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Synthesis and in vitro and in silico antimicrobial studies of novel piperine–pyridine analogs

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Abstract Pepper is used as a food additive and preservatives because of its antimicrobial activity. Piperine, an alkaloid amide derived from pepper, is the cause of its biological activity. Aminopyridine is a well-known antimicrobial agent. In silico studies proved that conjugation of piperine with substituted aminopyridine results in a new hybrid molecule with improved antimicrobial activity compared with the parent molecules. The present work describes design and synthesis of novel piperine analogs with substituted aminopyridine analogs (**PY1–8**). The synthesized compounds were characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR), infrared (IR), and mass spectroscopy and subjected to antimicrobial testing using bacterial strains *Bacillus subtilis*, *Streptobacillus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* and fungal strains *Aspergillus niger*, *A. flavus*, and *A. fumigatus*.

Keywords Piperine · Aminopyridine · Antimicrobial activity · In silico studies

Introduction

Piper nigrum, commonly known as black pepper, is considered as the king of spices and is a traditional drug used for many diseases. Black pepper is used as a traditional medicine and food preservative [1] due to its antibacterial and antifungal activities. Black pepper is reported to possess antibacterial activity [2, 3]. Zou et al. [4] studied the mechanism of antibacterial activity of black pepper chloroform extract (BPCE).

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The biological activities and pungency of pepper are due to the presence of an alkaloid, viz. piperine [5, 6], an amide of piperidine. On hydrolysis, it converts into piperic acid and piperidine. Piperine alkaloid is a potent antibacterial agent against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, and *Proteus* sp. [7, 8]. Antioxidant and antimicrobial activities of various solvent extracts of pepper, piperine, and piperic acid have been explored [9]. Piperine analogs also show excellent antibacterial activity compared with the parent molecule [10]. Zainab et al. [11] evaluated the antimicrobial activity of piperine purified from *Piper nigrum*. Piperine analogs were found to be more potent inhibitors of the NorA efflux pump of *Staphylococcus aureus* compared with the parent molecule [12]. Quantitative structure–activity relationship (QSAR) analysis of piperine analogs as inhibitors of efflux pump NorA from *Staphylococcus aureus* has been performed to obtain a highly accurate model enabling prediction of inhibition of *S. aureus* [13]. Piperine was tested for use in combination with mupirocin for antimicrobial activity against *Staphylococcus aureus* strains, including methicillin-resistant *S. aureus* (MRSA), which reduced the minimum inhibitory concentration (MIC) of mupirocin and also lowered the mutation frequency [14]. Piperine was evaluated for its immunomodulatory activity to enhance the efficacy of rifampicin in a murine model of *Mycobacterium tuberculosis* infection [15]. In one study on the combination of piperine with gentamicin prepared by dehydration–rehydration, novel strategies were developed to prevent bacterial growth, revealing that the liposomal combination is a powerful nano-antibacterial agent to eradicate MRSA infection [16]. Inhibitory effects of curcumin, capsaicin, and piperine against *Helicobacter pylori* were investigated, and the results suggested that capsaicin and piperine have antiinflammatory effects on *H. pylori*-induced gastritis in gerbils, independent of direct antibacterial effects [17]. The pro- and antioxidant and NOX-inhibiting qualities of four phytochemicals, viz. celastrol, resveratrol, apigenin, and piperine, were also studied [18]. *Piper nigrum* fruit isolations were prepared and their activity in vivo studied by oral administration to guinea pigs, revealing antitussive effect [19]. Extract of *Piper nigrum* was given to rats and an ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS–MS) method developed to determine their content in plasma, showing good linearity [20]. Piperine induced increase of mammalian target of rapamycin complex 1 (mTORC1) activity in resident peritoneal macrophages.

Pyridine is a versatile basic six-member aromatic heterocyclic molecule with chemical formula C_5H_5N, containing one nitrogen and five carbon atoms. The structure of pyridine is almost like that of benzene. Aminopyridines have been found to possess a broad spectrum of biological activities. Aminopyridines are potent pharmacophores with versatile applications in pharmaceutical industry. Drugs including aminopyridine moiety such as tenoxicam, piroxicam [nonsteroidal antiinflammatory drugs (NSAIDs)], sulfasalazine (used to treat rheumatoid arthritis, ulcerative colitis, and Crohn's disease), delavirdine [anti-human immunodeficiency virus (HIV) drug], sulfapyridine (antibacterial drug), and tripelennamine (antihistamine drug) are available on the market. 4-Aminopyridine is present in tacrine, a centrally acting anticholinesterase. Reports suggest that aminopyridines exhibit

various biological applications [21]. Derivatives of aminopyridines possess antibacterial and antimicrobial activity [22–30].

A recent approach for discovery of new pharmaceutical drugs involves linking two molecules and development of hybrid or conjugate molecules with individual essential activity into a single hybrid molecule with elevated biological activity. Such hybrid or conjugate molecules can show enhanced biological activity compared with both parent molecules and be very effective drugs. They have different alkylation sites, which are essential to and play a vital role in tumor treatment [31]. Literature review reveals that [32] designed and synthesized such novel hybrid molecules for malaria treatment, with decreased side effects and increased activity of the drug moiety. This approach of combining different active molecules for enhanced bioavailability is encouraging. We employed this strategy to synthesize piperine analogs [33], with positive results. Taking this as a precedent and considering the antimicrobial nature of both piperine and aminopyridine, in this work we designed and synthesized hybrid piperine–pyridine analogs as promising antimicrobial agents. All target molecules were designed using ChemDraw software and used for in silico molecular docking studies. To date, there are no reports on synthesis of novel piperine–pyridine analogs or study of their antimicrobial activity.

Materials and methods

All reagents used were of analytical reagent (AR) grade and used directly without further purification. Piperine was purchased from Sigma Aldrich chemicals with 97 % purity and used directly. Melting points were determined in open capillary tube. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 and 100 spectrometer, respectively, with tetramethylsilane (TMS) as internal standard; chemical shifts are given in parts per million (ppm). High-resolution mass data were obtained using a Bruker micro TOF-Q II ESI instrument operating at ambient temperature. An FTIR spectrum was recorded on a PerkinElmer FTIR spectrophotometer using KBr pellets. Reaction progress was monitored by thin-layer chromatography on Merck TLC silica gel plates using hexane:ethylacetate (6:4) as solvent. Spots were visualized under ultraviolet (UV) chamber.

Synthesis of piperine analogs

Hydrolysis of piperine to piperic acid

Piperine (10 g) and 300 ml 20 % ethanolic KOH were taken in a round-bottomed flask and refluxed for 10 h. The resulting potassium piperate was filtered and washed with anhydrous ethanol. The precipitate was dissolved in distilled water, and 0.1 M HCl was added to the solution, followed by filtering and washing with distilled water. Yellow crystals of piperic acid were recrystallized from ethanol [34] (yield 86.6 %, m.p. 214–216 °C).

Coupling of piperine with aminopyrimidine

Exactly weighed 2.18 g piperic acid in 20 ml dimethylformamide (DMF) was taken in a round-bottomed flask and stirred at 0–5 °C, followed by addition of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) with continuation of stirring at 0–5 °C for 30 min. To this mixture, 2.60 ml *N*,*N*-diisopropylethylamine (DIPEA) and 2 g substituted aminopyridine were added dropwise, followed by stirring at room temperature for 5 h; reaction completion was monitored by TLC using 6:4 hexane:ethylacetate. After reaction completion, the crude mixture was washed with HCl then NaOH solution and concentrated to give crude product. The crude product was column chromatographed using hexane:ethylacetate to obtain pure compounds.

PY1 (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)-*N*-(pyridin-4-yl)penta-2,4-dienamide M.p. 248 °C, yield: 65 %, brown crystalline solid. ^{1}H NMR (500 MHz, chloroform-*d*) δ 9.44 (s, 1H), 8.50–8.45 (m, 2H), 7.71–7.66 (m, 2H), 7.09–7.00 (m, 2H), 6.90 (dd, $J = 8.4$, 2.0 Hz, 1H), 6.80–6.72 (m, 2H), 6.69 (ddd, $J = 14.1$, 7.0, 0.8 Hz, 1H), 6.04 (d, $J = 2.4$ Hz, 1H), 5.99 (d, $J = 2.4$ Hz, 1H), 5.56 (dd, $J = 14.6$, 1.0 Hz, 1H) ^{13}C NMR (125 MHz, common NMR solvents) δ 165.29, 150.44, 147.52, 147.39, 143.61, 140.82, 138.78, 130.71, 125.85, 123.16, 123.08, 113.65, 108.52, 106.90, 101.00. Mass spectrum: *m*/*z* 295.1 (100 %), 296.11 (20 %). IR spectrum: 1710 (amide-CONH), 3100–3500 (amide NH stretching).

PY2 (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)-*N*-(pyridin-2-yl)penta-2,4-dienamide M.p. 238 °C, yield: 70 %, pale-yellow crystalline solid. ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.70 (dd, $J = 3.5$, 1.5 Hz, 1H), 8.55 (s, 1H), 8.23 (dd, $J = 7.9$, 1.4 Hz, 1H), 7.95 (td, $J = 7.7$, 1.6 Hz, 1H), 7.38–7.28 (m, 2H), 7.04 (d, $J = 2.0$ Hz, 1H), 6.90 (dd, $J = 8.4$, 2.0 Hz, 1H), 6.80–6.72 (m, 2H), 6.69 (ddd, $J = 14.1$, 7.1, 0.9 Hz, 1H), 6.04 (d, $J = 2.4$ Hz, 1H), 5.99 (d, $J = 2.4$ Hz, 1H), 5.63–5.56 (m, 1H). ^{13}C NMR (125 MHz, common NMR solvents) δ 166.30, 152.75, 147.92, 147.52, 147.39, 140.82, 138.78, 138.19, 130.71, 125.85, 123.16, 122.92, 118.85, 115.24, 108.52, 106.90, 101.00. Mass spectrum: *m*/*z* 295.1 (100 %), 296.11 (20 %). IR spectrum: 1675 (amide-CONH), 3100–3300 (amide NH stretching).

PY3 (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)-*N*-(pyridin-3-yl)penta-2,4-dienamide M.p. 262 °C, yield: 65 %, grey crystalline solid. ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.75 (t, $J = 1.8$ Hz, 2H), 7.90 (dt, $J = 7.9$, 1.9 Hz, 2H), 7.77 (dt, $J = 3.5$, 1.7 Hz, 2H), 7.51 (dd, $J = 7.8$, 3.6 Hz, 2H), 7.35 (dd, $J = 14.7$, 7.1 Hz, 2H), 7.04 (d, $J = 2.0$ Hz, 2H), 6.90 (dd, $J = 8.4$, 2.0 Hz, 2H), 6.80–6.72 (m, 4H), 6.69 (ddd, $J = 14.1$, 7.1, 0.9 Hz, 2H), 6.04 (d, $J = 2.4$ Hz, 2H), 5.99 (d, $J = 2.4$ Hz, 2H), 5.56 (dd, $J = 14.6$, 1.0 Hz, 2H). ^{13}C NMR (125 MHz, common NMR solvents) δ 164.94, 147.52, 147.39, 144.56, 143.89, 140.82, 138.78, 133.36, 130.71, 127.01, 125.85, 124.38, 123.16, 123.08, 108.52, 106.90, 101.00. Mass spectrum: *m*/*z* 295.1 (100 %), 296.11 (20 %). IR spectrum: 1650 (amide-CONH), 3100–3300 (amide NH stretching).

PY4 (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)-*N*-(6-methylpyridin-2-yl)penta-2,4-dienamide M.p. 256 °C, yield: 70 %, brownish crystalline solid. ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.40 (s, 1H), 7.54–7.43 (m, 2H), 7.33 (dd, J = 14.6, 7.1 Hz, 1H), 7.06–6.97 (m, 2H), 6.90 (dd, J = 8.4, 2.0 Hz, 1H), 6.80–6.72 (m, 2H), 6.69 (ddd, J = 14.1, 7.1, 0.9 Hz, 1H), 6.04 (d, J = 2.4 Hz, 1H), 5.99 (d, J = 2.4 Hz, 1H), 5.63–5.56 (m, 1H), 2.42 (d, J = 0.7 Hz, 3H). ^{13}C NMR (125 MHz, common NMR solvents) δ 166.21, 156.90, 151.07, 147.52, 147.39, 140.82, 138.78, 138.46, 130.71, 125.85, 123.16, 122.92, 118.68, 111.87, 108.52, 106.90, 101.00, 24.46. Mass spectrum: *m*/*z* 309.1 (100 %), 310.12 (20 %). IR spectrum: 1700 (amide-CONH), 3100–3500 (amide NH stretching).

PY5 (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)-*N*-(3-hydroxypyridin-2-yl)penta-2,4-dienamide M.p. 222 °C, yield: 63 %, colorless crystalline solid. ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.98 (s, 1H), 8.27 (dd, J = 3.5, 1.8 Hz, 1H), 7.88 (s, 1H), 7.39 (dd, J = 14.6, 7.2 Hz, 1H), 7.33 (dd, J = 7.9, 1.8 Hz, 1H), 7.25 (dd, J = 7.9, 3.5 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H), 6.90 (dd, J = 8.4, 2.0 Hz, 1H), 6.80–6.72 (m, 2H), 6.69 (ddd, J = 14.1, 7.1, 0.9 Hz, 1H), 6.04 (d, J = 2.4 Hz, 1H), 5.99 (d, J = 2.4 Hz, 1H), 5.60 (dd, J = 14.7, 0.9 Hz, 1H). ^{13}C NMR (125 MHz, common NMR solvents) δ 166.36, 147.52, 147.39, 144.95, 141.60, 140.82, 139.06, 138.78, 130.71, 125.85, 123.16, 122.83, 121.96, 120.57, 108.52, 106.90, 101.00. Mass spectrum: *m*/*z* 311.10 (100 %), 312.10 (20 %). IR spectrum: 1675 (amide-CONH), 3100–3300 (amide NH stretching).

PY6 (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)-*N*-(5-chloropyridin-2-yl)penta-2,4-dienamide M.p. 268 °C, yield: 74 %, pale-brown crystalline solid. ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.98 (s, 1H), 8.27 (dd, J = 3.5, 1.8 Hz, 1H), 7.88 (s, 1H), 7.39 (dd, J = 14.6, 7.2 Hz, 1H), 7.33 (dd, J = 7.9, 1.8 Hz, 1H), 7.25 (dd, J = 7.9, 3.5 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H), 6.90 (dd, J = 8.4, 2.0 Hz, 1H), 6.80–6.72 (m, 2H), 6.69 (ddd, J = 14.1, 7.1, 0.9 Hz, 1H), 6.04 (d, J = 2.4 Hz, 1H), 5.99 (d, J = 2.4 Hz, 1H), 5.60 (dd, J = 14.7, 0.9 Hz, 1H). ^{13}C NMR (125 MHz, common NMR solvents) δ 166.36, 147.52, 147.39, 144.95, 141.60, 140.82, 139.06, 138.78, 130.71, 125.85, 123.16, 122.83, 121.96, 120.57, 108.52, 106.90, 101.00. Mass spectrum: *m*/*z* 329.06 (100 %), 331.06 (35 %), 330.07 (20 %). IR spectrum: 1680 (amide-CONH), 3200–3500 (amide NH stretching).

PY7 (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)-*N*-(3-methylpyridin-2-yl)penta-2,4-dienamide M.p. 232 °C, yield: 75 %, colorless crystalline solid. ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.52–8.43 (m, 2H), 7.66 (ddd, J = 7.9, 1.8, 0.8 Hz, 1H), 7.37 (dd, J = 14.7, 7.1 Hz, 1H), 7.27 (dd, J = 7.9, 3.5 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H), 6.90 (dd, J = 8.4, 2.0 Hz, 1H), 6.80–6.72 (m, 2H), 6.69 (ddd, J = 14.1, 7.1, 0.9 Hz, 1H), 6.04 (d, J = 2.4 Hz, 1H), 5.99 (d, J = 2.4 Hz, 1H), 5.60 (dd, J = 14.6, 0.9 Hz, 1H), 2.34 (d, J = 0.7 Hz, 3H). ^{13}C NMR (125 MHz, common NMR solvents) δ 166.31, 150.46, 147.52, 147.39, 145.47, 140.82, 138.78, 138.37, 130.71, 125.85, 123.16, 123.07, 122.83, 118.49, 108.52, 106.90, 101.00, 16.34. Mass spectrum: *m*/*z* 309.1 (100 %), 310.12 (20 %). IR spectrum: 1670 (amide-CONH), 3100–3500 (amide NH stretching).

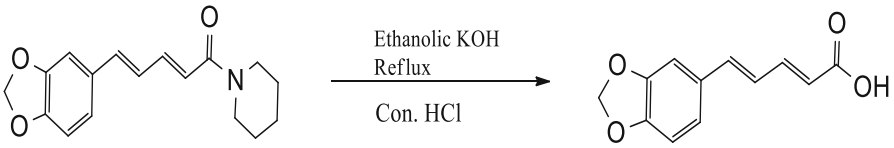
PY8 (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)-*N*-(5-nitropyridin-2-yl)penta-2,4-dienamide M.p. 252 °C, yield: 72 %, grey crystalline solid. ^{1}H NMR (500 MHz, chloroform-*d*) δ 9.42 (d, $J = 1.8$ Hz, 2H), 9.13 (s, 2H), 8.52 (dd, $J = 8.4$, 2.0 Hz, 2H), 7.82 (d, $J = 8.4$ Hz, 2H), 7.33 (dd, $J = 14.7$, 7.1 Hz, 2H), 7.04 (d, $J = 2.0$ Hz, 2H), 6.90 (dd, $J = 8.4$, 2.0 Hz, 2H), 6.80–6.72 (m, 4H), 6.69 (ddd, $J = 14.1$, 7.1, 0.9 Hz, 2H), 6.04 (d, $J = 2.4$ Hz, 2H), 5.99 (d, $J = 2.4$ Hz, 2H), 5.63–5.56 (m, 2H). ^{13}C NMR (125 MHz, common NMR solvents) δ 166.30, 155.57, 147.52, 147.39, 146.13, 140.82, 138.78, 138.29, 134.49, 130.71, 125.85, 123.16, 122.92, 113.78, 108.52, 106.90, 101.00. Mass spectrum: *m*/*z* 340.09 (100 %), 341.09 (20 %). IR spectrum: 1690 (amide-CONH), 3100–3400 (amide NH stretching).

Antimicrobial activity

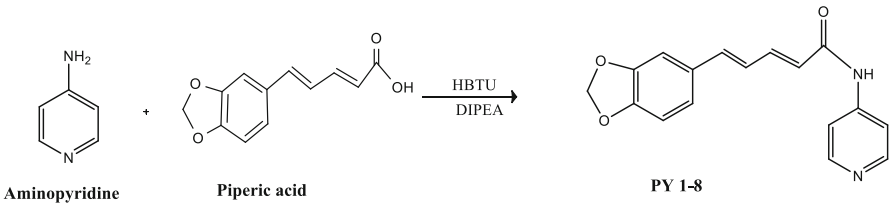
Antimicrobial activity was investigated against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus cereus*), Gram-negative bacteria (*Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*), and fungal strains (*Aspergillus niger*, *A. flavus*, *A. fumigatus*, and *Candida albicans*). The test was conducted by agar well diffusion method. In this method, 20 ml Müller–Hinton medium was spread on Petri plates, and these plates were spread with 24-h-cultured 108 colony-forming units (CFU)/ml of microbial strains. Wells of 6 mm diameter were made in the culture medium. Target compounds (50 µl) were added to the wells and incubated at 37 °C for 24 h. Streptomycin and tetracycline (30 µg/ml each) were taken as reference drugs for antibacterial activity, and carbendazim for antifungal activity. A positive control was kept for reference. The activity of the target compounds was measured based on the inhibition zone formed around the well. The assay was carried out in triplicate, and mean values were taken as readings. The diameter of the zone of inhibition was measured in millimeters.

In silico docking studies

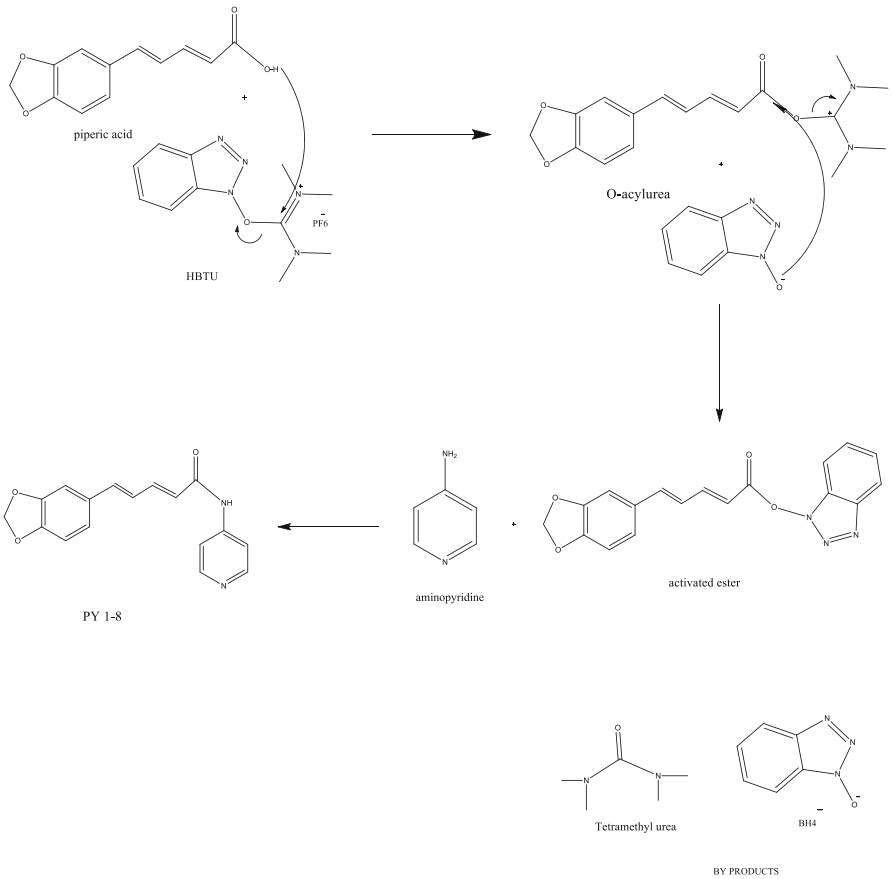
Possible docking modes of the piperine–pyridine analogs with 5XGT (*Staphylococcus aureus*) [35], 3ZIH (*Bacillus subtilis*) [36], 5NWT (*Escherichia coli*) [37], and 2W7Q (*Pseudomonas aeruginosa*) [38] were studied using AutoDock 2.0 software [39]. The target molecules were designed using ChemDraw software and used to analyze the binding affinity with the bacterial proteins. Crystal structures of all the bacterial proteins were downloaded from the Protein Data Bank website (http://www.rcsb.org) in PDB format and converted to PDBQT format. We chose the Lamarckian genetic algorithm (LGA) for ligand conformations. An AutoDock tool was used for creating PDBQT files [40]. The protocol was given in AutoDock Tools software. AutoDock was run several times to obtain various docked conformations and used to analyze the predicted docking energy. AutoDock Tools provide various parameters to analyze the results of docking simulations, such as binding energy, ligand efficiency, inhibition constant, and intramolecular energy.



Scheme 1 Preparation of piperic acid from piperine



Scheme 2 Coupling of pyridine with piperic acid



Scheme 3 Mechanism of amide bond formation

Table 1 Antimicrobial activity of piperine–pyridine analogs (zone of inhibition in mm)

S. no.	Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus cereus*	*E. coli*	*Enterococcus faecalis*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
1	**PY1**	15	10	14	**24**	18	**20**	**21**
2	**PY2**	11	**20**	18	16	**20**	17	18
3	**PY3**	12	11	**22**	13	15	**20**	18
4	**PY4**	14	9	13	15	**26**	17	**24**
5	**PY5**	18	**21**	**23**	19	**26**	**25**	18
6	**PY6**	**20**	17	**22**	15	17	19	17
7	**PY7**	**22**	10	14	**21**	19	18	**20**
8	**PY8**	11	16	15	18	15	19	**21**
9	Streptomycin (30 μg/ml)	26	21	**25**	24	26	25	26
10	Tetracycline (30 μg/ml)	25	24	**24**	22	25	26	24

Bold values indicate the highest activity than the standard drug

For each ligand, ten best conformations were generated and scored using Auto-Dock 4.2 scoring functions [41].

Results and discussion

Chemistry

Considering our interest in piperine and its analogs and our previous work on piperine analogs, piperine–pyridine analogs were designed using ChemDraw software and synthesized. Piperine was first hydrolyzed to piperic acid (Scheme 1), then coupled to different substituted aminopyridines using coupling agent HBTU and DIPEA as shown in Scheme 2. The mechanism of amide bond formation is shown in Scheme 3. The structures of all the synthesized compounds are presented in Table 3. All novel compounds were characterized using ^{1}H and ^{13}C NMR and mass spectra. The ^{1}H NMR spectrum of compound **PY1** showed a signal at chemical shift of δ 9.44, corresponding to amide N–H protons as singlet. The

Table 2 Antifungal activity of piperine–pyridine analogs (zone of inhibition in mm)

S. no.	Compound	*Aspergillus niger*	*A. flavus*	*A. fumigatus*	*Candida albicans*
1	**PY1**	15	18	16	**24**
2	**PY2**	**25**	**22**	18	16
3	**PY3**	22	22	**11**	13
4	**PY4**	13	19	20	15
5	**PY5**	20	**30**	**25**	**23**
6	**PY6**	20	**27**	12	16
7	**PY7**	**24**	12	14	**21**
8	**PY8**	13	6	15	20
9	Carbendazim (30 µg/ml)	30	35	28	30

Bold values indicate the highest activity than the standard drug

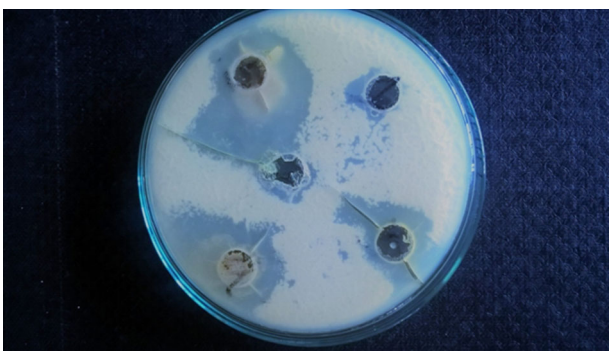


Fig. 1 Zone of inhibition for *Staphylococcus aureus*

multiplet signal in the range of 8.50–8.45 and 7.71–7.66 for four protons is related to aromatic pyridine ring. The doublet signal at δ 6.80–6.72 for two protons is related to CH_2–O group, and the 5.56 doublets are assigned to CH–CO group. The proton peaks for *trans*-double bond appear at 5.99, 6.90, and 6.69 ppm. The other C–H protons of benzene ring appeared as doublet at δ 6.90 ppm. Two protons on benzene ring appeared as multiplet located at 7.09–7.00 ppm. The ^{13}C NMR spectrum of the compound exhibited signals at different δ-chemical shifts including 165.29, 150.44, 147.52, 147.39, 143.61, 140.82, 138.78, 130.71, 125.85, 123.16, 123.08, 113.65, 108.52, 106.90, and 101.00 ppm. The IR spectrum showed bands at 3100–3500 for amide N–H stretching, 3048 for aromatic C–H stretching, 1710 for C=O amide stretching, 1594 for aromatic C=C, 1640 for C=C aliphatic double bond stretching, and 1125 cm^{-1} for C–O group.

Antimicrobial activity

Table 1 summarizes the antibacterial results of the tested compounds. The values are zones of inhibition measured in mm. All tested compounds were active against

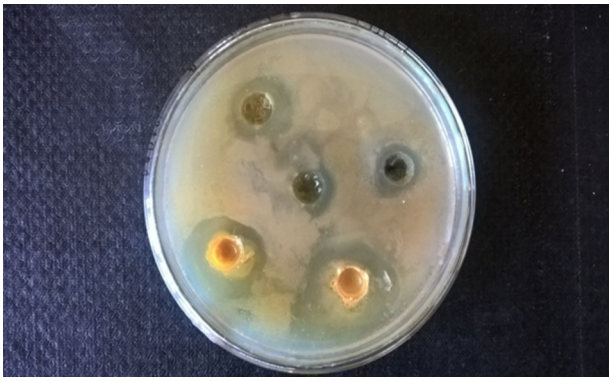


Fig. 2 Zone of inhibition for *Escherichia coli*

Fig. 3 Zone of inhibition for *Aspergillus niger*

all tested organisms. Compounds **PY1**, **PY4**, **PY8** were very active against Gram-negative bacteria *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, whereas compound **PY6** was only active against Gram-positive bacteria. Compounds **PY6** and **PY7** showed good activity against *Staphylococcus aureus* (20 and 22 mm). For *Bacillus subtilis*, compounds **PY2** and **PY5** were most active. Compounds **PY3** and **PY6** were active against *Bacillus cereus*. Among all target compounds, **PY5** showed very strong antibacterial activity, especially against *Enterococcus faecalis* and *Klebsiella pneumoniae*, which might be due to presence

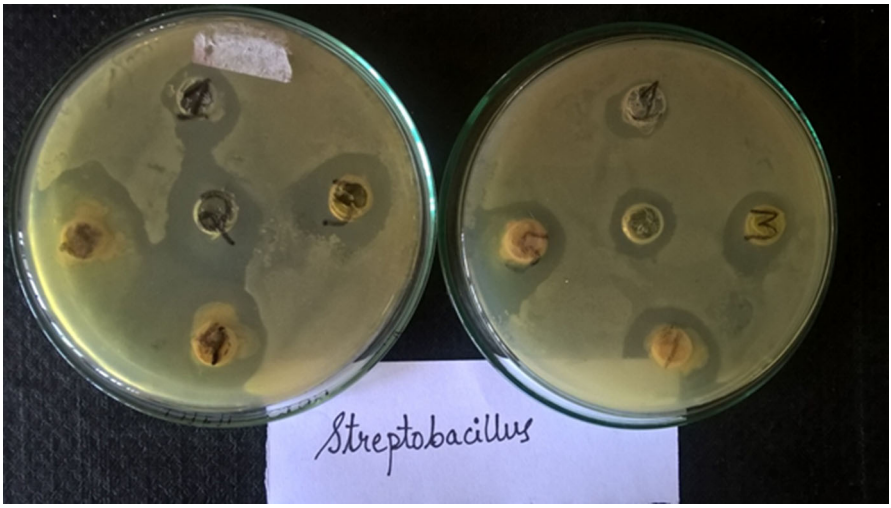


Fig. 4 Zone of inhibition for *Streptobacillus*

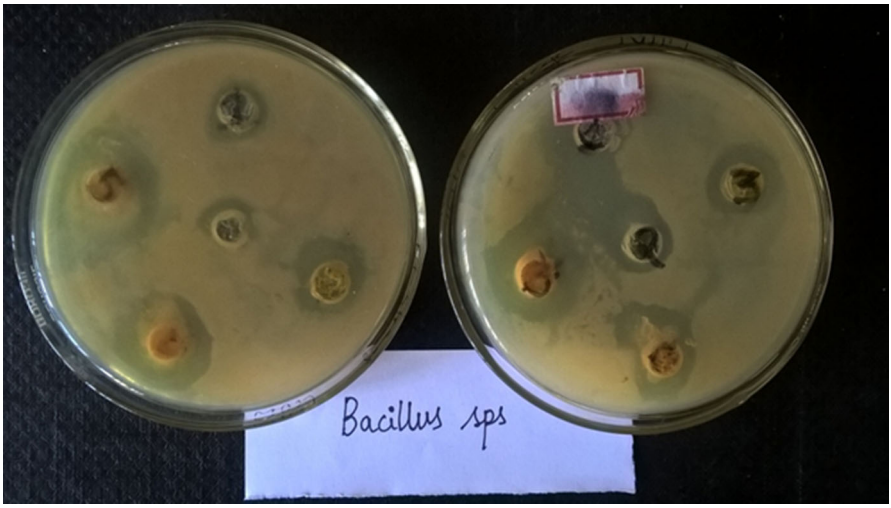


Fig. 5 Zone of inhibition for *Bacillus subtilis*

Table 3 Binding energies and structures of piperine–pyridine analogs

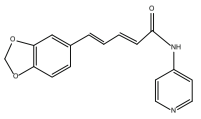
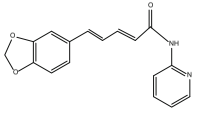
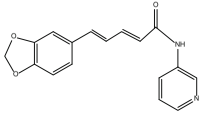
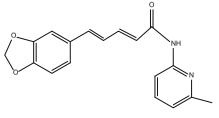
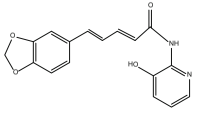
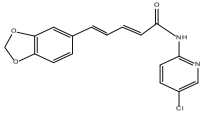
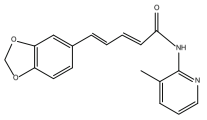
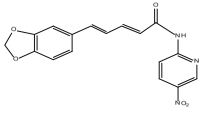
S. no.	Compound	Structure of compound	Binding energy (kcal/mol)			
			Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	**PY1**		−18.54	−20.35	−18.7	−18.82
2	**PY2**		−18.57	−22.52	−20.55	−19.55
3	**PY3**		−19.84	−21.57	−21.37	−19.53
4	**PY4**		−17.63	−20.45	−21.62	−19.11
5	**PY5**		−20.12	−23.37	−23.11	−21.33
6	**PY6**		−18.33	−20.48	−22.94	−22.29

Table 3 continued

S. no.	Compound	Structure of compound	Binding energy (kcal/mol)			
			Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
7	**PY7**		−17.22	−21.47	−20.67	−19.88
8	**PY8**		−13.14	−21.44	−20.35	−17.61

of ring-activating hydroxyl group. Most of the compounds were active against *Pseudomonas aeruginosa*, with compound **PY4** showing the highest activity (24 mm). The Gram-negative bacterium *Enterococcus faecalis* showed large inhibition zone (26 mm) for the applied standard drug. The antifungal activity of all target molecules is summarized in Table 2. All compounds were moderately active towards all fungi. Compound **PY5** with ring-activating hydroxyl group showed the highest antifungal activity against *Aspergillus flavus*, *A. fumigatus*, and *Candida albicans*, with compound **PY6** being the next most active against *Aspergillus flavus*. The zones of inhibition are shown in Figs. 1–5.

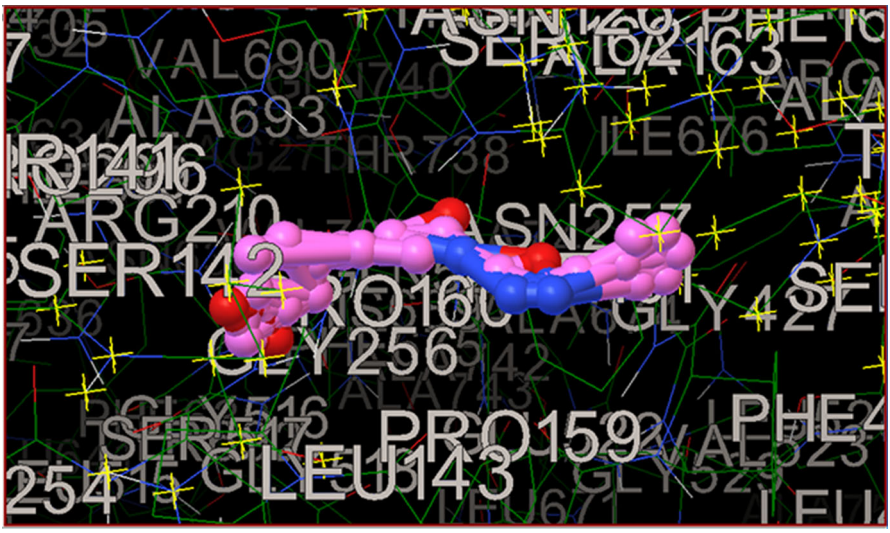


Fig. 6 Binding interactions for different amino acids with the target molecule

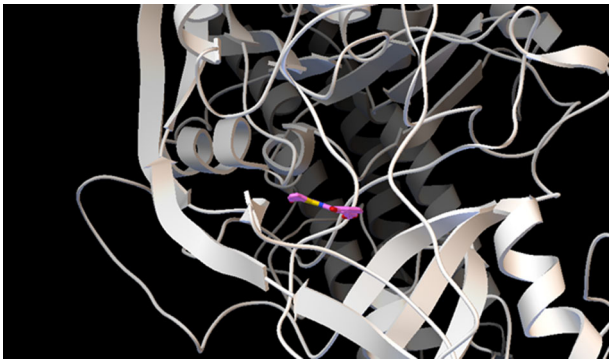


Fig. 7 In silico binding mode of piperine-pyridine analogs to protein molecule

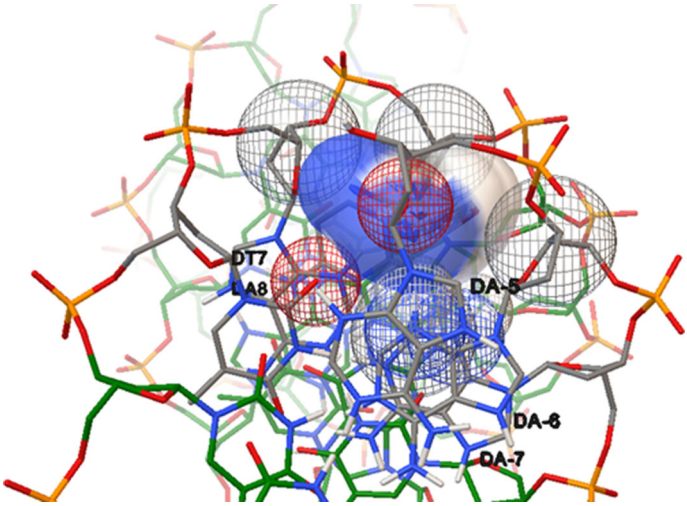


Fig. 8 Ball and stick model for the interactions of amino acids to the analogs

In silico studies

In silico studies of docking to 5XGT (*Staphylococcus aureus*), 3ZIH (*Bacillus subtilis*), 5NWT (*Escherichia coli*), and 2W7Q (*Pseudomonas aeruginosa*) were carried out using AutoDock 2.0 software. The binding energies of the target compounds with all microorganisms are expressed in kcal/mol in Table 3. Compound **PY5** showed the highest binding energy with all the organisms. Due to presence of hydroxyl group, this compound might bond to protein via strong hydrogen bonding (Figs. 6–8).

Conclusions

A novel series of piperine–pyridine analogs (**PY1**–**8**) were designed and synthesized by coupling piperic acid with substituted aminopyridines using HBTU as coupling agent. All synthesized molecules were novel and tested for their antibacterial and antifungal activities. In silico studies were also conducted to determine the binding efficiency of each synthesized molecule to proteins of microorganisms. All tested compounds showed moderate to high activity towards all tested organisms. Among all the compounds, **PY5** with ring-activating hydroxyl group exhibited the highest activity against both bacteria and fungi. There are no reports on synthesis of piperine–pyridine analogs, so this represents the first report on synthesis of novel piperine–pyridine analogs and their antimicrobial activity.

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