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Synthesis, molecular modeling and biological evaluation of PSB as targeted antibiotics

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ABSTRACT

We described here the design, synthesis, molecular modeling, and biological evaluation of a series of peptide and Schiff bases (PSB) small molecules, inhibitors of *Escherichia coli* β-Ketoacyl-acyl carrier protein synthase III (ecKAS III). The initial lead compound was reported by us previously, we continued to carry out structure–activity relationship studies and optimize the lead structure to potent inhibitors in this research. The results demonstrated that both *N*-(2-(3,5-dichloro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (**1f**) and 2-hydroxy-*N*-(2-(2-hydroxy-5-iodobenzylideneamino)propyl)-4-methylbenzamide (**3e**) posses good ecKAS III inhibitory activity and well binding affinities by bonding Gly152/Gly209 of ecKAS III and fit into the mouth of the substrate tunnel, and can be as potential antibiotics agent, displaying minimal inhibitory concentration values in the range 0.20–3.13 μg/mL and 0.39–3.13 μg/mL against various bacteria.

1. Introduction

Multi-drug-resistant Gram-positive and Gram-negative pathogens have become a serious problem in hospitals and the community.[1] Particularly alarming is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE) and vancomycin-resistant *Enteroccocus faecium* (VRE).[2,3] Drug discovery efforts have been significantly intensified in the past years to search for more effective antibacterial agents with a broader spectrum of activity and especially activity against resistant pathogens to fight infectious diseases.[4–6]

Bacterial type II fatty-acid synthesis (FASII) is an attractive target for antibacterial drug discovery.[7–9] The initiation condensing enzyme, *Escherichia coli* β-Ketoacyl-acyl carrier protein synthase III (ecKAS III), and elongation condensing enzymes, KAS I/II, are essential components of fatty-acid biosynthesis[10,11] and are highly conserved among key pathogens.[12] Notably, KAS III, regulates the fatty-acid biosynthesis rate via an initiation pathway and its substrate specificity is a key factor in membrane fatty-acid composition and this protein represents a promising target for the antimicrobial drugs design.[13] Moreover, the three-dimensional structure of the protein is highly conserved in various bacteria, and its inhibitors may thus act as potent antibiotics with broad-spectrum activity.[14]

Pioneering reports demonstrated that platencin,[15] thiolactomycin[16] and its analogues compounds[17] had low toxicity and some of which were highly active against KAS III. In particular, Schiff base compounds were reported as a potent inhibitor of *Helicobacter pylori* KAS I,[18] *Plasmodium falciparum* KAS III[19] and ecKAS III.[20] In the previous research, we performed that a kind of peptide and Schiff bases (PSB) small molecules with electron-withdrawing groups on B-ring and appropriate length of aliphatic chain ($n = 2$ or 3) were important factors responsible for the inhibitory activities against ecKAS III.[21]

In this study, our main objective is development of potential inhibitors by newly synthesized PSB as targeted antibiotics agents, based on molecular modeling and investigation of SAR between new inhibitors and ecKAS III. Firstly, we used structure-based design method to synthesize the PSB by introducing substitute group on A-ring and using 1,2-propanediamine as connect aliphatic chain (Scheme 1) and test their inhibitory activity against ecKAS III. And following, top 10 PSB compounds which posses good ecKAS III inhibitory activity (low IC_{50}) were picked out to test their antibacterial activities against two Gram-negative bacterial strains (*E. coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 13525) and two Gram-positive bacterial strains (*Bacillus subtilis* ATCC 6633 and *S. aureus* ATCC 6538), expecting to exploit potent antibacterial agent with broad-spectrum antibiotics activity. As expected, the results demonstrated that the antimicrobial compound, *N*-(2-(3,5-dichloro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (**1f**) and 2-hydroxy-*N*-(2-(2-hydroxy-5-iodobenzylideneamino)propyl)-4-methylbenzamide (**3e**), can be as an inhibitor of ecKAS III and as potential antibiotics agent, displaying MIC values in the

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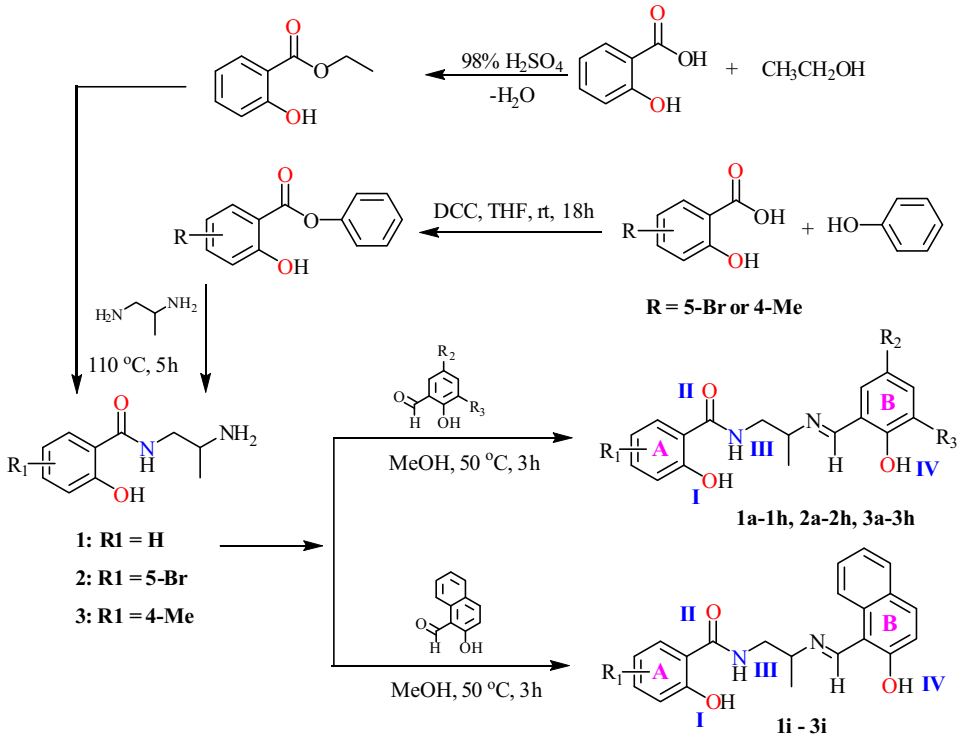

1a	$R_1 = R_2 = R_3 = H$	**2a**	R_1 = 5-Br, $R_2 = R_3 = H$	**3a**	R_1 = 4-Me, $R_2 = R_3 = H$
1b	$R_1 = R_3 = H$, $R_2 = F$	**2b**	R_1 = 5-Br, $R_3 = H$, $R_2 = F$	**3b**	R_1 = 4-Me, $R_3 = H$, $R_2 = F$
1c	$R_1 = R_3 = H$, $R_2 = Cl$	**2c**	R_1 = 5-Br, $R_3 = H$, $R_2 = Cl$	**3c**	R_1 = 4-Me, $R_3 = H$, $R_2 = Cl$
1d	$R_1 = R_3 = H$, $R_2 = Br$	**2d**	R_1 = 5-Br, $R_3 = H$, $R_2 = Br$	**3d**	R_1 = 4-Me, $R_3 = H$, $R_2 = Br$
1e	$R_1 = R_3 = H$, $R_2 = I$	**2e**	R_1 = 5-Br, $R_3 = H$, $R_2 = I$	**3e**	R_1 = 4-Me, $R_3 = H$, $R_2 = I$
1f	$R_1 = H$, $R_2 = R_3 = Cl$	**2f**	R_1 = 5-Br, $R_2 = R_3 = Cl$	**3f**	R_1 = 4-Me, $R_2 = R_3 = Cl$
1g	$R_1 = H$, $R_2 = R_3 = Br$	**2g**	R_1 = 5-Br, $R_2 = R_3 = Br$	**3g**	R_1 = 4-Me, $R_2 = R_3 = Br$
1h	$R_1 = H$, $R_2 = R_3 = I$	**2h**	R_1 = 5-Br, $R_2 = R_3 = I$	**3h**	R_1 = 4-Me, $R_2 = R_3 = I$
1i	$R_1 = H$	**1i**	R_1 = 5-Br	**3i**	R_1 = 4-Me

Scheme 1. The general procedure of the synthesized compounds. 'I', 'II', 'III', 'IV' represent the atom number bonding to the amino acid active site.

range 0.20–3.13 μg/mL and 0.39–3.13 μg/mL against various bacteria. This is much better than or compare to the result reported previously.[21] Computational docking simulation study for the top 10 inhibitors docked into the active sites of ecKAS III also have been carried out.

2. Result and discussion

2.1. Chemistry

In this study, 27 PSB were subjected by reacting salicylamide, 5-bromo salicylamide, 4-methyl salicylamide primary amines with salicylaldehyde or its derivatives. The general method for preparing the compounds is outlined in Scheme 1. Salicylamide type's primary amines, which were not commercially available, were synthesized using modified procedures of Kido et al.[22] The crude products were purified using silica gel column chromatography, preparative TLC and recrystallization. All the compounds gave satisfactory chemical analyses (±0.4%). ^{1}H NMR, ESI-MS spectra and elemental analyses were consistent with the assigned structures.

Compound **1f** was successfully crystallized and its structure was determined by single-crystal X-ray diffraction analysis. The crystal data are presented in Table 1, and Figure 1 gives a perspective view of this compound together with the atomic labeling sys-

Table 1
Crystal and experimental data for complexes **1f**

Compound	**1f**
Empirical formula	$C_{17}H_{16}Br_2N_2O_3$
Mr	456.12
T (K)	298(2)
Radiation (Mo Kα), λ (Å)	0.71073
Crystal shape/color	Needle/yellow
Crystal size (mm^3)	0.31 × 0.20 × 0.12
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	14.860(3)
b (Å)	16.964(3)
c (Å)	7.066(1)
α (°)	90.00
β (°)	95.71(3)
γ (°)	90.00
V (Å^3)	1772.4(6)
Z	4
D_{calcd} (g/cm^{-3})	1.709
μ (mm^{-1})	4.591
F(0 0 0)	904
Goodness-of-fit on F^2	0.944
R_1, wR [$I \geq 2\sigma(I)$][a]	0.0608, 0.1213
R_1, wR (all data) [a]	0.0993, 0.1490

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$, $w_1 = [\sigma^2(F_o)^2 + (0.1009(F_o^2 + 2F_c^2)/3)^2]^{-1}$, $w_2 = [\sigma^2(F_o)^2 + (0.0962(F_o^2 + 2F_c^2)/3)^2]^{-1}$.

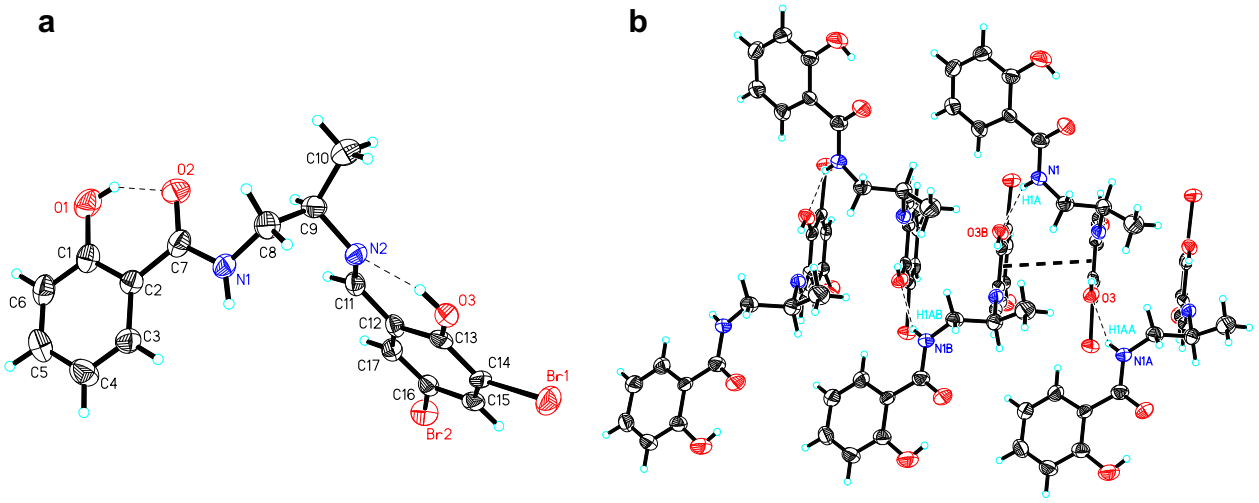

Figure 1. Molecular structure of compound **1f** with atoms and hydrogen bonds labeling (**1a**) and π···π interaction between the two benzyl rings (**1b**).

tem. In the structure of compound **1f**, there are intramolecular O–H···O (O1–H1B···O2, 2.506(3) Å, 146.6°), O–H···N hydrogen bond (O3–H3B···N2: 2.558(7) Å, 136.9°) and an intermolecular N–H···O hydrogen bond (N1–H1A···O3^{i}: 2.909(3) Å, 149.9° (**1a**), symmetry codes: i, *x*, −*y* + 1/2, *z* + 1/2) in the structure. The dihedral angle between the two benzene rings is 34.1°. The bond length of N1–C7 (1.344(8) Å) indicates a single bond and bond length of N2=C10 (1.284(8) Å) exhibits a double bond. The two benzyl rings have strong π···π interaction with distance of 3.566(6) Å (**1b**), making the structure stable in the crystal configuration.

Table 2
Inhibition constant (IC_{50}) against ecKAS III

Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)
1a	8.14	**2a**	>50	**3a**	7.88
1b	12.11	**2b**	22.12	**3b**	5.66
1c	5.46	**2c**	>50	**3c**	8.64
1d	6.03	**2d**	16.71	**3d**	6.33
1e	0.78	**2e**	9.32	**3e**	0.45
1f	0.31	**2f**	8.75	**3f**	1.75
1g	3.13	**2g**	>50	**3g**	5.37
1h	2.56	**2h**	>50	**3h**	14.95
1i	19.75	**2i**	10.94	**3i**	4.68

2.2. Inhibitory activities of PSB against ecKAS III

All the synthesized compounds (**1a–1i, 2a–2i** and **3a–3i**) were tested for inhibitory activity against ecKAS III. The inhibition constant (IC_{50}) of the compounds are presented in Table 2 and Figure 2 (IC_{50} more than 50 μg/mL not demonstrated in Fig. 2). It was observed that many compounds have been found to show fairly good inhibitory activity displaying IC_{50} values between 0.31 and 22.12 μg/mL. Inspection of the chemical structure of the

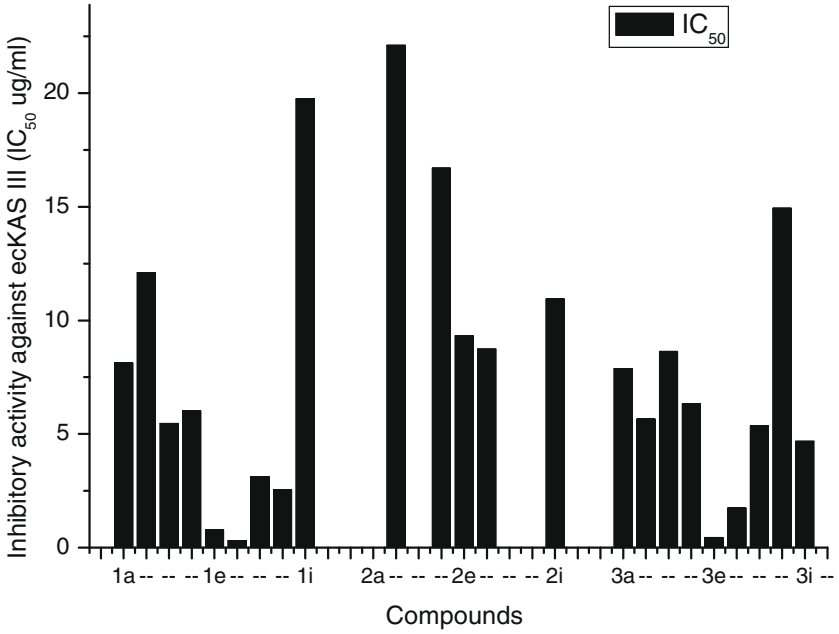

Figure 2. Inhibitory activities of the compounds. IC_{50} more than 50 μg/mL not demonstrated.

compounds (Scheme 1) suggested that it could be divided into two subunits: A- and B-rings.

The initial lead compound was reported by us previously. Here we used 1,2-propanediamine as connect aliphatic chain for the appropriate length of aliphatic chain ($n = 2$ or 3) showed better inhibitory activities.[21] Subsequently SAR studies were performed by modification of the parent compound to determine how the substituents of the subunits affected the inhibitory activities. Replacement of H atom (**1a** IC_{50} 8.14 μg/mL) at R_2-position by one halogen atom or R_2- and R_3-position of B-ring by two halogen atoms resulted in the improving of their inhibitory activity (**1c–1h**). Introduced the bromo substitution group on A-ring (**2a**), the inhibitory activity was remarkably decreasing (IC_{50} >50 μg/mL) compared to **1a**. When modified the substituents on B-ring with one or two halogen atoms (**2b–2i**), the inhibitory activity had a few improving, but the best compounds **2e** and **2f** still turned out to be less potent than **1a**. For more extensive evidences, we attempted further modification of the substituent on A-ring by a methyl group, and the results are summarized in Table 2 and Figure 2. As shown, **3a** had an increase in the IC_{50} value compared to **1a**. Halogen substituents at the B-ring also re-

Table 3
Antimicrobial activity and Docking parameters of the top 10 compounds

Top 10 compounds	Minimum inhibitory concentrations (μg/mL)				Docking parameters	
	Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	ΔG_b (kcal/mol)	Hydrogen bond
1f	0.39	0.20	1.56	3.13	−9.21	N^{III}–H···O/Gly209 O^{IV}–H···O/Gly152
3e	0.39	3.13	1.56	1.56	−9.77	N^{III}–H···O/Gly152 O^{IV}–H···O/Gly209
1e	6.25	3.13	0.78	12.5	−9.41	N^{III}–H···O/Gly152 O^{IV}–H···O/Gly209
3f	3.13	25.00	1.56	6.25	−8.17	O^{I}–H···N/Gly152 O^{IV}–H···O/Gly209
1h	>50.00	6.25	12.50	12.50	−7.05	O^{IV}–H···O/Gly152
1g	25.00	0.78	12.50	6.25	−7.13	O^{I}–H···O/Gly152 O^{II}···H–N/Arg36
3i	12.5	>50.00	6.25	>50.00	−8.39	O^{I}–H···O/Gly209 O^{II}···N–H/Asn247
3g	0.78	12.50	3.13	>50.00	−7.93	O^{IV}–H···O/Gly152
1c	12.5	3.13	>50.00	>50.00	−7.12	O^{I}–H···O/Gly209
3b	6.25	>50.00	3.13	12.50	−6.73	O^{I}–H···O/Asn247
Kanamycin	3.13	3.13	1.56	1.56		

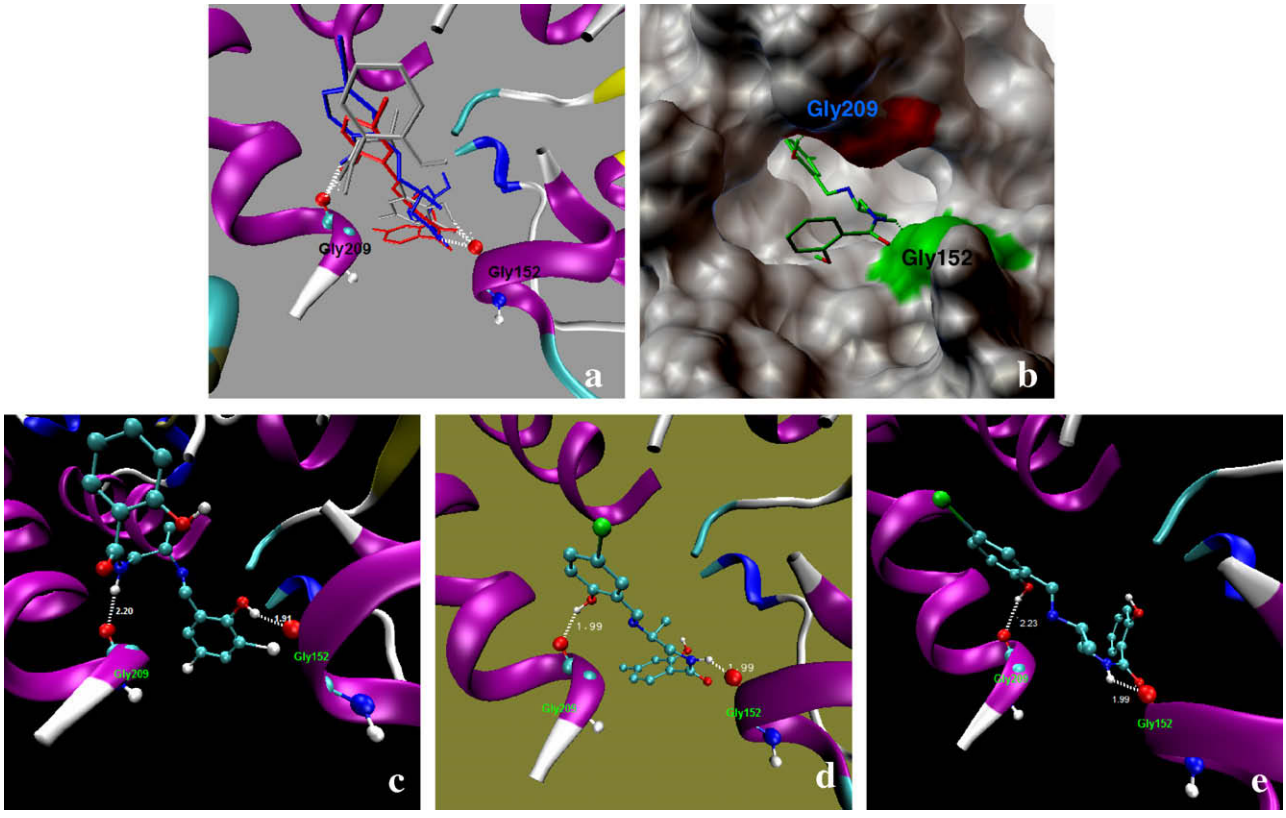

Figure 3. Compounds **1f**, **3e** and **1e** bound into ecKAS III receptor site via hydrophilic binding. (a) **1f** is in grey, **3e** is in red and **1e** is in blue. (b) eg. **1e** is bonding into the mouth of the substrate tunnel. (c) **1f** bonding into esKAS III via N^{III}–H···O/Gly209 (2.20 Å, 158.5°) and O^{IV}–H···O/Gly152 (1.91 Å, 163.9°). (d) **3e** bonding into esKAS III via N^{III}–H···O/Gly152 (1.99 Å, 136.9°) and O^{IV}–H···O/Gly209 (1.99 Å, 139.3°). (e) **1e** bonding into esKAS III via N^{III}–H···O/Gly152 (2.23 Å, 136.8°) and O^{IV}–H···O/Gly209 (1.99 Å, 151.3°).

sulted in increased IC_{50} values (**3b**–**3f**, **3i**). This suggested that compounds with electron-withdrawing groups on R_1 and R_2 and none of the electron-withdrawing groups on A-ring showed better inhibitory activities. The introduction of naphthyl substitute of phenyl group shows improvement in potency (**2i** IC_{50} 10.95 μg/mL, **3i** IC_{50} 4.68 μg/mL) compared to **2a** and **3a**. Among them, two chloro in B-ring at R_2- and R_3-positions (**1f**) displayed the most potent activity with IC_{50} 0.31 μg/mL, which had a little improvement compared to the best compound reported previously. So is **3e** (IC_{50} 0.45 μg/mL) with methyl group at A-ring and iodo group at R_2 position.

2.3. Molecular docking study

Here, we investigated the AutoDock-binding affinities of the synthesized PSB into ecKAS III. We defined the active site of ecKAS III, based on the center and radius of the binding substrate in an X-ray structure of ecKAS III complexed with CoA or inhibitor.[23] Towards optimization of the aforementioned compounds of the promising antimicrobial activities, the docking program AutoDock 4.0[24] was used to evaluate the binding free energies as potential inhibitors into the target ecKAS III macromolecule.

2.3.1. Validation of the accuracy and performance of AutoDock

According to the method of validation cited in literature,[25] where if the RMSD (root mean square deviation) of the best docked conformation is ≤2.0 Å from the experimental one, the used scoring function is successful. The obtained success rates of AutoDock (Morris et al.)[24] are highly excellent as illustrated in Table 3 for the native ligand, which was docked into its KAS III (PDB code: 1HNJ).

2.3.2. AutoDock-binding affinities of the synthesized and designed compounds into KAS III

The binding affinity was evaluated by the binding free energies (ΔG_b, kcal/mol) and hydrogen bonding. The compounds which revealed the highest binding affinities, that is, lowest binding free energies, within KAS III and the hydrogen bond interactions into the target macromolecule are represented in Table 3. These compounds include *N*-(2-(3,5-dichloro-2-hydroxybenzylideneamino)-propyl)-2-hydroxybenzamide (**1f**), 2-hydroxy-*N*-(2-(2-hydroxy-5-iodobenzylideneamino)propyl)-4-methylbenzamide (**3e**), 2-hydroxy-*N*-(2-(2-hydroxy-5-iodobenzylideneamino)propyl)benzamide (**1e**), and *N*-(2-(3,5-dichloro-2-hydroxybenzylideneamino)propyl)-2-hydroxy-4-methylbenzamide (**3f**). Many of these derivatives exhibited one or two hydrogen bonds between O^{I}, O^{II}, O^{IV} or N^{III} of PSB and different amino acids of the target ecKAS III including Gly152, Gly209, Arg36 and Asn247 as cited in Table 3. The molecular docking study revealed that the majority of the compounds docked into the ecKAS III (PDB code: 1HNJ) exhibited hydrogen bonds via N^{III}–H, O^{IV}–H···O/Gly152 or Gly209 as illustrated for compounds **1f**, **3e** and **1e** in Figure 3.

The global results are listed in Table 3, and we selected the top 10 compounds (**1f**, **3e**, **1e**, **3f**, **1h**, **1g**, **3i**, **3g**, **1c** and **3b**) which have better inhibitory activity to test their antibacterial activities against Gram-negative bacterial strains (*E. coli* ATCC 35218 and *P. aeruginosa* ATCC 13525) and two Gram-positive bacterial strains (*B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538), expecting to exploit potent antibacterial agent with broad-spectrum antibiotics activity. Out of the top 10 compounds, the results demonstrate that the antimicrobial compounds, *N*-(2-(3,5-dichloro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (**1f**) and 2-hydroxy-*N*-(2-(2-hydroxy-5-iodobenzylideneamino)propyl)-4-methylbenzamide (**3e**), can be as inhibitor of ecKAS III and a potential antibiotics agent, displaying MIC of 0.39, 0.20, 1.56, 3.13 μg/mL and 0.39, 3.13, 1.56, 1.56 μg/mL against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*, respectively, which was similar to the broad spectrum antibiotic kanamycin with corresponding MIC of 3.13, 3.13, 1.56 and 1.56 μg/mL. Compounds **1e** and **3f** showed similar moderate antibacterial activity with MIC of 0.78–25.00 μg/mL against all the tested bacterial strains.

From the binding model, we can see compounds **1f**, **3e** and **1e** (Fig. 3a) are bound into ecKAS III receptor site (Fig. 3b) via hydrophilic binding by hydrogen bond between N^{III}–H···O/Gly209 and O^{IV}–H···O/Gly152 (Fig. 3c), N^{III}–H···O/Gly152 and O^{IV}–H···O/Gly209 (Fig. 3d), N^{III}–H···O/Gly152 and O^{IV}–H···O/Gly209 (Fig. 3e). Gly152/Gly209 are very important residues in ecKAS III,[26,20] effecting to these amino acid resides may greatly influence, inhibit or even stop its catalytic activity. Compounds **1f**, **3e** and **1e** bond two Gly active sites (Gly152 and Gly209) of ecKAS III and fit into the mouth of the substrate tunnel. This fact made **1f** and **3e** have excellent inhibitory activity than other compounds and can be acted as an inhibitor of KAS III and as a potential antibiotics agent in theory. The antimicrobial activity (minimum inhibitory concentrations are the average values of triplicates experiment results) against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* proved this point in practice. The binding energy indicates that **1f** is somewhat smaller than **2d**,[26] but the antibacterial activity has a few improvements against the tested bacterial strains.

3. Conclusion

A series of novel PSB derivatives were prepared and tested for their inhibitory activity against ecKAS III, and the Autodock investigation also carried out by docking them into the active site of ecKAS III. Both the theory and practice demonstrate that both *N* -(2-(3,5-dichloro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (**1f**) and 2-hydroxy-*N*-(2-(2-hydroxy-5-iodobenzylideneamino)propyl)-4-methylbenzamide (**3e**), can be as an inhibitor of ecKAS III and as a potential antibiotics agent, displaying MIC values in the range 0.20–3.13 μg/mL and 0.39–3.13 μg/mL against various bacteria.

4. Experimental

4.1. Materials

All chemicals (reagent grade) used were commercially available. All the ^1H NMR and spectra were recorded on a Bruker DRX 500 or DPX 300 model Spectrometer at 25 °C with TMS and solvent signals allotted as internal stands. Chemical shifts were reported in parts per million (δ). ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within 0.4% of the theoretical values. Melting points were measured on a Boetius micro melting point apparatus.

4.2. General method of synthesis PSB

Ethyl salicylate: Concentrated sulfuric acid (1.0 mL) was added slowly with vigorous agitation to a mixture of 20.0 g (144 mmol) of salicylic acid and 63.0 mL (1.08 mol) of anhydrous ethanol. The mixture was then reflux for 15 h. Excess ethanol was removed by evaporation and the residue was washed with water. The organic layer was again treated with water and concentrated sodium bicarbonate solution to an alkaline reaction, and then again with water. The product was dried over anhydrous $MgSO_4$, collected by filtration, and distilled (yield: 55.0%).

Phenyl 5-bromo-2-hydroxybenzoate: 5-Bromo salicylic acid 4.32 g (20 mmol), phenol 3.72 g (40 mmol), dicyclohexylcarbodiimide 0.08 g and THF 10 mL were mixed and stirred at room

temperature for 18 h. The mixture was subsequently concentrated under reduced pressure giving the crude product as a yellow oil. The crude product was purified by means of flash chromatography on silica and 25% EtOAc/hexanes as eluent to obtain as a yellow oil (57%).

Phenyl 4-methyl-2-hydroxybenzoate: The same as phenyl 5-bromo-2-hydroxybenzoate preparation. Only use 4-methyl salicylic acid 3.04 g (20 mmol) instead of 5-bromo salicylic acid, obtained a colorless oil (60%).

Salicylamide types primary amines: Equimolar quantities (10 mmol) of 1,2-propanediamine, ethyl salicylate, or phenyl 5-bromo-2-hydroxybenzoate, or phenyl 4-methyl-2-hydroxybenzoate and isopropanol (20 mL) were mixed and vigorously stirred at 110 °C under oil bath for 5 h to give a melicera solution. The crude product was then purified by column chromatography in 10% MeOH/CH_2Cl_2 to afford the desired compound (yield: 62.0%).

Equimolar quantities (0.5 mmol) of salicylamide type's primary amines and salicylaldehyde or its derivatives, or 2-hydroxy-1-naphthaldehyde were dissolved in methanol (10 mL) and stirred at 50 °C for 3 h to give a clear solution. The crude product was then purified by column chromatography in 25% EtOAc/hexanes to afford the desired compound.

4.2.1. General data of 1a–1i

4.2.1.1. (*E*)-2-Hydroxy-*N*-(2-(2-hydroxybenzylideneamino)propyl)benzamide (1a). Yellow powder, yield 91%, mp: 39–41 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.35 (d, J = 6.6 Hz, 3H); 3.39 (m, 1H); 3.66 (m, 1H); 3.91 (m, 1H); 6.53 (s, 1H); 6.78 (m, 1H); 6.89 (m, 1H); 6.97 (m, 2H); 7.22 (m, 2H); 7.35 (m, 2H); 8.37 (s, 1H); 12.97 (s, 2H). ESI-MS: 299.1 ($C_{17}H_{19}N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.45; H, 6.04; N, 9.35.

4.2.1.2. (*E*)-*N*-(2-(5-Fluoro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (1b). Yellow powder, yield 87%, mp: 45–47 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.36 (d, J = 6.4 Hz, 3H); 3.39 (m, 1H); 3.71 (m, 1H); 3.84 (m, 1H); 6.48 (s, 1H); 6.80 (d, J = 6.8 Hz, 1H); 6.94 (m, 2H); 7.21 (m, 2H); 7.39 (m, 1H); 8.31 (s, 1H); 12.61 (s, 2H). ESI-MS: 317.1 ($C_{17}H_{18}FN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{17}FN_2O_3$: C, 64.55; H, 5.42; N, 8.86. Found: C, 64.45; H, 5.40; N, 8.75.

4.2.1.3. (*E*)-*N*-(2-(5-Chloro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (1c). Yellow powder, yield 87%, mp: 41–44 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.37 (d, J = 6.3 Hz, 3H); 3.38 (m, 1H); 3.69 (m, 1H); 3.82 (m, 1H); 6.47 (s, 1H); 6.80 (m, 1H); 6.91 (d, J = 8.8 Hz, 1H); 6.98 (d, J = 8.3 Hz, 1H); 7.22 (m, 2H); 7.38 (m, 1H); 8.30 (s, 1H); 12.16 (s, 1H); 13.05 (s, 1H). ESI-MS: 333.1 ($C_{17}H_{18}ClN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{17}ClN_2O_3$: C, 61.36; H, 5.15; N, 8.42. Found: C, 61.35; H, 5.11; N, 8.45.

4.2.1.4. (*E*)-*N*-(2-(5-Bromo-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (1d). Yellow powder, yield 85%, mp: 75–77 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.35 (d, J = 6.9 Hz, 3H); 3.40 (m, 1H); 3.69 (m, 1H); 3.84 (m, 1H); 6.53 (s, 1H); 6.79 (m, 1H); 6.90 (d, J = 5.3 Hz, 1H); 6.97 (d, J = 5.2 Hz, 1H); 7.20 (m, 1H); 7.27 (m, 2H); 7.38 (m, 1H); 8.30 (s, 1H); 12.11 (s, 1H); 13.15 (s, 1H). ESI-MS: 377.0 ($C_{17}H_{18}BrN_2{}_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{17}BrN_2O_3$: C, 54.13; H, 4.45; N, 7.42. Found: C, 54.35; H, 4.41; N, 7.45.

4.2.1.5. (*E*)-2-Hydroxy-*N*-(2-(2-hydroxy-5-iodobenzylideneamino)propyl)benzamide (1e). Yellow powder, yield 79%, mp: 97–98 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.34 (d, J = 6.8 Hz, 3H); 3.41 (m, 1H); 3.71 (m, 1H); 3.83 (m, 1H); 6.52 (s, 1H); 6.77 (m, 1H); 6.91 (d, J = 5.3 Hz, 1H); 6.98 (d, J = 5.2 Hz, 1H); 7.22 (m, 1H); 7.27 (m, 1H); 7.38 (m, 2H); 8.31 (s, 1H); 12.10 (s, 1H); 13.11 (s, 1H). ESI-MS: 425.0 ($C_{17}H_{18}IN_2O_3^+C_{17}H_{18}IN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{17}IN_2O_3$: C, 48.13; H, 4.05; N, 6.60. Found: C, 48.15; H, 4.01; N, 6.55.

4.2.1.6. (*E*)-*N*-(2-(3,5-Dichloro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (1f). Yellow crystal, yield 90%, mp: 125–126 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.37 (d, J = 6.4 Hz, 3H); 3.34 (m, 1H); 3.82 (m, 2H); 6.64 (s, 1H); 6.79 (m, 1H); 6.97 (d, J = 8.2 Hz, 1H); 7.12 (s, 1H); 7.29 (m, 1H); 7.40 (m, 2H); 8.27 (s, 1H); 12.06 (s, 1H); 14.22 (s, 1H). ESI-MS: 367.0 ($C_{17}H_{17}Cl_2N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}Cl_2N_2O_3$: C, 55.60; H, 4.39; N, 7.63. Found: C, 55.60; H, 4.37; N, 7.62.

4.2.1.7. (*E*)-*N*-(2-(3,5-Dibromo-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (1g). Yellow powder, yield 89%, mp: 163–165 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.37 (d, J = 6.3 Hz, 3H); 3.32 (m, 1H); 3.84 (m, 2H); 6.56 (s, 1H); 6.80 (m, 1H); 6.97 (d, J = 7.8 Hz, 1H); 7.31 (m, 2H); 7.38 (m, 1H); 7.70 (s, 1H); 8.24 (s, 1H); 12.05 (s, 1H); 14.38 (s, 1H). ESI-MS: 456.9 ($C_{17}H_{17}Br_2N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}Br_2N_2O_3$: C, 44.76; H, 3.54; N, 6.14. Found: C, 44.70; H, 5.56; N, 6.12.

4.2.1.8. (*E*)-2-Hydroxy-*N*-(2-(2-hydroxy-3,5-diiodobenzylideneamino)propyl)benzamide (1h). Yellow powder, yield 84%, mp: 155–156 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.36 (d, J = 6.3 Hz, 3H); 3.31 (m, 1H); 3.82 (m, 2H); 6.55 (s, 1H); 6.81 (m, 1H); 6.96 (d, J = 7.4 Hz, 1H); 7.33 (m, 2H); 7.38 (m, 1H); 7.71 (s, 1H); 8.25 (s, 1H); 12.04 (s, 1H); 14.41 (s, 1H). ESI-MS: 550.9 ($C_{17}H_{17}I_2N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}I_2N_2O_3$: C, 37.12; H, 2.93; N, 5.09. Found: C, 37.11; H, 2.92; N, 5.12.

4.2.1.9. (*E*)-2-Hydroxy-*N*-(2-((2-hydroxynaphthalen-1-yl)methyleneamino)propyl)benzamide (1i). Yellow powder, yield 81%, mp: 171–173 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.46 (d, J = 6.4 Hz, 3H); 3.36 (m, 1H); 3.93 (m, 2H); 6.71 (m, 1H); 6.81 (d, J = 9.3 Hz, 1H); 6.96 (d, J = 8.4 Hz, 1H); 7.23 (m, 1H); 7.39 (m, 4H); 7.54 (m, 2H); 7.78 (d, J = 8.4 Hz, 1H); 8.83 (s, 1H); 12.24 (s, 1H); 14.52 (s, 1H). ESI-MS: 349.1 ($C_{21}H_{22}N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04. Found: C, 72.32; H, 5.88; N, 5.69.

4.2.2. General data of 2a–2i

4.2.2.1. (*E*)-5-Bromo-2-hydroxy-*N*-(2-(2-hydroxybenzylideneamino)propyl)benzamide (2a). Yellow powder, yield 86%, mp: 59–61 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.38 (d, J = 6.4 Hz, 3H); 3.41 (m, 1H); 3.71 (m, 1H); 3.89 (m, 1H); 6.53 (s, 1H); 6.91 (m, 2H); 6.99 (d, J = 5.4 Hz, 1H); 7.25 (m, 1H); 7.35 (m, 1H); 7.42 (m, 1H); 7.46 (dd, J = 2.3 Hz, J = 8.9 Hz, 1H); 8.39 (s, 1H); 12.56 (s, 2H). ESI-MS: 377.0 ($C_{17}H_{18}BrN_2{}_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{17}BrN_2O_3$: C, 54.13; H, 4.54; N, 7.43. Found: C, 53.10; H, 5.56; N, 7.45.

4.2.2.2. (*E*)-5-Bromo-*N*-(2-(5-fluoro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (2b). Yellow powder, yield 91%, mp: 130–133 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.36 (d, J = 6.3 Hz, 3H); 3.42 (m, 1H); 3.71 (m, 1H); 3.85 (m, 1H); 6.45 (s, 1H); 6.89 (m, 2H); 7.20 (s, 1H); 7.27 (m, 1H); 7.37 (s, 1H); 7.45 (d, J = 8.9 Hz, 1H); 8.30 (s, 1H); 12.52 (s, 2H). ESI-MS: 395.0 ($C_{17}H_{17}BrFN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}BrFN_2O_3$: C, 51.66; H, 4.08; N, 7.09. Found: C, 51.55; H, 4.96; N, 7.15.

4.2.2.3. (*E*)-5-Bromo-*N*-(2-(5-chloro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (2c). Yellow powder, yield

89%, mp: 130–133 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.35 (d, J = 6.3 Hz, 3H); 3.43 (m, 1H); 3.72 (m, 1H); 3.85 (m, 1H); 6.46 (s, 1H); 6.88 (m, 2H); 7.21 (s, 1H); 7.28 (m, 1H); 7.37 (s, 1H); 7.46 (d, J = 8.7 Hz, 1H); 8.31 (s, 1H); 12.53 (s, 2H). ESI-MS: 413.0 ($C_{17}H_{17}BrClN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}BrClN_2O_3$: C, 49.60; H, 3.93; N, 6.80. Found: C, 49.56; H, 3.96; N, 6.78.

4.2.2.4. (*E*)-5-Bromo-*N*-(2-(5-bromo-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (2d). Yellow powder, yield 86%, mp: 120–121 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.35 (d, J = 6.4 Hz, 3H); 3.41 (m, 1H); 3.69 (m, 1H); 3.85 (m, 1H); 6.43 (s, 1H); 6.87 (m, 2H); 7.36 (m, 3H); 7.45 (m, 1H); 8.30 (s, 1H); 12.73 (s, 2H). ESI-MS: 456.9 ($C_{17}H_{17}Br_2N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}Br_2N_2O_3$: C, 44.76; H, 3.54; N, 6.14. Found: C, 44.66; H, 3.56; N, 6.08.

4.2.2.5. (*E*)-5-Bromo-2-hydroxy-*N*-(2-(2-hydroxy-5-iodobenzylideneamino)propyl)benzamide (2e). Yellow powder, yield 79%, mp: 90–92 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.34 (d, J = 6.3 Hz, 3H); 3.42 (m, 1H); 3.68 (m, 1H); 3.84 (m, 1H); 6.44 (s, 1H); 6.88 (m, 2H); 7.38 (m, 3H); 7.46 (m, 1H); 8.31 (s, 1H); 12.74 (s, 2H). ESI-MS: 502.9 ($C_{17}H_{17}BrIN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}BrIN_2O_3$: C, 40.58; H, 3.21; N, 5.57. Found: C, 40.61; H, 3.16; N, 5.53.

4.2.2.6. (*E*)-5-Bromo-*N*-(2-(3,5-dichloro-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (2f). Yellow powder, yield 88%, mp: 161–163 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.38 (d, J = 6.2 Hz, 3H); 3.41 (m, 1H); 3.81 (m, 2H); 6.53 (s, 1H); 6.88 (d, J = 8.8, 1H); 7.14 (d, J = 2.2, 1H); 7.43 (m, 3H); 8.28 (s, 1H); 11.95 (s, 1H); 14.13 (s, 1H). ESI-MS: 446.9 ($C_{17}H_{16}BrCl_2N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}BrCl_2N_2O_3$: C, 45.77; H, 3.39; N, 6.28. Found: C, 45.68; H, 3.37; N, 6.19.

4.2.2.7. (*E*)-5-Bromo-*N*-(2-(3,5-dibromo-2-hydroxybenzylideneamino)propyl)-2-hydroxybenzamide (2g). Yellow powder, yield 94%, mp: 188–190 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.37 (d, J = 6.3 Hz, 3H); 3.38 (m, 1H); 3.82 (m, 2H); 6.54 (s, 1H); 6.87 (d, J = 8.9, 1H); 7.32 (s, 1H); 7.44 (m, 2H); 7.70 (m, 1H); 8.25 (s, 1H); 11.97 (s, 1H); 14.32 (s, 1H). ESI-MS: 534.8 ($C_{17}H_{16}Br_3N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}Br_3N_2O_3$: C, 38.16; H, 2.83; N, 5.24. Found: C, 38.12; H, 2.79; N, 5.22.

4.2.2.8. (*E*)-5-Bromo-2-hydroxy-*N*-(2-(2-hydroxy-3,5-diiodobenzylideneamino)propyl)benzamide (2h). Yellow powder, yield 91%, mp: 171–173 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.36 (d, J = 6.2 Hz, 3H); 3.39 (m, 1H); 3.81 (m, 2H); 6.54 (s, 1H); 6.88 (d, J = 8.8, 1H); 7.33 (s, 1H); 7.45 (m, 2H); 7.71 (m, 1H); 8.26 (s, 1H); 11.93 (s, 1H); 14.33 (s, 1H). ESI-MS: 628.8 ($C_{17}H_{16}BrI_2N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}BrI_2N_2O_3$: C, 32.47; H, 2.40; N, 4.45. Found: C, 32.48; H, 2.39; N, 4.36.

4.2.2.9. (*E*)-5-Bromo-2-hydroxy-*N*-(2-((2-hydroxynaphthalen-1-yl)methyleneamino)propyl)benzamide (2i). Yellow powder, yield 95%, mp: 88–90 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.46 (d, J = 6.4 Hz, 3H); 3.32 (m, 1H); 3.93 (m, 2H); 6.67 (d, J = 9.1, 1H); 6.81 (d, J = 9.0, 1H); 7.19 (m, 1H); 7.41 (m, 4H); 7.72 (d, J = 8.2, 1H); 7.78 (s, 1H); 8.48 (s, 1H); 8.71 (s, 1H); 14.51 (s, 2H). ESI-MS: 429.0 ($C_{21}H_{20}BrN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{21}H_{19}BrN_2O_3$: C, 59.03; H, 4.48; N, 6.56. Found: C, 58.98; H, 4.49; N, 6.61.

4.2.3. General data of 3a–3i

4.2.3.1. (*E*)-2-Hydroxy-*N*-(2-(2-hydroxybenzylideneamino)propyl)-4-methylbenzamide (3a). Yellow powder, yield 81%, mp: 38–41 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.35 (d, J = 6.5 Hz, 3H); 2.29 (s, 3H); 3.35 (m, 1H); 3.68 (m, 1H); 3.87 (m, 1H); 6.51 (s, 1H); 6.59 (d, J = 8.1 Hz, 1H); 6.77 (s, 1H); 6.87 (m, 1H); 6.96 (d, J = 8.4 Hz, 1H); 7.13 (d, J = 8.4 Hz, 1H); 7.21 (m, 1H); 7.32 (m, 1H); 8.36 (s, 1H); 12.51 (s, 2H). ESI-MS: 313.4 ($C_{18}H_{21}N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{20}N_2O_3$: C, 69.21; H, 6.45; N, 8.97. Found: C, 69.10; H, 6.46; N, 8.95.

4.2.3.2. (*E*)-*N*-(2-(5-Fluoro-2-hydroxybenzylideneamino)propyl)-2-hydroxy-4-methylbenzamide (3b). Yellow powder, yield 81%, mp: 61–62 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.35 (d, J = 6.6 Hz, 3H); 2.29 (s, 3H); 3.36 (m, 1H); 3.70 (m, 1H); 3.83 (m, 1H); 6.59 (m, 2H); 6.77 (s, 1H); 6.90 (d, J = 8.5 Hz, 1H); 7.14 (d, J = 8.1 Hz, 1H); 7.19 (m, 1H); 7.25 (m, 1H); 8.28 (s, 1H); 12.19 (s, 2H). ESI-MS: 331.3 ($C_{18}H_{20}FN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{19}FN_2O_3$: C, 65.44; H, 5.80; N, 8.48. Found: C, 65.26; H, 5.75; N, 8.45.

4.2.3.3. (*E*)-*N*-(2-(5-Chloro-2-hydroxybenzylideneamino)propyl)-2-hydroxy-4-methylbenzamide (3c). Yellow powder, yield 81%, mp: 54–56 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.36 (d, J = 6.5 Hz, 3H); 2.29 (s, 3H); 3.35 (m, 1H); 3.69 (m, 1H); 3.82 (m, 1H); 6.56 (s, 1H); 6.60 (d, J = 8.2 Hz, 1H); 6.78 (s, 1H); 6.89 (d, J = 8.5 Hz, 1H); 7.14 (d, J = 8.2 Hz, 1H); 7.18 (m, 1H); 7.24 (m, 1H); 8.29 (s, 1H); 12.13 (s, 1H); 13.10 (s, 1H). ESI-MS: 349.1 ($C_{18}H_{20}ClN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{19}ClN_2O_3$: C, 62.34; H, 5.52; N, 8.08. Found: C, 62.31; H, 5.55; N, 8.07.

4.2.3.4. (*E*)-*N*-(2-(5-Bromo-2-hydroxybenzylideneamino)propyl)-2-hydroxy-4-methylbenzamide (3d). Yellow powder, yield 89%, mp: 69–70 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.35 (d, J = 6.4 Hz, 3H); 2.30 (s, 3H); 3.37 (m, 1H); 3.68 (m, 1H); 3.83 (m, 1H); 6.48 (s, 1H); 6.60 (d, J = 8.5 Hz, 1H); 6.78 (s, 1H); 6.90 (d, J = 8.9 Hz, 1H); 7.13 (d, J = 7.9 Hz, 1H); 7.19 (m, 1H); 7.27 (m, 1H); 8.29 (s, 1H); 12.13 (s, 1H); 13.12 (s, 1H). ESI-MS: 391.1 ($C_{18}H_{20}BrN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{19}BrN_2O_3$: C, 55.26; H, 4.89; N, 7.16. Found: C, 55.31; H, 4.84; N, 7.13.

4.2.3.5. (*E*)-2-Hydroxy-*N*-(2-(2-hydroxy-5-iodobenzylideneamino)propyl)-4-methylbenzamide (3e). Yellow powder, yield 81%, mp: 121–123 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.34 (d, J = 6.3 Hz, 3H); 2.30 (s, 3H); 3.35 (m, 1H); 3.69 (m, 1H); 3.84 (m, 1H); 6.48 (s, 1H); 6.61 (d, J = 8.3 Hz, 1H); 6.78 (s, 1H); 6.91 (d, J = 8.8 Hz, 1H); 7.14 (d, J = 7.8 Hz, 1H); 7.20 (m, 1H); 7.28 (m, 1H); 8.29 (s, 1H); 12.14 (s, 1H); 13.22 (s, 1H). ESI-MS: 439.1 ($C_{18}H_{20}IN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{19}IN_2O_3$: C, 49.33; H, 4.37; N, 6.39. Found: C, 49.31; H, 4.33; N, 6.42.

4.2.3.6. (*E*)-*N*-(2-(3,5-Dichloro-2-hydroxybenzylideneamino)propyl)-2-hydroxy-4-methylbenzamide (3f). Yellow powder, yield 85%, mp: 118–120 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.51 (d, J = 6.1 Hz, 3H); 2.21 (s, 3H); 4.61 (m, 3H); 6.17 (s, 1H); 7.68 (m, 3H); 7.80 (m, 1H); 7.88 (m, 1H); 8.34 (s, 1H); 12.25 (m, 2H). ESI-MS: 381.1 ($C_{18}H_{19}Cl_2N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}Cl_2N_2O_3$: C, 56.71; H, 4.76; N, 7.35. Found: C, 56.62; H, 4.75; N, 7.40.

4.2.3.7. (*E*)-*N*-(2-(3,5-Dibromo-2-hydroxybenzylideneamino)propyl)-2-hydroxy-4-methylbenzamide (3g). Yellow powder, yield 85%, mp: 171–172 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.52 (d, J = 6.2 Hz, 3H); 2.22 (s, 3H); 4.61 (m, 3H); 6.18 (s, 1H); 7.66 (m, 2H); 7.81 (s, 1H); 7.88 (m, 1H); 7.98 (s, 1H); 8.34 (s, 1H); 12.25 (m, 2H). ESI-MS: 470.9 ($C_{18}H_{19}Br_2N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}Br_2N_2O_3$: C, 45.98; H, 3.86; N, 5.96. Found: C, 46.01; H, 3.73; N, 5.92.

4.2.3.8. (*E*)-2-Hydroxy-*N*-(2-(2-hydroxy-3,5-diiodobenzylideneamino)propyl)-4-methylbenzamide (3h). Yellow powder, yield 93%, mp: 157–158 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.50 (d, J = 6.1 Hz, 3H); 2.23 (s, 3H); 4.58 (m, 3H); 6.17 (s, 1H); 7.68 (m, 2H); 7.80 (s, 1H); 7.86 (m, 1H); 7.99 (s, 1H); 8.34 (s, 1H); 12.25 (m, 2H). ESI-MS: 564.9 ($C_{18}H_{19}I_2N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}I_2N_2O_3$: C, 38.32; H, 3.22; N, 4.97. Found: C, 38.23; H, 3.23; N, 4.92.

4.2.3.9. (*E*)-2-Hydroxy-*N*-(2-((2-hydroxynaphthalen-1-yl)methyleneamino)propyl)-4-methylbenzamide (3i). Yellow powder, yield 95%, mp: 66–67 °C, ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.42 (d, J = 6.3 Hz, 3H); 2.23 (s, 3H); 3.31 (m, 1H); 3.92 (m, 2H); 6.49 (d, J = 8.0 Hz, 1H); 6.74 (m, 2H); 7.16 (m, 1H); 7.35 (m, 2H); 7.49 (m, 2H); 7.71 (d, J = 8.2 Hz, 1H); 7.90 (s, 1H); 8.72 (s, 1H); 12.36 (m, 2H). ESI-MS: 363.1 ($C_{22}H_{23}N_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73. Found: C, 72.86; H, 6.13; N, 7.66.

4.3. *E. coli* KAS III purification and activity assay

Native *E. coli* KAS III protein was expressed in *E. coli* strain BL21 (DE3) using the pET30 vector. Harvested cells containing KAS III were lysed by sonication in 20 mM Tris, pH 7.6, 5 mM imidazole, 0.5 M NaCl and centrifuged at 20,000 rpm for 30 min. The supernatant was applied to a Ni–NTA agarose column, washed, and eluted using a 5–500 mM imidazole gradient over 20 column volumes. Eluted protein was dialyzed against 20 mM Tris, pH 7.6, 1 mM DTT, and 100 mM NaCl. Purified ecKAS III were concentrated up to 2 mg/mL and stored at −80 °C in 20 mM Tris, pH 7.6, 100 mM NaCl, 1 mM DTT, and 20% glycerol for enzymatic assay.

In a final 20 μL reaction, 20 mM Na_2HPO_4/NaH_2PO_4, pH 7.0, 0.5 mM DTT, 0.25 mM $MgCl_2$, and 2.5 μM holo-ACP were mixed with 1 nM ecKAS III, and H_2O was added to 15 μL. After 1 min incubation, a 2 μL mixture of 25 μM acetyl-CoA, 0.5 mM NADH, and 0.5 mM NADPH was added for ecKAS III reaction for 25 min. The reaction was stopped by adding 20 μL of ice-cold 50% TCA, incubating for 5 min on ice, and centrifuging to pellet the protein. The pellet was washed with 10% ice-cold TCA and resuspended with 5 μL of 0.5 M NaOH. The incorporation of the ^{3}H signal in the final product was read by liquid scintillation. When determining the inhibition constant (IC_{50}), inhibitors were added from a concentrated DMSO stock such that the final concentration of DMSO did not exceed 2%.

4.4. Antimicrobial activity

The antibacterial activity of the synthesized compounds was tested against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* using MH medium (Mueller–Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs of the test compounds were determined by a colorimetric method using the dye MTT.[27] A stock solution of the synthesized compound (50 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid medium (MH medium for antibacterial activity and RPMI-1640 medium for antifungal activity). A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h for bacterial. After the MICs were visually determined on each of the microtitration plates, 50 L of PBS (Phosphate Buffered Saline 0.01 mol/L, pH 7.4: $Na_2HPO_4{\cdot}12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm. The observed MICs were presented in Table 3.

4.5. Experimental protocol of docking study

The automated docking studies were carried out using AutoDock version 4.0. First, AutoGrid component of the program precalculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. The cubic grid box of 60 Å size (x, y, z) with a spacing of 0.375 Å and grid maps were created representing the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitors within the macromolecules. The GALS search algorithm (genetic algorithm with local search) was chosen to search for the best conformers. The parameters were set using the software ADT (Auto-Dock Tool Kit) on PC which is associated with Auto-Dock 4.0. For all docking parameters, default values were used with 20 independent docking runs for each docking case. The AutoDock performs the task of the docking, where the ligand moves randomly in any one of six degrees of freedom, and the energy of the new ligand 'state' is calculated. If the energy of the new state is lower than that of the old state, the new one is automatically accepted as the next step in docking.

4.5.1. Preparation of ligands and target ecKAS III

The compounds were studied for their binding activities into protein ecKAS III. The three-dimensional structures of the aforementioned compounds were constructed using Chem 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2009)], then they were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger–Hückel charges of ligands were assigned. The crystal structures of protein ecKAS III (PDB code: 1hnj) complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All bound waters and ligands were eliminated from the protein and the polar hydrogens and the Kollman-united charges were added to the proteins. The amino acids of the ligand-target-binding site were defined using data in pdbsum (http://www.ebi.ac.uk/pdbsum/).

4.5.2. Molecular modeling and analysis of the docked results

The predicted binding free energy that includes the intermolecular energy and torsional free energy was used as the criterion for ranking. Furthermore, the intermolecular hydrogen bonds, whose effect has already been counted in the binding energy, were also investigated in order to find useful information for drug design. Cluster analysis was performed on the docked results using a root mean square (RMS) tolerance of 0.5 Å. Each of the clusters that exhibited significant negative interaction energies was examined by Accelrys, DS visualizer 2.0 [Accelrys Inc., San Diego CA (2007)] to determine their binding orientations, molecular modeling, and evaluation of the hydrogen bonds.

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Supplementary data

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