved singlets at 2.4 ppm corresponding to the methyl group of the tolyl derivative (6i). singlet at 2.12 ppm of methyl group of the tolyl derivative (6j). The methoxy derivatives (6k), (6l) and (6m) showed a methyl proton at 3.81, 2.8 and 3.82 ppm respectively. In case of compounds (6n) and (6o) showed characteristic -OH broad singlets at 10.89 and 12.52 ppm respectively. All the compounds displayed the number of carbon atoms as per the molecular formula and the characteristic signals were observed for the compounds like in (2) the carboxyl carbon signals at 167.5 ppm, in compound (3) there are two carboxyl carbons at 167.1 and 168.1 respectively, in compound (4) the amide carbons show signal at 161.0 ppm. All compounds were subjected to determination of molecular weight following a mass spectral analysis; all compounds were found to be in agreement with the theoretical values.

The synthesised compounds were then subjected to enzyme kinetics assay using the biotin carboxylase enzyme obtained as per the protocol mentioned in the experimental section. Compounds were tested against the *E. Coli* of wild type and tolC mutant resistant stain, *P. aeruginosa* wild type and δ-RND mutant resistant variety, *S. aureus* wild and Moiramide resistant mutant strain, *S. epidermidis* wild type and *E. faecalis* wild type strains. All the compounds were subjected to the assay in varying concentrations in the reaction mixture consisting of BC and other reagents (Figure 3). The UV absorptions of these compounds were recorded in the computer acquisition system and the readings were calculated using kinetic analysis formula provided by Cleland. It was observed that compounds 6d, 6l, 6n and 6o shows non-competitive inhibition with ATP and showed the K_{is} around 29.8 ± 12.8 nM and a K_{ii} of 33.8 ± 7.4 nM. The inhibitory concentrations for compounds 6a-r is presented in (Table 1).

Table 1: Antibacterial activity of synthesised compounds

	MIC (µg/mL)																				
Strain type	Rifampin	Gentamicin	Chloramphenicol	Kanamycin	6a	6b	6c	6d	6e	6f	6g	6h	6i	6j	6k	6l	6m	6n	6o	6p	6q	6r
E. coli (wild type)	32	0.15	9	3	>35	>35	>35	>35	>35	>65	>65	>65	>65	>65	>65	>35	>65	>35	>35	>65	>65	>65
E. coli (tolC; mutant)	0.5	≤0.05	1	0.5	>25	>25	25	17	>25	>25	>25	>25	>25	>25	090	055	1	>35	5	12	12	26
P. aeruginosa (wild type)	32	3	>65	130	>35	>35	>35	>35	>35	>35	>35	>35	>35	>35	>35	>35	>35	>35	>35	>35	>35	>35
P. aeruginosa (δ-RND; mutant)	32	0.7	3	65	>35	9	>35	>35	>35	>35	>35	>35	>35	>35	51	9	17	>35	>35	>35	>35	>35
S. aureus (wild type)	≤0.04	0.6	9	5	>35	>35	4	>35	>35	>35	>35	>35	>35	>35	>35	1	>35	>35	4	>35	>35	>35

S. aureus (moir-R; mutant) *	≤0.04	0.5	8	4	>35	>35	10	>35	>35	>35	>35	>35	>35	>35	>35	5	>35	>35	5	>35	>35	>35
S. epidermidis (Wild type)	≤0.04	9	9	>129	>35	>35	1	>35	>35	>35	>35	>35	>35	>35	>35	025	>35	>35		>35	>35	>35
E. faecalis (wild type)	0.5	0.5	9	35	>35	>35	>35	>35	12	>35	>35	>35	>35	>35	>35	9	>35	>35		>35	>35	>35

*moir-R: Moiramide resistant strains

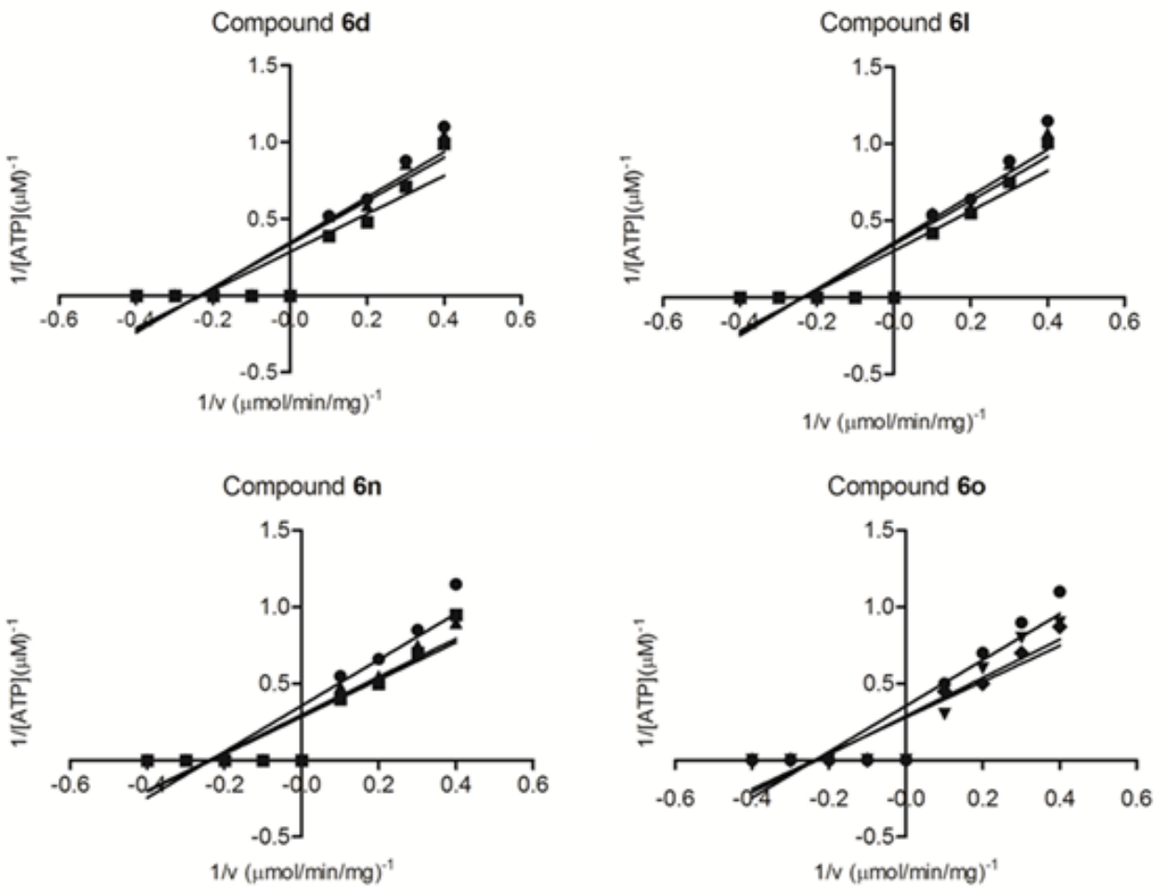


Fig. 3: Compound 6d: Inhibition at 0 (●), 0.1 (■), 0.2 (▲) and 0.3 (♦) nM. Compound 6l: Inhibition at 0 (●), 0.1 (■), 0.2 (▲) and 0.3 (♦) nM. Compound 6n: Inhibition at 0 (●), 0.1 (■), 0.2 (▲) and 0.3 (♦) nM. Compound 6o: Inhibition at 0 (●), 0.1 (■), 0.2 (▲) and 0.3 (♦) nM. The substrate ATP was varied, HCO3- and acetyl-CoA was held constant.

The compound 6d, 6l, 6n and 6o were selected from the kinetic assay for determination of their antibacterial activity. The Minimum Inhibitory Concentrations (MICs) were evaluated following the standard protocol, the details are mentioned in experimental section and results are given in table 1. Compound 6d exhibited antibacterial activity against mutant type *E. coli* at 17µg/mL, which is higher compared to standard drug used in therapy. Compound 6l exhibited comparative antibacterial activity against mutant type of *E. coli* at 0.55µg/mL which is similar to rifampin and kanamycin. Compound 6l showed inhibitory activity at concentration of 9µg/mL against *P.aeruginosa* better than rifampin. Compounds 6l and 6o exhibited comparative inhibition of wild type and mutant type of S. aureus. Compound 6l showed a good inhibitory activity against *S. epidermidis* at 0.25 µg/mL and E. faecalis at 9µg/mL. Compound 6o showed good activity against wild and mutant type of *S. aureus* at 4 and 5µg/mL respectively, it exhibited superior activity against wild type of *S. epidermidis* and wild type of *E. faecalis* at 2 and 5µg/mL respectively. Thus, the MICs of compounds showed a comparative activity against the wild and mutant types of *E. coli*, *P. aeruginosa, S. aureus, S. epidermidis* and *E. faecalis*. The details of MIC for remaining compounds are provided in the supplementary information (Table S2).

Table 2: Single step resistance studies on E. Coli

Compound	MIC (µg/mL)	MPC µg/mL	MSW	Frequency of resistance
Rifampin	8	>64	>8	7×10^{-9}
6l	1	32	32	$<3 \times 10^{-9}$
6n	16	84	>5	$<3 \times 10^{-9}$
6o	16	84	>5	$<3 \times 10^{-9}$

MIC: minimum inhibitory concentration,
MPC: mutant prevention concentration,
MSW: mutant selection windows

To determine the potential of these compounds we carried out an experiment to determine the single step resistance study and frequency of developing resistance for three compounds 6l, 6n and 6o with rifampin as a reference standard. Compound 6n and 6o was found to have higher mutant prevention concentration (MPC) at 84 µg/mL and compound 6l showed the MPC of 32µg/mL. The MPC dose of drug at which resistant mutant strain were no longer obtained after using 4 x 109 CFU. The mutant selection windows (MSWs) is a ratio of MPC: MIC, it was determined for the three compounds under study. It was found that MSW values for compound 6l was 32, for compound 6n and 6o it was >5. Compound 6n and 6o showed better MSW compared to compound 6l. The results suggest that compounds 6n and 6o have low potential to develop spontaneous resistance.

All the compounds were subjected to molecular docking studies to determine the mode of binding and energy changes. The crystal structure of biotin carboxylase (PDB: 2V58) was used for this study[34] and ligands were prepared for docking by method described elsewhere. The biotin carboxylase is co-crystallised with 6-(2,6-dibromophenyl) pyrido[2,3-d]pyrimidine-2,7-diamine, which we have used as reference ligand for molecular docking studies. It was observed that compound 6d, 6l, 6n and 6o showed highest interaction with the enzyme in their molecular docking studies, the details of docking studies are mentioned in the table S2 (Supplementary material). The bound ligand in the active site forms covalent nitration with Glu201A, Lys202A, aromatic interactions with Leu204A and Lys159A, it shows weaker interactions with Ile437A and His438. The docked compound 6d, show a high docking score of -7.58 and Glide energy of -44.50. It forms a salt bridge between pyridopyridine nucleus and HIS438 (Figure 4 a and b). It forms an aromatic interaction with the Lys159, the presence of electronegative substituent like chlorine on the phenylpyridopyrimidine nucleus contributes to its biological activity. Compound 6l, showed a high docking score of -7.34 and Glide energy of -45.06, the pyridopyridine nucleus forms a salt bridge with the residue HIS430 and the phenyl ring formed a salt bridge with HIS209 (Figure 4c and d), this may be due to the presence of electron donating group like methoxy on the phenylpyridopyrimidine nucleus. Compound 6n, show a docking score of -7.76 and Glide energy of -47.34. It forms a salt bridge between pyridopyridine nucleus and HIS438, the phenyl ring of substituent forms another salt bridge with HIS209 and a hydrogen bond is formed between hydroxy group with GLU276 (Figure 5a and b). Compound 6o, showed a high docking score of -7.96 and Glide energy of -46.71, the pyridopyridine nucleus forms a salt bridge with the residue HIS438 and the OH group forms a hydrogen bond with GLY165 (Figure 5c and d). In case of compounds 6l and 6n there is hydroxyl group (-OH) on the 4^{th} and 2nd positions respectively. Their biological activity, high dock score and hydrogen bond formation with Glu276 and Gly165 suggest importance of strong electron donating character of hydroxyl functional group on the phenylpyridopyrimidine nucleus.

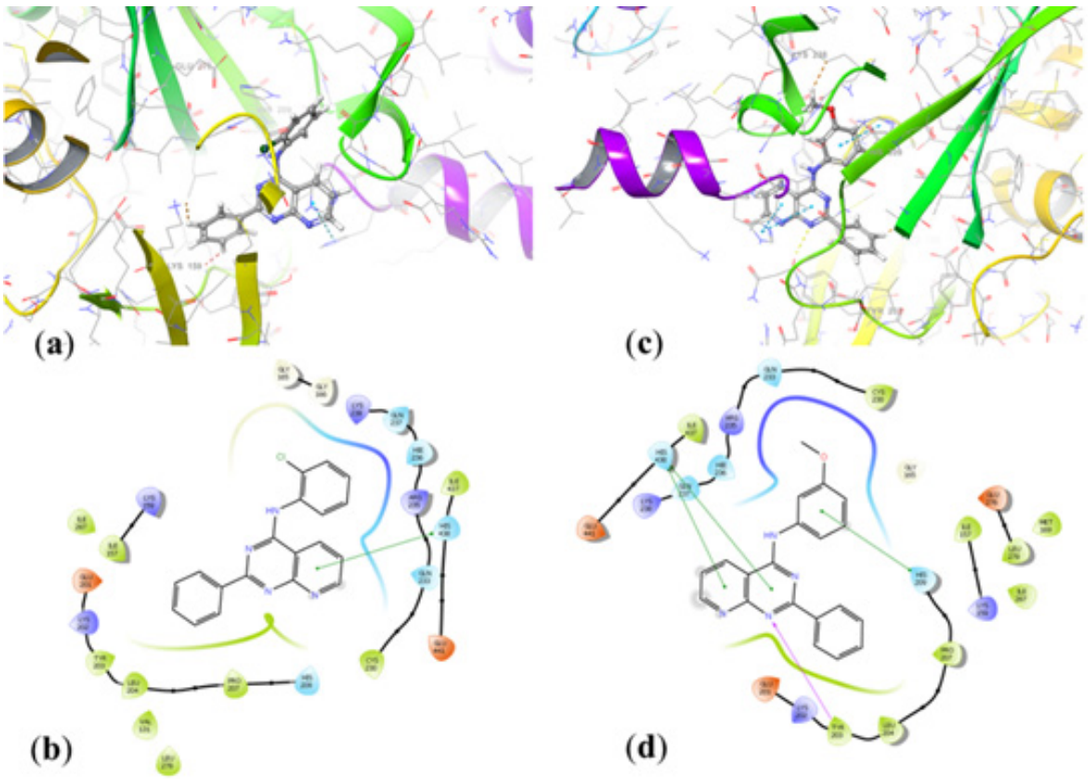


Fig. 4: (a, b) Molecular interaction between the biotin carboxylase and compound 6d, (c, d) Molecular interaction between the biotin carboxylase and compound 6l

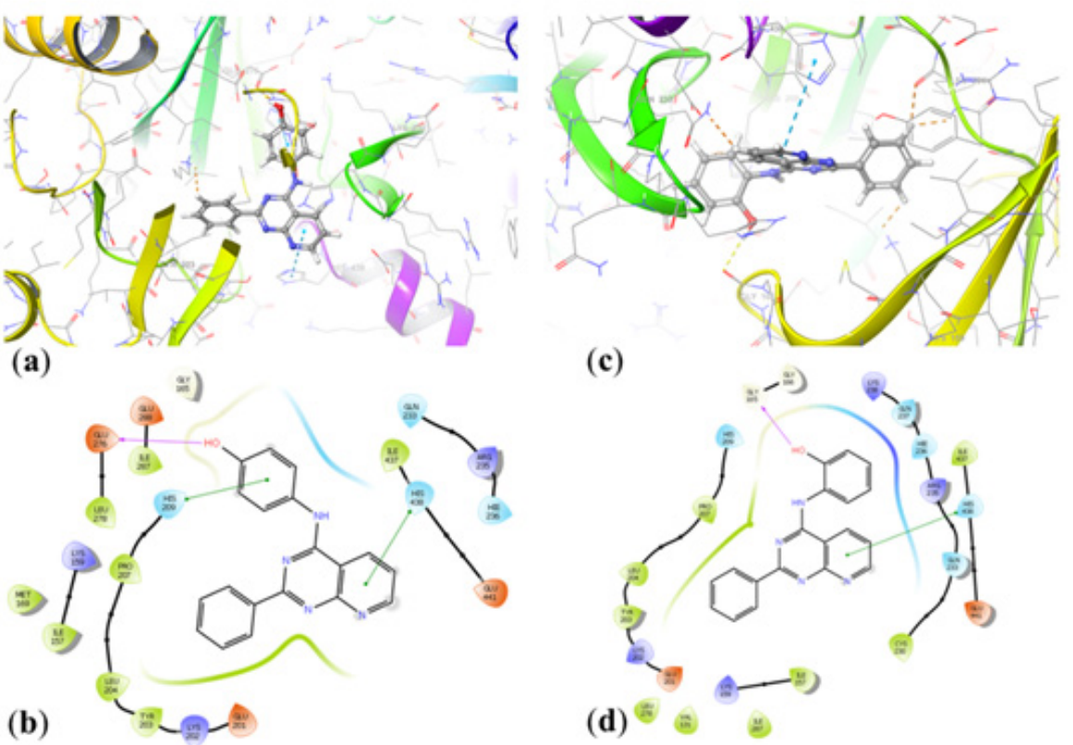


Fig. 5: (a, b) Molecular interaction between the biotin carboxylase and compound 6n, (c, d) Molecular interaction between the biotin carboxylase and compound 6o

CONCLUSION

BC is an important intermediate in the synthesis of malonyl CoA. Present study reports the development of novel pyridopyrimidine derivatives with potent antibacterial activity with less possibility for developing of resistance by targeting this biotin carboxylase enzyme. The series of pyridopyrimidines yielded eighteen compounds. These compounds were characterized on the basis of spectral data and further tested for their antibacterial potential by the enzyme kinetic assay against the BC and then MIC. Compounds 6d, 6l, 6n and 6o were showed promising activity. These compounds were then tested for the single step resistance study; compound 6o was found to be a promising lead in the development of antibacterial with greater potential and lesser possibility for bacteria to be resistant to this compound. This compound can be studied further and developed into a potential antibacterial lead molecule.

ACKNOWLEDGEMENTS

VBP acknowledges the School of Pharmacy, Swami Ramanand Teerth Marathwada University for all the research facilities provided.

CONFLICT OF INTEREST

There are no conflicts of interest.

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