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RESEARCH ARTICLE

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Synthesis, in vitro and in silico anti-bacterial analysis of piperine and piperic ester analogues

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Abstract

A set of 12 analogues of piperine was designed, replacing the amide functional group of the molecule with different aliphatic and aromatic ester functional groups. Molecular docking studies of these molecules with FDA-approved target proteins for anti-bacterial drugs were done. The binding energy of the proteins and the ligands were low and the analogues were found to be drug-like based on the ADME results; hence, the molecules were synthesized. The synthesized compounds were tested for their anti-bacterial property against six bacterial species via Agar well-diffusion method. *Acinetobacter baumannii*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus epidermidis* were the strains tested. The overall susceptibility is higher in gram-positive. The analogues showed better activity than piperine. The analogues, propyl piperic ester (P3) and 2-fluorophenyl piperic ester (P9) and 4-fluorophenyl piperic ester (P10) showed comparatively bigger inhibition zones for all the strains.

KEYWORDS

ADME, anti-microbial, bacteria, docking, piperine, piperine analogues

1 | INTRODUCTION

A constant update in the library of anti-microbial is must as bacteria tend to mutate. A mutated bacterium, otherwise called a Super bug, is considered highly dangerous, and it can withstand a broad range of anti-bacterial drugs at once. For instance, mutated *Staphylococcus aureus,* methicillin resistant *S. aureus* (MRSA), can be effectively encountered with new antibiotics that the bacterium or its antecedent had not fought with before. Introducing 'Molecule with new pharmacophore' as antibiotics is one of the efficient ways to address this problem. Natural products that are known for their bioactivity provides such perfect new molecular bases. Herein, we report the bactericidal effect of a natural product piperine and its structural analogues.

Piperine is a bio-active component obtained from Indian black pepper, *Piper nigrum*. Piperine is reported for its anti-microbial property, bioavailability enhancement, GABA modulation property, anti-cancerous property, TRPV1 inhibition etc. Focussing on the anti-bacterial activity, there have been quite a number of articles that discuss the synergistic anti-microbial effect, the most studied bacteria for the synergistic effect of piperine and its analogues are *Staphylococcus aureus* with NorA efflux pump and MdeA efflux pump (Khan et al., 2006; A. Kumar et al., 2008; Mgbeahuruike et al., 2019; Mirza et al., 2011; Raja et al., 2015; Sangwan et al., 2008) of piperine analogues and just very few reports on the bactericidal effect of the compounds.

In 2013, the anti-bacterial activity of piperine analogues against four Gram-negative and three Gram-positive bacteria was tested by Umadevi et.al. They have targeted the piperidine ring moiety and replaced it with 4-chloro aniline, 4-bromo aniline, histidine, phenylalanine and tryptophan amines. These amide analogues are reported to have better activity than piperine (Umadevi et al., 2013). When the piperidine moiety is replaced with α-aminoacyl phenylalanine pinanediol boronic

ester in the place of piperidine ring, the anti-bacterial activity reduces. Here, piperine shows better activity than the boronic ester analogues (Venugopal, 2014). Then, using the theory of Hybrids or Conjugate molecule, piperidine ring has been replaced with substituted pyridine moieties and 1,2,4 triazole rings retaining the amide functionality. These analogues exceeded the activity of piperine successfully against *S. aureus* (Amperayani et al., 2018; Kumar et al., 2019).

In this study, the analogues were synthesized in an orderly fashion with alkyl and aryl esters. The focus is on the bactericidal efficacy of the molecules and to find the plausible reason for the activity, and the synergism is not checked. The objective is to change the functional group of the molecule and record the change in activity. Firstly, the tertiary amide functional group in the molecule is converted to piperic acid which is then converted to different aliphatic and aromatic esters. Esters are labile functional groups that are used as prodrugs and drugs (Lavis, 2008; Roll et al., 2007; Wollina, 2011). Since there is no research explicitly on the anti-bacterial properties of alkyl/aryl piperic carboxylic esters, we took the opportunity to study them.

Six bacterial species, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia faecalis*, *Escherichia coli, Staphylococcus epidermidis* and *Staphylococcus aureus,* were taken for the screening. The bactericidal activity of the analogues is considerable against the third generation bacteria, which are substantially stronger than the first sub-culture. This proves the analogues to be potent. Also, all the analogues are more potent than the mother molecule piperine.

2 | MATERIALS AND METHODS

All reagents used were of analytical grade (AR) and used directly without further purification. Piperine was a kind gift from the commercial producers Polyhedron Laboratories Pvt. Ltd, Tamilnadu, India. AV-500-Bruker 500 MHz high-resolution multinuclear FT-NMR spectrometer was used for ^{1}H &^{13}C Spectroscopic measurements, and chemical shifts are given in parts per million (ppm). FTIR spectrum was recorded on a PerkinElmer FT-IR attenuated total reflectance (ATR) spectroscopy. Reaction progress was monitored by thin-layer chromatography on Merck TLC silica gel plates with either hexane: ethyl acetate (6:4) or methanol: chloroform (1:9) as mobile phase. Spots were visualized under ultraviolet (UV) chamber. The anti-bacterial experiments were carried out in Thermo Fischer Bio Safety Cabinet of B2 series, model number 1300. HiMedia Sterile disposable petri plates were used to grow the culture.

2.1 | Chemistry

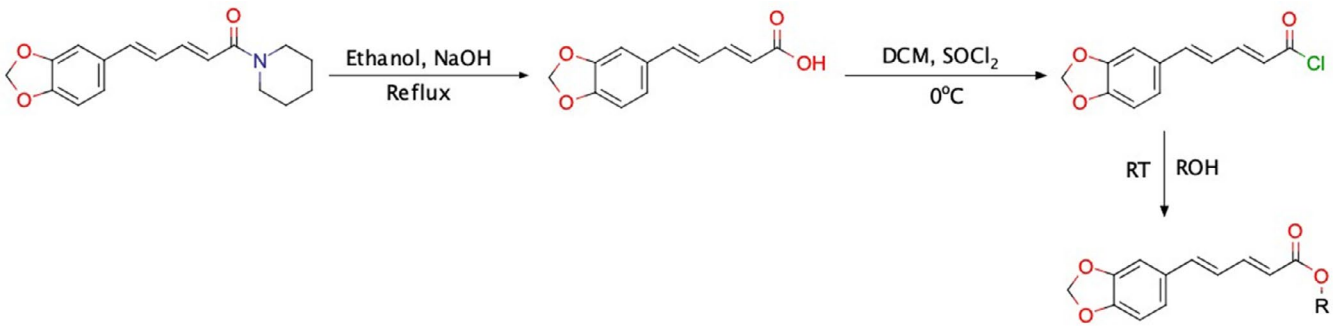
The amide functional group of piperine was converted to a better reactive carboxylic acid functional group via base hydrolysis, to piperic acid. Piperic acid is the first analogue of piperine, and this molecule was used as the starting material for the synthesis of all the other ester analogues in this study. Piperic acid was treated with thionyl chloride to form piperic acyl chloride which is then treated with alcoholic or phenolic -OH to form respective esters. The structures of the compounds are validated by ^{1}H, ^{13}C NMR and IR spectroscopic techniques.

2.2 | Synthesis of piperic acid

Piperine (10 g) was dissolved in 200 ml of 15% ethanolic NaOH in a single neck RB flask and refluxed. The reaction was monitored through TLC. The reaction got over after 24 hr. The ethanol in the medium was completely evaporated, and the resulting sodium piperate is dissolved in ice cold distilled water. To this, 0.1N HCl was added slowly, till the precipitation of piperic acid was complete. The precipitate was then filtered and washed with distilled water. The product was recrystallized in ethanol.

2.3 | Synthesis of piperic esters

Scheme 1 represents the general synthetic procedure followed. In a vacuum dried RBF, piperic acid (500 mg, 2.29 mmol)



SCHEME 1 General Scheme of synthesis of ester analogues of piperine

dissolved in 15 ml of DCM was added. An ice bath was used to cool this set up, and $SOCl_2$ (1.5 mmol) was added slowly. After the addition of $SOCl_2$, the temperature was gradually raised to room temperature. The formation of acyl chloride was confirmed through TLC. The reaction was completed after 7 hr. In a separate vacuum-dried RBF, alcohol/phenol of interest (1.1 mmol) and tri ethyl amine (1 mmol) were taken and dissolved in 10 ml of DCM. These contents were transferred slowly into the RBF containing acyl chloride at 0°C. The reaction was let to run and monitored via TLC. After the completion, the reaction was quenched with water and the organic layer was collected. The collected DCM layer was washed with water, brine solution, dried with magnesium sulphate and evaporated to get respective esters. The crude product was then column chromatographed using hexane and ethyl acetate (9:1) to get pure compounds. Figure 1 enlists the structure of the analogues.

2.4 | Computational analysis

The analogues were studied theoretically for their antibacterial property via chemometric studies. The compounds were docked with the crystal structures of 7 proteins obtained from RCSB Protein Data bank (Berman et al., 2000). Uridylate kinase, Penicillin-binding protein 1B, Membrane-bound lytic murein transglycosylase F, Acyl carrier protein, tRNA dimethyl allyl transferase and Teichoic acid D-alanine hydrolase proteins (PDB ID: 4a7x, 3vma, 5a5x, 6dfl, 3crm, 5zh8, 1amp) were taken as targets, which are popular anti-bacterial targets. The proteins were prepped by removing the water molecules and the ligands from their crystal structures. Flexible blind docking was employed. The target sites in the protein structures were confirmed via Metapocket 2.0 (Zhang et al., 2011). Here, the receptor is protein molecule, and the ligand is piperine analogue. Ligands were drawn using ChemDraw suite.

Autodock Vina with Python GUI was used for the Docking studies (Trott & Olson, 2010). Conjugate gradient algorithm and MMFF94 were the forcefield used for the energy minimization of the ligands, throughout the docking studies (Developed by the Resource for Biocomputing; Morris et al., 2009). The exhaustiveness was set to 8, total number of steps involved in the energy minimization to 200 steps and the process to end when the energy difference between the structures was found to be <0.1 for the whole process. For each protein used, the dimension of the Grid box differed. The binding score of the docked model with the least Root Mean Square Deviation (RMSD) was considered. Discovery Studio was used to view the Autodock results.

The ADME properties of the molecules were calculated theoretically. The theoretical calculations of physicochemical properties like partition co-efficient (Log P) (Cheng et al., 2007; Wildman & Crippen, 1999), Topological Polar Surface Area (TPSA), number of hydrogen Bonds, number of hydrogen acceptors, molar refractivity, gastro intestinal absorption, cytochrome P450 interaction along with the compliance or violation of the drug-likeness rules of Lipinski, Ghose, Veber, Egan and Muegge were considered before synthesis (Daina et al., 2017).

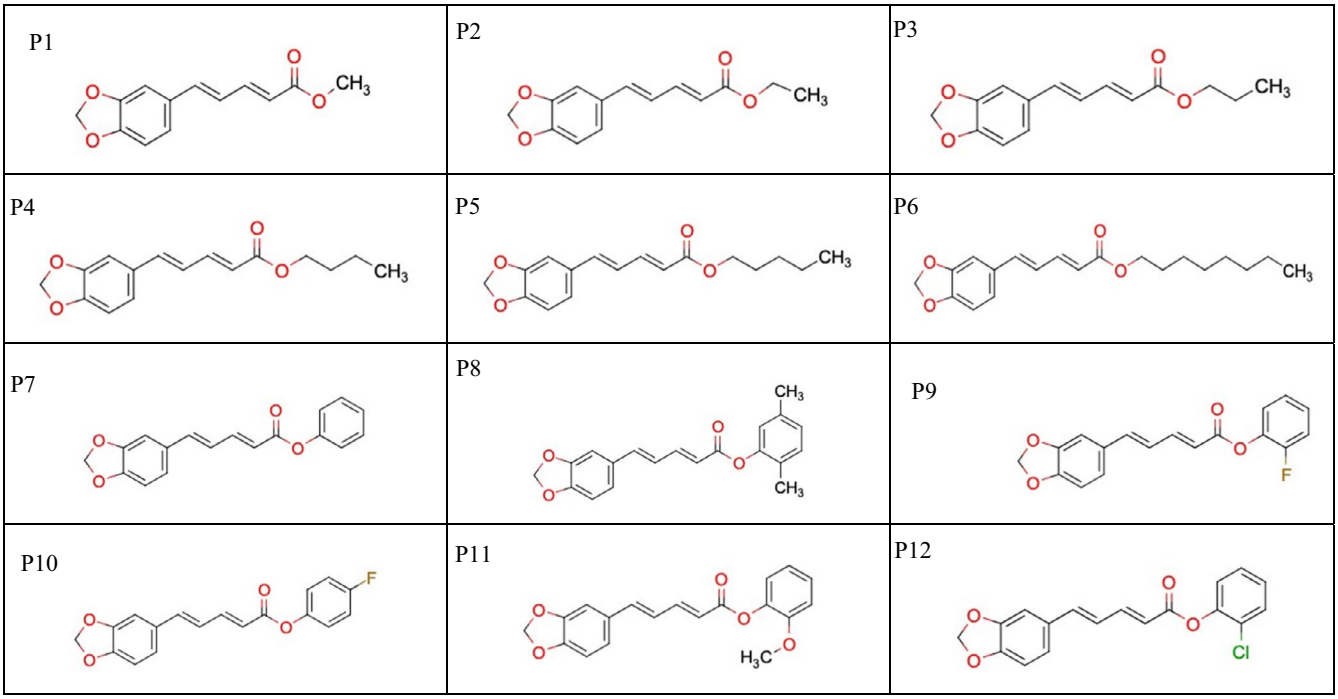


FIGURE 1 Structure of Piperine analogues

2.5 | Anti-bacterial assay

Commercially obtained three gram-positive and three gram-negative bacterial strains were taken for the anti-bacterial test. *Escherichia coli*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* were the bacteria. The obtained bacteria were sub-cultured to their third generations and then used. Agar well-diffusion method was employed. 20 ml of Muller–Hinton Agar (MHA) was spread on Petri plates and led to solidify in the room temperature. The cultured bacteria were diluted with sterile solution to 10^5 CFU/ml prior to the even application on the MHA Petri plates. The stock solution of piperine and the analogues was prepared in the concentration of 50 mg/ml in DMSO solvent. Serial dilution was avoided to reduce the volume of DMSO used in the well. The concentration variation of the compounds was achieved by using different volumes (30 µl = 1.5 mg/well, 40 µl = 2 mg/well, 50 µl = 2.5 mg/well) of the same stock solution, which is one of our major variations from the literature. Wells of 6 mm diameter were made in the MHA petri plates. The target compounds were added to the wells and incubated at 37°C for 24 hr. Gentamycin and Ampicillin (30 µg/ml) were used as reference drugs. A blank well (DMSO) was kept for reference. The activity of the target compounds was measured based on the measure of the zone of inhibition after 24 hr of incubation. The diameter of the zone of inhibition was measured in millimetres.

3 | RESULTS AND DISCUSSION

3.1 | Spectroscopic analysis

The spectral data of the synthesized compounds clearly show the formation of the analogues. The shift of peak in IR from 1674 for piperic acid to around 1,700 cm^{-1} for the ester analogues is clear indication of formation of carboxylic ester functional group, while the peak for dioxy methylene group appears around 1,492 cm^{-1}. In the ^{1}H NMR, the dioxy methylene group (-O-CH_2-O-) gives a singlet around δ 6 ppm, as the alkyl chain is in conjugation with the aromatic ring, the peaks for the protons in the alkyl chain falls under the aromatic region too, except for one proton next to the carbonyl group which gives a doublet around δ 6 ppm. In ^{13}C NMR, the peak around δ 167 ppm indicates the carboxylic ester functional group and peak around δ 101 ppm indicates the dioxy methylene (-O-CH_2-O-) carbon.

Structure of P2 in Figure 2 is taken for single compound discussion, 1,703 cm^{-1} indicates carbonyl ester stretching, peak around 1,489 cm^{-1} indicates -CH_2- bending from 1,3-benzodioxole ring, peak around 1,253 cm^{-1} is the indication of asymmetric -CO stretching and peak at 930 cm^{-1}

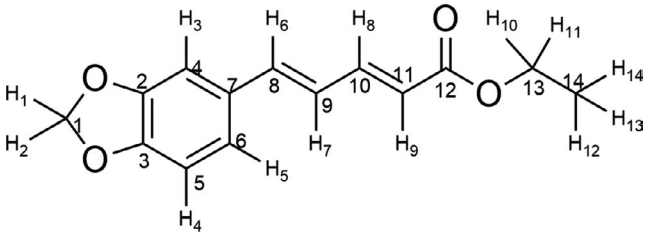


FIGURE 2 Structure of P2 with H numbering

is another characteristic of CO stretching. These peaks are observed in all the analogue's IR spectrum, which is one of the clear indications for the formation of the compounds. In ^{1}H NMR, the unsaturated chain protons and the benzene protons come in the aromatic region of δ 7.43–δ 6.67; breaking that down, quartet at δ 7.43 ppm is due to H_8 with $J = 15$ Hz indicating trans coupling with the protons H_6 and H_7; singlet at δ 6.99 ppm is due to H_3; a doublet with coupling constant 10 Hz indicates cis coupling of H_4 with H_5; peaks by H_5 and H_6 are merged to give peaks around δ 6.79 ppm, with coupling constant values 15, 20 and 5 Hz respectively; H_7 gives a quartet at δ 6.72–δ 6.67 with coupling constant 15 Hz indicating trans coupling; the high trans coupling constant values indicate the flexible unsaturated chain; further, singlet at δ 5.98 ppm is due to H_1 and H_2; H_9 gives rise to a doublet at δ 5.939 away from the other alkyl chain protons due to its position near the carbonyl group which de-shields the H_9; a quartet at δ 4.24–δ 4.20 ppm is due to the ethyl protons $H_{11}$and H_{10} with J = 10 Hz; and triplet at δ 1.31 ppm is due to H_{11}, H_{12} and H_{13} of the ethyl protons, the coupling constant value 10Hz indicated vicinal coupling. The same pattern can be observed in all the other analogues too.

For ^{13}C NMR (Figure 2), the carbonyl carbon in P2 gives its peak at δ 167 ppm; C_2 and C_3 give peaks at δ 148.55 and 148.29 ppm respectively; C_{10} gives peak at δ 140 ppm; C_6, C_7, C_8 and C_9 give peaks at δ 130.59, 124.56, 122.94 and 120.46 ppm; C_4 and C_5 at δ 108.55 and 105.88 ppm; C_1 at δ 101.40 ppm and the ethyl carbons C_{13} and C_{14} came at δ 60.31 and δ 14.35 ppm, respectively. The trend is observed in all the analogues which proves formation of the compounds.

3.2 | Computational analysis

The analogues were given ester functional group, as esters are well-known prodrugs. The pharmacophore of the molecule maintained for this work is given in Figure 3. The pharmacophore contains three hydrogen donors (HYD) separated by the distance of 1.2 A^0 : two aromatic ring moieties (AR) at distance 2.1 A^0 from each other and one aromatic ring from the HYD is at the distance 2 A^0, and all these are surrounded by 4 hydrogen acceptors (ACC). Similar arrangement could be found in molecules like gingerol, curcumin and quercetin which are known for their anti-microbial properties.

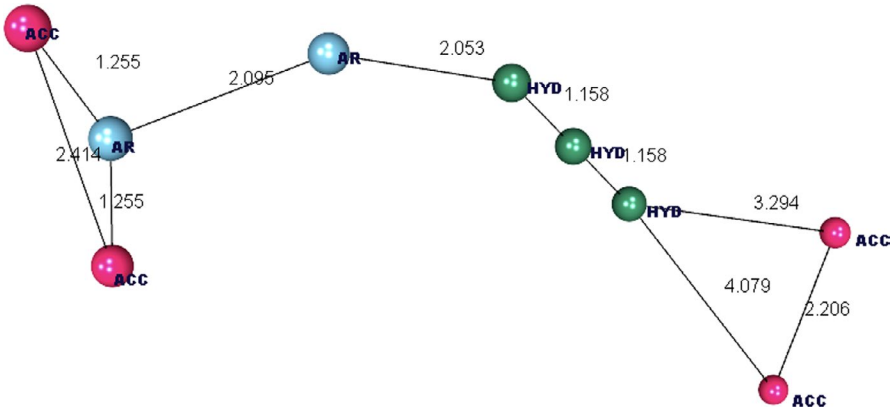


FIGURE 3 Pharmacophore of the analogues generated by overlapping the 15 analogues and the Piperine molecule. Here, Acc is Acceptor, AR is Aromatic Ring, HYD is H Donor, Bond length is in A^0

TABLE 1 Binding energies in kcal/mol of the analogues with different proteins

Compound	4a7x	3vma	5a5x	6dfl	3crm	5zh8	1amp
Pip	−7.3	−8.7	−7.3	−7.8	−8.2	−7.6	−6.7
PA	−6.1	−6.9	−7.3	−9.2	−7.3	−6.9	−5.4
P1	−7.2	−7	−7.5	−9.2	−7.2	−7.3	−5.1
P2	−6.1	−5.9	−7.1	−6.4	−6.9	−6.6	−5.1
P3	−6.2	−7.7	−7.3	−9.5	−7.2	−6.8	−5.8
P4	−6.2	−7.5	−6.9	−9.8	−7.2	−6.9	−6.1
P5	−6.4	−7.7	−6.8	−6.5	−7	−6.8	−5.2
P6	−6.4	−7.4	−7.2	−7.9	−6.7	−7.9	−5.8
P7	−7.2	8.5	−7.6	−11.3	−8	−7.9	−6.6
P8	−7.5	−9.1	−8.6	−8	−8.5	−7.6	−6.7
P9	−7	−8.9	−8.2	−9.2	−7.9	−7.9	−6.3
P10	−7.1	−8.9	−9.3	−10.5	−8.1	−8.1	−6.4
P11	−7.1	−8.8	−8.5	−11.8	−8.4	−8.9	−6.8
P12	−7.2	−8.3	−8	−10.7	−8.2	−9.2	−6.6

The compounds were docked with 7 proteins with PDB ID: 4a7x, 3vma, 5a5x, 6dfl, 3crm, 5zh8 and 1amp. UridylateAQ6 kinase is involved in UDP synthesis. Penicillin-binding protein 1B is involved in the inhibition of cell wall synthesis. Membrane-bound lytic murein transglycosylase F was involved in cell wall synthesis. Acyl carrier and lipopolysaccharide core heptose (I) kinase protein, tRNA dimethylallyl transferase and Teichoic acid D-alanine hydrolase proteins were taken as targets.

From Swiss ADME, the ADME properties of the compounds were calculated and it was made sure the analogues were drug-like theoretically. We have taken WLogP and XLogP3 (atomistic LogP values), which are proven to be most relevant to the Experimental LogP (Pyka et al., 2006). The XLogP3 of the analogues ranges from 3.23 to 6.44, while WLogP ranges from 1.96 to 4.78. The bioavailability score predicted by the software is 0.55–0.56 which is equivalent to the predicted bioavailability of the commercial antibiotics (for example, Ciprofloxacin: WLogP –1.18, Bioavailability Score- 0.55, with GI absorption, Ampicillin: WLogP -−0.39, Bioavailability Score- 0.55, with GI absorption). The analogues are predicted to be CYP inhibitors which again supports the drug bioavailability. It is noted that the molecules follow the Rule of five by Lipinski, drug-like rules by Ghose, Veber, Egan and Muegge. As the ADME properties were satisfactory and the Binding Energy of the molecules was found to be good, it was proceeded to the synthesis of the molecules. The binding energy of the compounds is in Table 1, and the ADME results are furnished in the supporting information.

The binding of the ligands that showed highest inhibition zones and nil inhibition zones against Protein (PDB ID: 6dfl) whose binding energy best correlated with the experimental results is exhibited in Figures 4–7. The aliphatic and the aromatic esters are divided and analysed for better understanding. Overall, in the aliphatic ester analogues P3 and P6 were the highest and nil inhibition zone producers respectively in almost all the strains. Similarly, P9 and P10 are best among aromatic esters, while P8 being the least inhibition zone producer in the aromatic esters. The analogues are not similar in action for all the bacterial strains. For the strains *E. faecalis* and *P. aeruginosa*, *A. baumannii* analogue P3 has

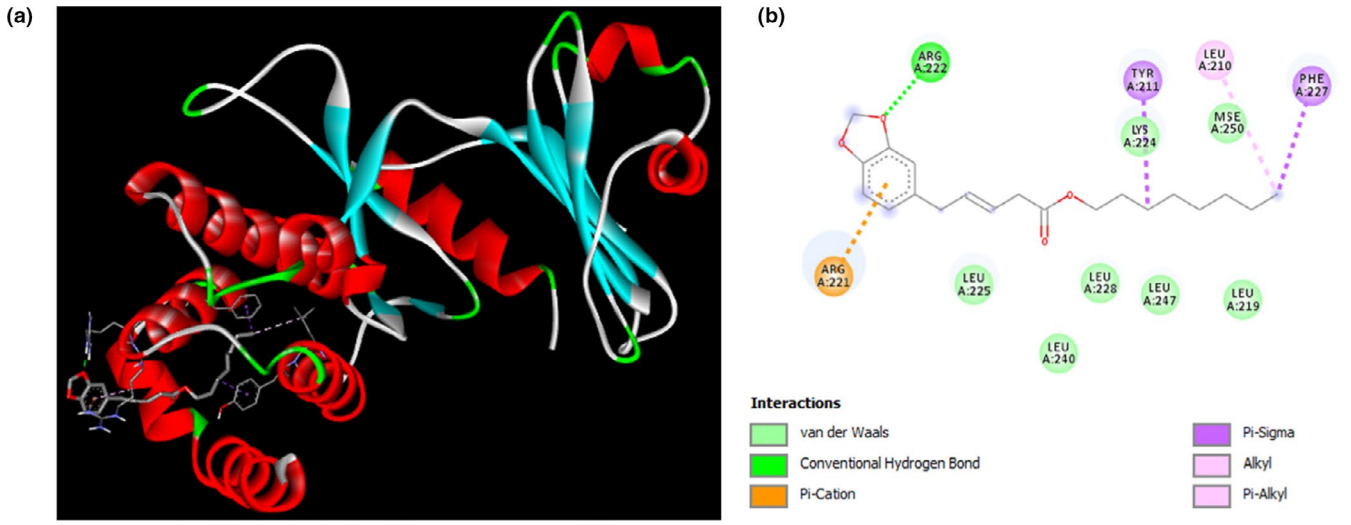


FIGURE 4 Binding mode of P6 with protein (PDB ID: 6dfl). (a) 3d binding mode (b) 2d binding mode

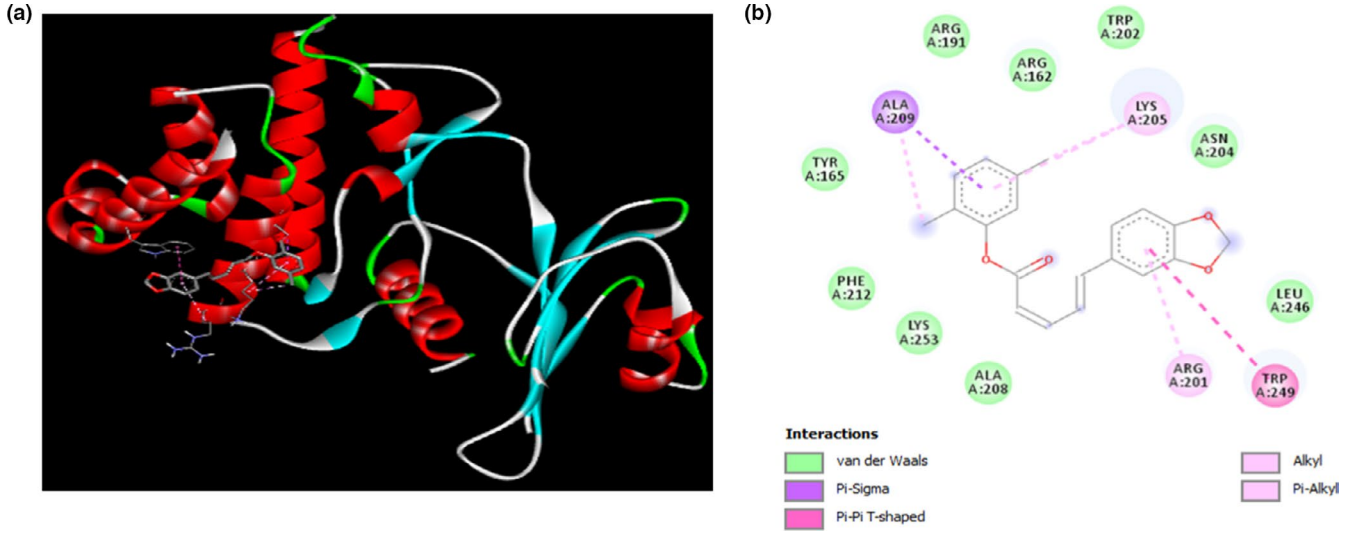


FIGURE 5 Binding mode of P8 with protein (PDB ID: 6dfl). (a) 3d binding mode (b) 2d binding mode

produced comparatively higher inhibition zone. Similarly for the strains *S. epidermidis* and *S. aureus*, the F substituted analogues P9 and P10 have shown better inhibition zones. It is to be noted that *A. baumanni* and *P. aeruginosa* fall under the order Pseudomonadales and strains *S. epidermidis* and *S. aureus* fall under the order Bacillales.

In the 3D view, we can observe that the better working analogues P3, P9 and P10 pass through the hydrophobic tunnels of the α-helix system of the protein. The binding mode of P3, P9 and P10 is similar in the 1,3-benzodioxole ring region of the analogue. In the figures, an inactive analogue from aliphatic ester (P6) and an inactive analogue from aromatic ester (P8) are shown to highlight the difference in the binding modes. In all active analogues, P3, P9 and P10, Pi–Pi staking is observed between the 1,3-benzodioxole ring and the pocket atoms PHE A:227 and TYR A:211. Similarly, these analogues show a Pi–alkyl interaction between 1,3-benzodioxole ring and Leu A:210 of the protein molecule. The fluoro phenyl ring of the P9 and P10 is observed to form a Pi–alkyl interaction with LYS A:224 and ARG A:221, along with a Pi–sigma interaction with LEU A:223. The residues LEU A:219, VAL A:144, LEU A:240, GLU A:251, LEU A:228, *MSE* A:250 and ALA A:214 are involved in the Van der waal's interaction with the molecule. In P3, other than the common interaction observed as with P9, alkyl interactions between the propyl chain and the residues LEU A:123, LEU A:143 and TRP

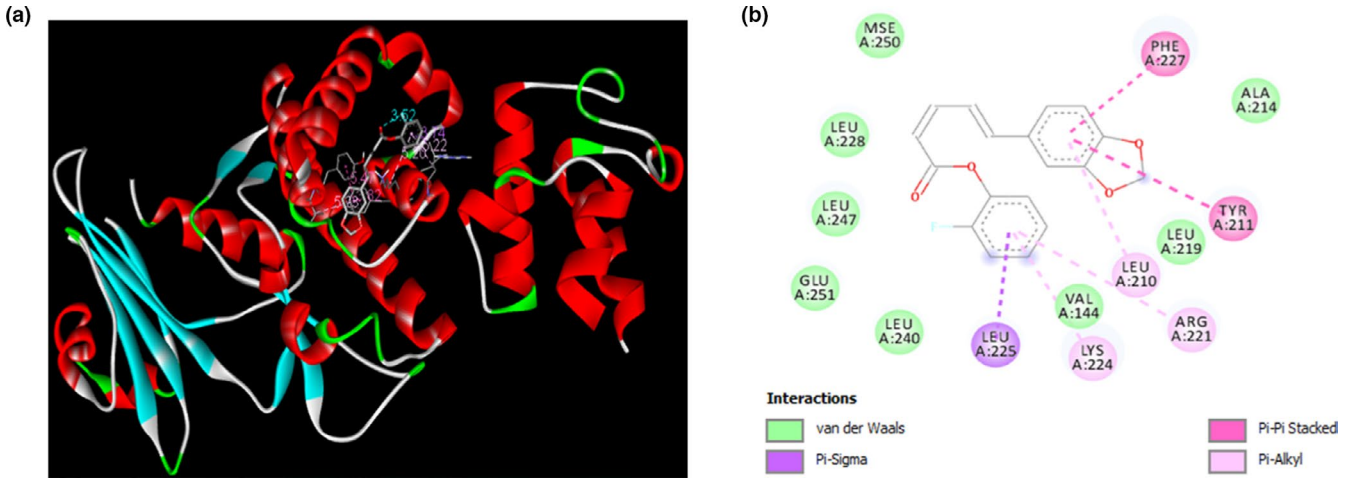


FIGURE 6 Binding mode of P9 with protein (PDB ID: 6dfl). (a) 3d binding mode (b) 2d binding mode

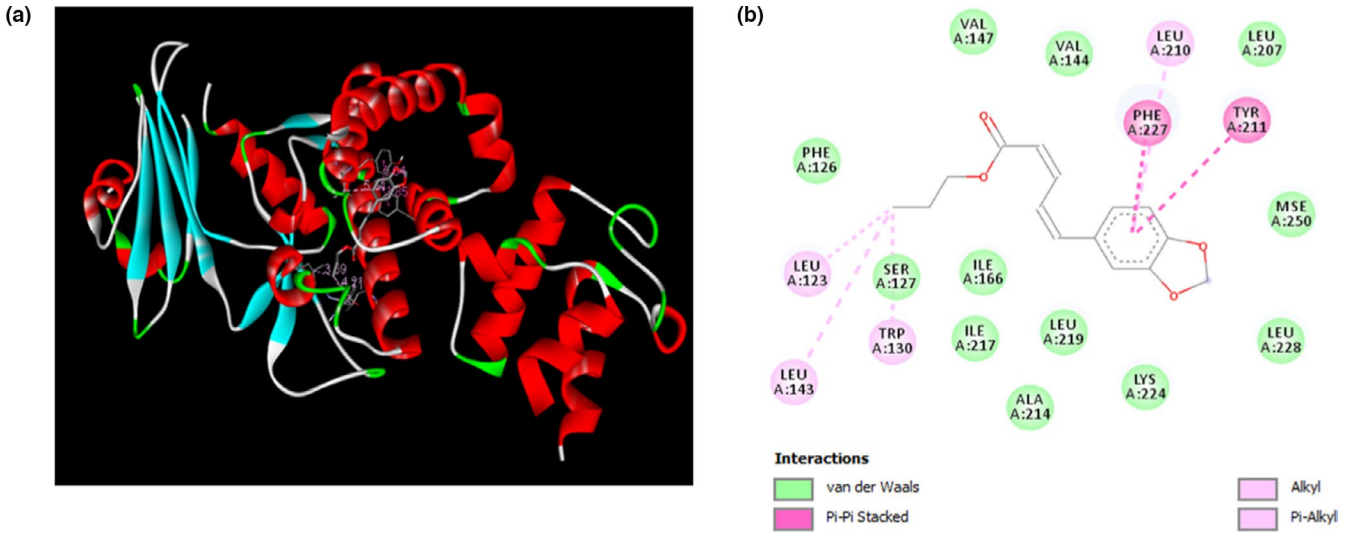


FIGURE 7 Binding mode of P3 with protein (PDB ID: 6dfl). (a) 3d binding mode (b) 2d binding mode

A:130 can be observed. Also VAL A:147, VAL A:144, LEU A:207, *MSE* A:250, LEU A:228, LYS A:224, ALA A:214, ILE A:217, ILE A:166, SER A:127 and PHE A:126 are involved in the Van der waal's interaction with the molecule P3.

3.3 | Anti-bacterial activity

Having the piperine binding sites in mind, we started the work by classifying the bacteria based on the thickness of their cell walls. Three gram-positive and three gram-negative bacteria were taken, and the action of the analogues over the bacteria was observed. Gram-positive bacteria have thick peptidoglycan layer while the gram-negative bacteria have thinner layer, but gram-negative bacteria have lipid bilayer which provides an extra protection to the cell making it less susceptible. Here, we used third generation bacteria which are substantially stronger that increases the ability of the bacteria to mutate or resist the drug much better, than the weaker bacteria. The results are given in the Tables 2 and 3. The Anti-bacterial assay plates after 24 hr of incubation with compounds P1, P2, P3 and P4 numbered as 1, 2, 3 and 4 respectively with Positive (+) and Negative (−) controls against the six bacterial strains are shown in Figure 8.

Based on our anti-bacterial result, we observed that,

1. The analogues show better activity than piperine at any concentration.
2. Piperine showed no inhibition zones for any strains but for *S. aureus* and *P. aeruginosa*.

TABLE 2 Inhibition zones produced by the analogues for Gram Positive Bacteria

	Inhibition Zone in mm (Gram positive)								
	S. aureus			*E. faecalis*			*S. epidermidis*		
Compound ID	**30 µl**	**40 µl**	**50 µl**	**30 µl**	**40 µl**	**50 µl**	**30 µl**	**40 µl**	**50 µl**
Piperine	–	–	10.57 ± 0.51	–	–	–	–	–	–
Piperic acid	–	–	8.66 ± 0.77	–	–	–	–	–	12.12 ± 0.45
P1	–	–	9.53 ± 0.51	–	–	–	–	–	11.6 ± 0.035
P2	–	–	9.12 ± 0.601	–	–	–	–	–	–
P3	–	8.71 ± 0.85	8.96 ± 0.403	–	10.88 ± 0.176	12.83 ± 0.233	–	–	11.18 ± 0.54
P4	–	–	8.955 ± 0.39	–	–	–	–	–	–
P5	–	–	8.81 ± 0.62	–	–	–	–	–	–
P6	–	–	–	–	–	–	–	–	–
P7	–	–	–	–	–	–	–	–	–
P8	–	–	–	–	–	–	–	–	–
P9	8.34 ± 0.52	8.70 ± 0.83	9.83 ± 0.86	–	–	8.33 ± 0.47	–	9.05 ± 0.64	10.09 ± 0.59
P10	9.3 ± 0.83	9.33 ± 0.45	10.03 ± 0.31	–		9.22 ± 1.011	–	11.31 ± 0.72	11.905 ± 0.13
P11	–	7.895 ± 0.32	7.9 ± 0.98	–	–	–	–	–	–
P12	–	–	–	–	–	–	–	–	9.9 ± 1.08
(Ref) Ampicillin	22.05 ± 1.11	NA	NA	23.48 ± 0.71	NA	NA	21.41 ± 0.87	NA	NA

TABLE 3 Inhibition zones produced by the analogues for Gram Negative Bacteria

	Inhibition Zone in mm (Gram Negative)								
	A. baumannii			*P. aeruginosa*			*E. coli*		
Compound ID	**30 µl**	**40 µl**	**50 µl**	**30 µl**	**40 µl**	**50 µl**	**30 µl**	**40 µl**	**50 µl**
Piperine	–	–	–	–	–	8.32 ± 0.45	–	–	–
Piperic acid	–	9.485 ± 0.39	9.88 ± 0.44	–	–	9.51 ± 0.70	–	–	–
P1	–	–	9.8 ± 0.28	–	9.125 ± 0.671	9.64 ± 0.65	–	–	8.74 ± 0.74
P2	–	–	9.24 ± 0.36	–	-	8.57 ± 0.38	–	–	8.61 ± 0.50
P3	–	–	9.95 ± 0.636	–	7.53 ± 0.39	9.59 ± 0.431	–	–	-
P4	–	–	9.25 ± 0.36	–	–	7.61 ± 0.56	–	–	–
P5	–	–	8.5 ± 0.55	–	–	8.54 ± 0.65	–	–	–
P6	–	–	-	–	–	-	–	–	–
P7	–	–	8.92 ± 0.59	–	–	-	–	–	–
P8	–	–	–	–	–	–	–	–	8.86 ± 0.48
P9	–	–	10.34 ± 0.44	–	–	7.84 ± 0.212	–	–	10.1 ± 0.21
P10	–	–	9.70 ± 0.43	–	–	7.49 ± 0.056	–	–	10.21 ± 0.46
P11	–	–	9.04 ± 1.15	–	–	–	–	7.46 ± 0.65	8 ± 0.47
P12	–	–	8.85 ± 0.22	–	–	–	–	–	–
(Ref) Gentamycin	23.02 ± 0.36	NA	NA	22.33 ± 0.43	NA	NA	27.53 ± 3.04	NA	NA

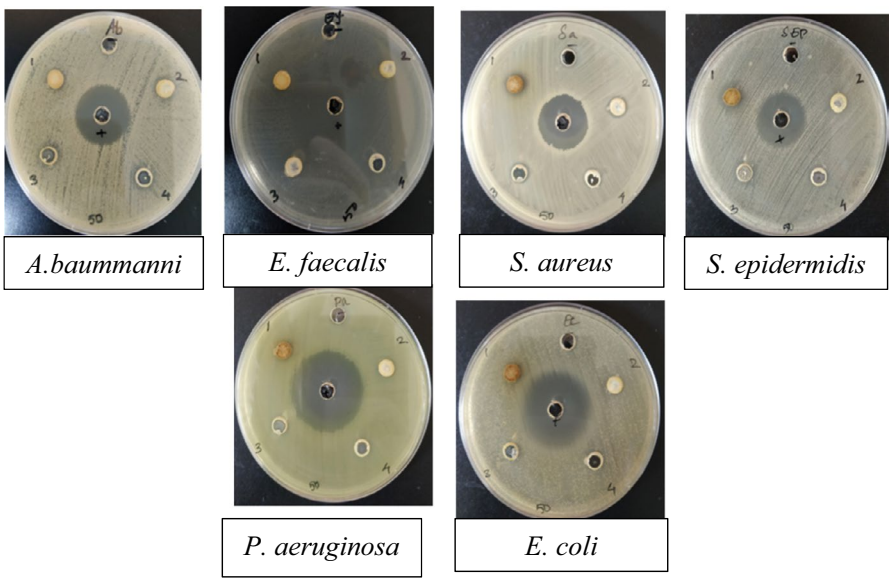


FIGURE 8 Anti-bacterial assay plates after 24 hr of incubation. Results of compounds P1, P2, P3 and P4 numbered as 1, 2, 3 and 4 respectively with Positive (+) and Negative (−) controls against the six bacterial strains

3. PA, P3, P9 and P10 showed the best activity among all the analogues for all the bacteria.
4. The inhibition zones for the Gram-positive bacteria are slightly higher than the Gram-negative bacteria. The inhibition of gram-negative is usually higher due to its thick outer lipid layer.
5. P3 works best for the strains under the order Pseudomonadales, and P9 and P10 for the strains under the order Bacillales

The theoretical interaction of the analogues with the Hydrolases protein (PDP ID: 6dfl) in Figures 4–7 shows the possible interaction mode of the ligands. From which it is clear that P3, P9 and P10 have comparatively more number of non-covalent interactions with the α-helix structure of the protein than other analogues.

4 | CONCLUSION

The synthesized structural analogues of piperine were tested against six bacterial strains, *A. baumannii*, *E. coli*, *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. Faecalis*. The cultures used were of third generation (3rd sub-culture), which considerably increases the strength of the bacteria than the first sub-culture. To maintain low carrier solvent level, the concentration variation was achieved by using different volume of dissolved analogues in the agar well from the same stock solution. Most of the analogues show better activity than piperine. The activity of the analogues increases with increase in the concentration. Proteins involved in the Hydrolases show better correlation with the activity of the molecules. Propyl substituted piperic ester analogue among the aliphatic esters category and fluoro phenyl piperic esters among the aromatic esters category show better activity. The theoretical binding model of the ligands to the protein PDB ID: 6dfl shows similar binding pattern among the best active analogues P3, P9 and P10 at 1,3-benzodioxole ring of the molecules along with the increased number of non-covalent interaction overall. This could be due to the optimum flexibility, bond energy and the rotatable bonds in the analogue. Additionally, the analogue P3 produces better inhibition zones for the bacterial strains under the order Pseudomonadales, while the fluoro phenyl esters P9 and P10 work best for the strains under the order Bacillales. From this, it is clear that the molecule has not lost its activity with the change in the functional group, that is, from amide to ester, but enhancement in the activity is observed. Yet, to increase the activity of the molecules to be equivalent to the commercial drugs, further modifications have to be done.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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