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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Fusaric acid and derivatives as novel antimicrobial agents

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Abstract

The synthesis and screening of several fusaric acid (FA) and analogues against five common clinical pathogens (gram-positive and gram-negative) and in vitro hemolytic activity assay in human red blood cells, for the first time, were reported. The biological results reveal that FA and its analogues exhibited moderate antimicrobial activities. Compounds **2**–**5** and **7** showed growth inhibitory activity in gram-positive bacteria. Compounds **6** and **9** showed growth inhibitory activity in both gram-positive and gram-negative bacteria. None of the compounds induce hemolysis, which is potent for future drug development on this template. In addition, the structure–activity relationship and docking studies are discussed.

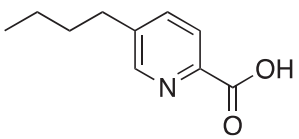
Keywords Fusaric acid · Antimicrobial · Docking

Introduction

Infectious diseases affecting to an estimated a quarter of the global population, and along with an increase in antimicrobial drug resistance, are the top leading causes of death annually (Brown et al. 2012). Finding new antibacterial and antifungal compounds is hence urgently needed for the development of effective drugs to treat these diseases (Rafique et al. 2017; Ashraf et al. 2017).

Fusaric acid (FA) is a well-known mycotoxin produced by *Fusarium* species (Yabuta et al. 1934). This compound can be isolated in a decent amount from 20 to 1080 µg per gram of *Fusarium* strain (Bacon et al. 1996). As one of the main chemical components of *Fusarium* sp., many research groups have focused on revealing the biological role of FA in the internal activities as well as external effects toward other microorganism (López-Díaz et al. 2018). In fact, recent studies have shown that FA exhibit moderate toxicity toward bacteria, plants, and animals (D'Alton and Etherton 1984; Fakhouri et al. 2003; Wang et al. 2013; Li et al. 2013; Asano and Hidaka 1977). For example, FA is known as a key role in wilt disease in plants (Bacon et al. 1996), inhibiting plant proton pump (Fakhouri et al. 2003), damaging roots, and leaves of banana (Li et al. 2013). In mammals, the relaxation effect of FA in an isolated aorta of the rabbit was observed (Asano and Hidaka 1977). Yin et al. (2015) reported that FA/copper chelate can cause malformation in zebrafish. Metal complexes of FA also found to have antimycobacterial activity in a report by Pan et al. (2011). Besides several reports, the mechanism of action of FA is not fully understood (Fig. 1).

Recently, we have discovered that FA and analogues are potent quorum sensing (QS) inhibitors of gram-negative bacteria (Tung et al. 2017). Compounds showed moderate QS inhibitory activity from 6.20 to 100.00 µg/mL concentration ranges without interrupting the growth of the bacteria. Interestingly, some compounds, especially acid derivatives (including FA) (Fig. 2) exhibited the growth inhibitory effect of *Escherichia coli* under our reported condition (*luxI-gfp*, *E. coli*) (Tung et al. 2017).



Fusaric acid

Fig. 1 Structure of fusaric acid

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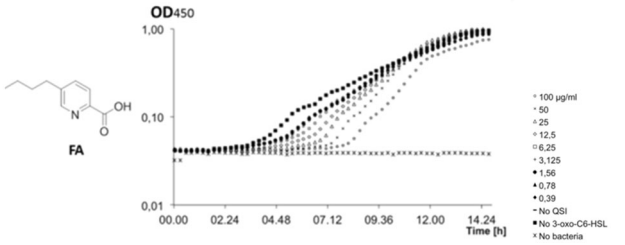


Fig. 2 Growth inhibitory activity of fusaric acid (FA) in *E. coli* as previously reported (Tung et al. 2017)

Intriguing from this finding, we report here the synthesis and screening of some potent FA analogues toward five clinical pathogens (for both gram-negative and gram-positive) in an effort of fighting antibiotic resistance.

Materials and methods

Chemistry

General information

Reagents were purchased from a commercially available vendor (Aldrich) and were used without further purification. NMR-spectra were recorded using a 400/600 MHz Bruker Avance (cryogenically cooled 5 mm dual-probe optimized for ^{13}C and ^{1}H is equipped). NMR solvents: DMSO-d_6, $CDCl_3$, MeOH-d_4, samples ran at 300 K. ^{1H}-NMR, COSY, HMBC, HSQC are recorded at 400 or 600 MHz. ^{13}CNMR were recorded at 151 or 101 MHz. Chemical shifts (δ) are reported in ppm relative to the residual solvent peak (^{1}HNMR) or the solvent peak (^{13}CNMR) as the internal standard. J values are reported in Hertz. Solvents for reaction were of analytical grade (water content <25 ppm). TLC was performed using silica gel 60 F_{254} plates (pre-coated) and visualized under UV light. Column chromatography was performed using silica 60 (Merck). Melting points were recorded on a MP70 Mettler Toledo. All tested compounds possess at least 95% purity. Purities were checked on a Waters 2795 system equipped with a Waters 996 PDA detector and a Waters Symmetry C18 Column (2.1 × 50 mm, 3.51 m), flow rate 0.2 ml/min. HRMS data were recorded on an electrospray (ESI) mass spectrometer.

Biology

Bacterial and fungal strains

Standard laboratory test strains: *Staphylococcus aureus* ATCC29213, *Enterococcus faecalis* ATCC29212, *E. coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853 (purchased from ATCC, Manassas, VA, USA), and *Candida albicans* were chosen for antimicrobial activity experiments.

In vitro antibacterial and antifungal assay

Minimum inhibitory concentrations (MIC) were determined as previously described (Broth microdilution) (Mygind et al. 2005). The MIC value was measured as the lowest concentration where no visible growth was observed as compared to the control (no compound added).

In vitro hemolytic activity

The lysis of human red blood cells (hRBCs) was measured as previously described (Jahnsen et al. 2014; Schmitt et al. 2007). The freshly drawn hRBCs were, first, washed with PBS buffer (twice, Sigma Aldrich, DK: 0.01 M Phosphate, 2.7 mM KCl, 0.137 M NaCl, pH 7.4) then centrifuged for 8 min (×2) at 3000 rpm (116 g) and 4000 rpm (206 g). FA and analogues were diluted in PBS buffer. Samples were then added to sterile polypropylene V-bottom 96-well plate (Whatman, UK), to give a total volume of 75 µL. 75 µL of 0.5% v/v hRBC suspension in PBS buffer was added to reach a final volume of 150 µL in each well. The plate was incubated at 37 °C for 1 h. Then, the cells were pelleted by centrifugation at 4000 rpm for 10 min. The 75 µL of supernatants was transferred to clear, flat-bottomed plastic 96-well plates. The concentration of hemoglobin was determined by recording the OD at 414 nm (VERSAmax microplate reader, Molecular Devices, USA). Hundred percent hemolysis was defined as the OD of cells incubated with melittin (400 µg/mL), and 0% hemolysis was defined as the OD of cells incubated with PBS buffer.

Results and discussion

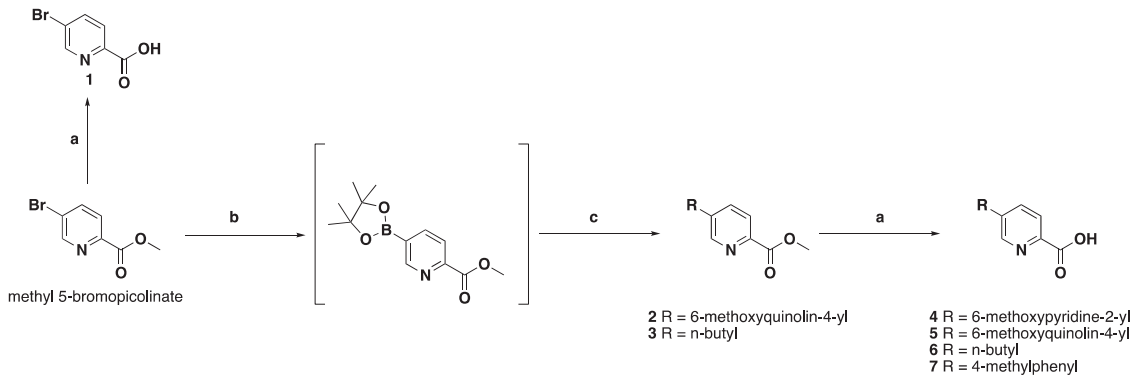
Synthesis and characterization

General procedure for the synthesis of library compounds

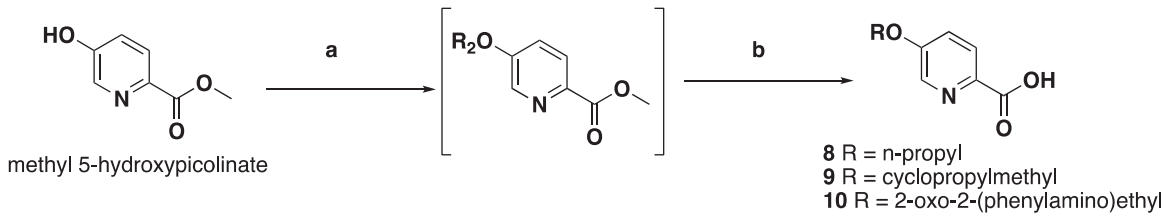
Synthesis of fusaric and analogues was prepared as previously described (depicted in Schemes 1, 2) (Tung et al. 2017). In brief, compounds **1**–**7** were prepared from methyl 5-bromopicolinate as starting material via Suzuki coupling reaction and saponification (Scheme 1). Compounds **8**–**10** were prepared from the alkylation of methyl 5-hydroxypicolinate followed by saponification (Scheme 2).

Characterization of 1–7

5-Bromopicolinic acid (1) Yellow solid; MP 176.9–177 °C; >98% purity; ^{1}HNMR (600 MHz, DMSO-d_6) δ 13.41 (br, 1H), 8.84 (d, $J = 2.3$ Hz, 1H), 8.25 (d, $J = 2.4$ Hz, 1H), 7.98 (d, $J = 8.3$ Hz); ^{13}CNMR (151 MHz, DMSO-d_6) δ 166.0,



Scheme 1 Synthetic route for the preparation **1**–**7**. **a** NaOH(aq) (5 equiv.), rt, 12 h, then HCl (1 M); **b** bis(pinacolato)diboron, potassium acetate, 10 mol % $Pd(dppf)Cl_2$ complex with chloroform, MW, 30 min; **c** R-Br, $Pd(dppf)Cl_2$ complex with chloroform 10%, Cs_2CO_3, 1,4-Dioxane/water (1/0.5), MW, 90 min



Scheme 2 Synthetic route for the preparation **8**–**10**. **a** R-Br, DMF, rt, 18 h; then **b** NaOH(aq) (5 equiv.), rt, 12 h, then HCl (1 M)

150.8, 147.6, 140.5, 126.8, 124.6; HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_6H_4BrNO_2Na^+$ 223.9323, found 223.9323.

Methyl 5-(6-methoxyquinolin-4-yl)picolinate (2) White solid; MP 121.8–122.6 °C; >98% purity; ^{1}HNMR (600 MHz, CDCl3) δ 8.94 (d, $J = 2.1$ Hz, 1H), 8.86 (d, $J = 4.3$ Hz, 1H), 8.34 (d, $J = 7.9$ Hz, 1H), 8.11 (d, $J = 9.2$ Hz, 1H), 8.03 (dd, $J = 8.0$, 2.2 Hz, 1H), 7.43 (dd, $J = 9.1$, 2.7 Hz, 1H), 7.31 (d, $J = 4.3$ Hz, 1H), 7.01 (d, $J = 2.7$ Hz, 1H), 4.09 (s, 3H), 3.79 (s, 3H); 13 C NMR (151 MHz, CDCl3) δ 165.4, 158.6, 149.9, 147.7, 147.4, 144.9, 142.0, 137.8, 137.6, 131.8, 127.1, 125.1, 122.5, 121.9, 102.4, 55.6, 53.2; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{17}H_{15}N_2O_3^+$ 295.1083, found 295.1083.

Ethyl 5-butylpicolinate (3) Yellow oil; >98% purity; ^{1}HNMR (600 MHz, $CDCl_3$) δ 8.57 (d, $J = 2.2$ Hz, 1H), 8.05 (d, $J = 7.8$ Hz, 1H), 7.63 (dd, $J = 2.2$, 7.8 Hz, 1H), 4.47 (q, $J = 7.1$ Hz, 2H), 2.69 (t, $J = 7.6$ Hz, 2H), 1.62 (m, 2H), 1.44 (t, $J = 7.1$, 3H), 1.37 (qt, $J = 7.3$, 7.6 Hz, 2H), 0.94 (t, $J = 7.3$ Hz, 3H); ^{13}CNMR (151 MHz, $CDCl_3$) d 165.4, 150.1, 145.9, 141.9, 136.5, 124.8, 61.7, 32.9, 32.7, 22.2, 14.4, 13.8; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{12}H_{18}NO_2^+$ 208.1338, found 208.1340.

6-Methoxy-[2,3′-bipyridine]-6′-carboxylic acid (4) Off-white solid; MP 210–215 °C; >98% purity; ^{1}HNMR (600 MHz, DMSO-d_6) δ 13.23 (s, 1H, OH), 9.39 (d, $J = 1.6$, 1H), 8.61 (dd, $J = 8.2$, 2.2 Hz, 1H), 8.15 (dd, $J = 8.2$, 0.6 Hz, 1H), 7.88 (t, $J = 7.4$, 1H), 7.76 (d, $J = 7.3$ Hz, 1H), 6.91 (d, $J = 8.2$ Hz, 1H), 3.99 (s, 3H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 166.4, 164.0, 150.8, 148.8, 148.0, 148.8, 136.7, 135.3, 125.1, 114.8, 111.5, 53.6; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{12}H_{11}N_2O_3^+$ 231.0770, found 231.0768.

5-(6-Methoxyquinolin-4-yl)picolinic acid (5) White solid; MP 246–247 °C; >98% purity; ^{1}HNMR (600 MHz, DMSO-d_6) δ 13.36 (s, 1H), 8.93 (q, $J = 1.1$, 0.7 Hz, 1H), 8.86 (d, $J = 4.4$ Hz, 1H), 8.25 (m, 2H, H3), 8.08 (d, $J = 9.2$ Hz, 1H), 7.54 (d, $J = 4.4$ Hz, 1H), 7.51 (dd, $J = 9.2$, 2.8 Hz, 1H), 7.10 (d, $J = 2.7$ Hz, 1H), 3.85 (s, 3H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 166.3, 159.6, 149.8, 149.4, 147.9, 144.1, 138.9, 138.5, 135.6, 128.1, 126.7, 125.5, 125.2, 123.4, 104.1, 56.3; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{16}H_{13}N_2O_3^+$ 281.0926, found 281.0927.

Fusaric acid (6) Slightly yellow solid; MP 97.0–98.0 °C; >99% purity; ^{1}HNMR (600 MHz, DMSO-d_6): δ 8.46 (d, $J = 2.2$ Hz, 1H), 8.16 (d, $J = 8.1$ Hz,1H), 7.76 (dd, $J = 8.1$, 2.2 Hz,1H), 2.74 (t, $J = 7.7$ Hz, 2H), 1.66 (m, 2H), 1.4 (tq, $J = 7.7$, 7.3 Hz, 2H), 0.96 (t, $J = 7.3$ Hz, 3H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 164.2, 148.2, 143.8, 143.5, 138.1, 123.4, 32.9, 32.8, 22.2, 13.8; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{10}H_{14}NO_2^+$ 180.1025, found 180.1025.

5-(p-Tolyl)picolinic acid (7) Off-white solid; MP 192.7–195.0 °C; >97% purity; ^{1}HNMR (600 MHz,

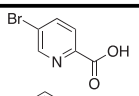
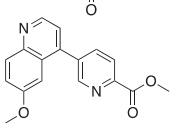
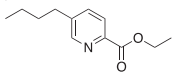
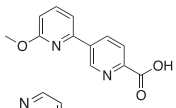
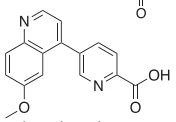
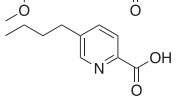
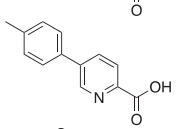
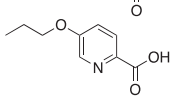
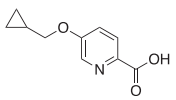
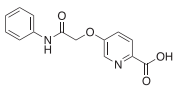
DMSO-d_6) δ 13.15 (b. s, 1H), 9.0 (d, $J = 1.6$ Hz, 1H), 8.23 (dd, $J = 8.1$, 2.1 Hz, 1H), 8.09 (dd, $J = 8.1$, 0.7 Hz, 1H), 7.72 (m, 2H), 7.35 (d, $J = 8.1$ Hz, 2H), 2.38 (s, 3H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 165.9, 147.2, 146.8, 138.5, 138.2, 134.7, 133.0, 129.8(2 C), 127.0(2 C), 124.8, 20.7; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{13}H_{12}NO_2^+$ 214.0868, found 214.0872.

Characterization of 8–10

5-Propoxypicolinic acid (8) Off-white solid; MP 129.0–129.2 °C; >98% purity; ^{1}HNMR (600 MHz, DMSO-d_6) δ 12.82 (s, 1H), 8.35 (s, 1H), 8.01 (dd, $J = 8.6$, 0.8 Hz, 1H), 7.48 (dt, $J = 8.7$, 2.6 Hz, 1H), 4.03 (td, $J = 6.5$, 1.7 Hz, 2H), 1.73–1.78 (m, 2H), 0.98 (td, $J = 7.4$, 1.7, 3H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 166.2, 157.7, 140.7, 138.3, 126.7, 121.1, 70.2, 22.3, 10.7; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_9H_{12}NO_3^+$ 182.0817, found 182.0817.

5-(Cyclopropylmethoxy)picolinic acid (9) Off-white solid; MP 138–139.5 °C; >98% purity; ^{1}HNMR (600 MHz, DMSO-d_6) δ 12.81 (s, 1H), 8.36 (d, $J = 2.7$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 1H), 7.48 (dd, $J = 8.8$, 2.9 Hz, 1H), 3.99 (d, $J = 7.1$ Hz, 2H), 1.23–1.29 (m, 1H), 0.59–0.62 (m, 2H), 0.35–0.38 (m, 2H); ^{13}CNMR δ (151 MHz, DMSO-d_6) 166.2, 157.7, 140.7, 138.4, 126.6, 121.2, 73.4, 10.3, 3.6 (2 C); HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{10}H_{12}NO_3^+$ 194.0817, found 194.0816.

Table 1 Minimal inhibitory concentration (in μg/mL) against several pathogens

Cpds	Structure	MIC (μg/mL)[a]				
		S. aureus ATCC29213	*E. faecalis* ATCC29212	*E. coli* ATCC25922	*P. aeruginosa* ATCC27853	*C. albicans*
1		>150	>150	>150	>150	>150
2		>150	121	>150	>150	>150
3		>150	112	>150	>150	>150
4		>150	100	>150	>150	>150
5		68	64	>150	>150	>150
6		>150	98	128	>150	>150
7		>150	100	>150	>150	>150
8		>150	>150	125	>150	>150
9		>150	107	116	>150	>150
10		>150	>150	>150	>150	>150
Ampicillin[b]		8	2.5	5.5	–[c]	–
Fluconazole[b]		–	–	–	–	0.5

[a]Experiments were performed in triplicate

[b]Positive control

[c]Not determined

5-(2-Oxo-2-(phenylamino)ethoxy)picolinic acid (10) Light yellow solid; MP 225–226 °C; >98% purity; ^{1}HNMR (600 MHz, DMSO-d_6) δ 12.9 (s, 1H), 10.18 (s, 1H), 8.45 (d, J = 2.9 Hz, 1H), 8.04 (d, J = 8.7 Hz, 1H), 7.62 (m, 2H), 7.53 (dd, J = 8.7, 2.9 Hz, 1H), 7.31–7.35 (m, 2H), 7.09 (m, 1H), 4.91 (s, 2H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 166.1, 166.1, 157.0, 141.5, 138.7, 138.6, 129.2, 126.6, 124.3, 121.6, 120.2(2 C), 67.6; HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{14}H_{13}N_2O_4^+$ 273.0875, found 273.0873.

Biological screening

FA and analogues were screened against four common clinical bacteria (*S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*) and fungi (*C. albicans*) (Table 1). Except for **5** (MIC of 68 µg/mL) none of the compounds affected the growth of *S. aureus*. The library compounds do not inhibit the growth of *P. aeruginosa* and *C. albicans* under the concentration tested. However, except for **1** and **10**, other compounds showed moderate growth inhibitory activity toward *E. faecalis* and *E. coli*.

In *E. faecalis*, the MIC values range from 64 to 121 µg/mL with **5** being the most potent inhibitor. Interestingly, the acid derivatives (**5**, **6**) are slightly better in activity compared to its corresponding esters (**2**, **3**). This observation is in accordance with our previous study where ester derivatives showed a QS effect without interrupting the growth (Tung et al. 2017).

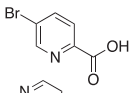
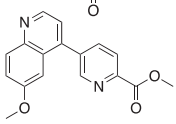
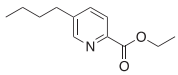
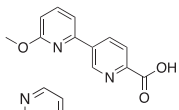
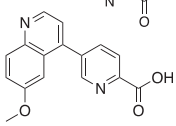
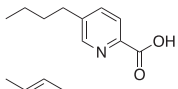
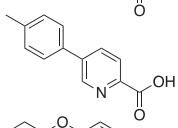
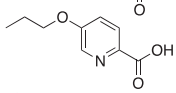
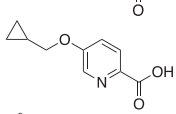
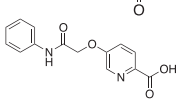
In *E. Coli*, compounds **6**, **8**, **9** exhibited a weak inhibitory activity with MIC values of 128, 125, and 116 µg/mL, respectively.

From a different perspective, compounds **2**–**5** and **7** are selective for gram-positive bacteria (*E. faecalis)*. In contrast, **6** and **9** are selective for both gram-positive and gram-negative (*E. faecalis and E. coli*, respectively). Noteworthy, compounds **2**, **4**, **5**, and **7** having an aromatic side chain attach to pyridine core, while **6** and **9** are an acyclic saturated hydrocarbon group. This structure–activity relationship could possibly be related to the polarity of the compounds and the physical properties of the peptidoglycan layer of the bacteria. In this series, **8** is the only substance that shows (weak) selective inhibition of gram-negative bacteria.

Compound-induced hemolysis can cause serious health problems when used in humans (Sharma et al. 1978). Therefore, it is important that the compounds do not cause any hemolysis effects. To best of our knowledge, none of the literature reports the hemolytic activities to demonstrate the drug's ability of FA. In this work, the in vitro hemolytic activity assay of the synthesized compounds has been performed (Table 2).

None of the compounds caused damage to the hRBCs at the concentration tested. The result indicated that the compounds are generally safe to humans. Moreover, FA could be a good template for future drug design and development. In order to demonstrate the drug-likeness of this scaffold, the prediction of ADME properties of the compounds was showed in Table 3.

Table 2 Hemolytic activity[a]

Compounds	Structure	ClogP	Hemolytic activity[b]	5% blood agar[c]
1		1.45	(−)	>10^6
2		1.91	(−)	>10^6
3		2.83	(−)	>10^6
4		1.51	(−)	>10^6
5		2.28	(−)	>10^6
6		2.67	(−)	>10^6
7		2.98	(−)	>10^6
8		1.57	(−)	>10^6
9		1.48	(−)	>10^6
10		1.12	(−)	>10^6

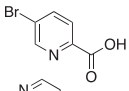
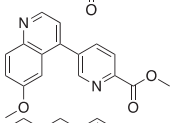
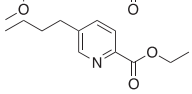
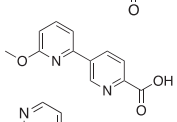
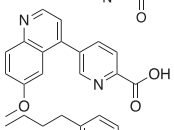
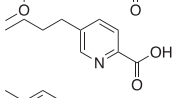
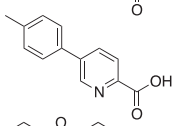
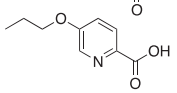
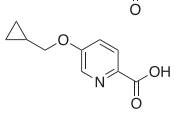
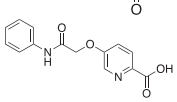
[a]Experiments were performed in triplicate

[b](−) negative, (+) positive

[c]Number of hemoglobin in 5% blood agar

The calculation of the key pharmacokinetic properties of the library compounds showed that none of them violated Lipinski "rule of five" (Table 3). In brief, the octanol–water partition coefficient values of the compounds were within acceptable range (<5). Number of acceptors, donors, and ROT bonds are perfectly suitable for future drug development on this scaffold compared to ampicillin (Tables 2, 3). Noteworthy, topological polar surface area of all compounds is <90, which demonstrated the excellent permeating cell membranes ability compared to that of ampicillin (Pajouhesh and Lenz 2005; Hitchcock and Pennington 2006).

Table 3 Drug-likeness of the library compounds

Compounds	Structure	MW	TPSA[a]	Number of atoms	Number of acceptors	Number of donors	Number of ROT bonds[b]	Number of violations[c]
1		202.01	50.19	10	3	1	1	0
2		294.31	61.32	22	5	0	4	0
3		207.27	39.20	15	3	0	6	0
4		230.22	72.32	17	5	1	3	0
5		280.28	72.32	21	5	1	3	0
6		175.19	53.09	13	3	2	1	0
7		213.24	50.19	16	3	1	2	0
8		181.19	59.42	13	4	1	4	0
9		193.20	59.42	14	4	1	4	0
10		272.26	88.52	20	6	2	5	0
Ampicillin		349.41	112.73	24	7	4	4	0

[a]Topological polar surface area

[b]Number of rotatable bonds

[c]According to the Lipinski's rule of five

Docking studies

Compound **5** is the only one that showed good growth inhibitory activities toward gram-positive bacteria tested (*S. aureus and E. faecalis*). In addition, an earlier report indicated that *S. aureus* tyrosyl-*t*RNA synthetase is a good template for studying the possible mechanism of actions (Xiao et al. 2011). Therefore, in order to gain some possible mechanism of actions, docking studies have been performed for hit compound **5** and ampicillin to the crystal structure of *S. aureus* tyrosyl-*t*RNA synthetase (Protein Data Bank ID: 1JIJ) (Fig. 3) (Qiu et al. 2001).

After removed from the crystal structure of *S. aureus* tyrosyl-*t*RNA synthetase, SB-239629 was re-docked as a control docking experiment using AutoDock Vina (Trott and Olson 2010). Then compound **5** and ampicillin were docked. Interestingly, compound **5** and ampicillin located perfectly to the active site of *S. aureus* tyrosyl-*t*RNA synthetase with the same stable energy of −8.9 kcal/mol. Noteworthy, Pi-Anion interaction of ASP-195 with Quinoline moiety was seen (Fig. 3). Thus, the docking results indicated that compound **5** is equal affinities to that of ampicillin toward *S. aureus* tyrosyl-*t*RNA synthetase. Overall results indicated that even though compounds exhibited moderate antimicrobial activities, they are good candidates for further drug development.

Overall, the structure–activity relationship observed in this study was summarized in Fig. 4.

Conclusion

In this work, 10 FA and analogues were synthesized and screened toward five common clinical pathogens. The library compounds possess the most sensitive toward *E.*

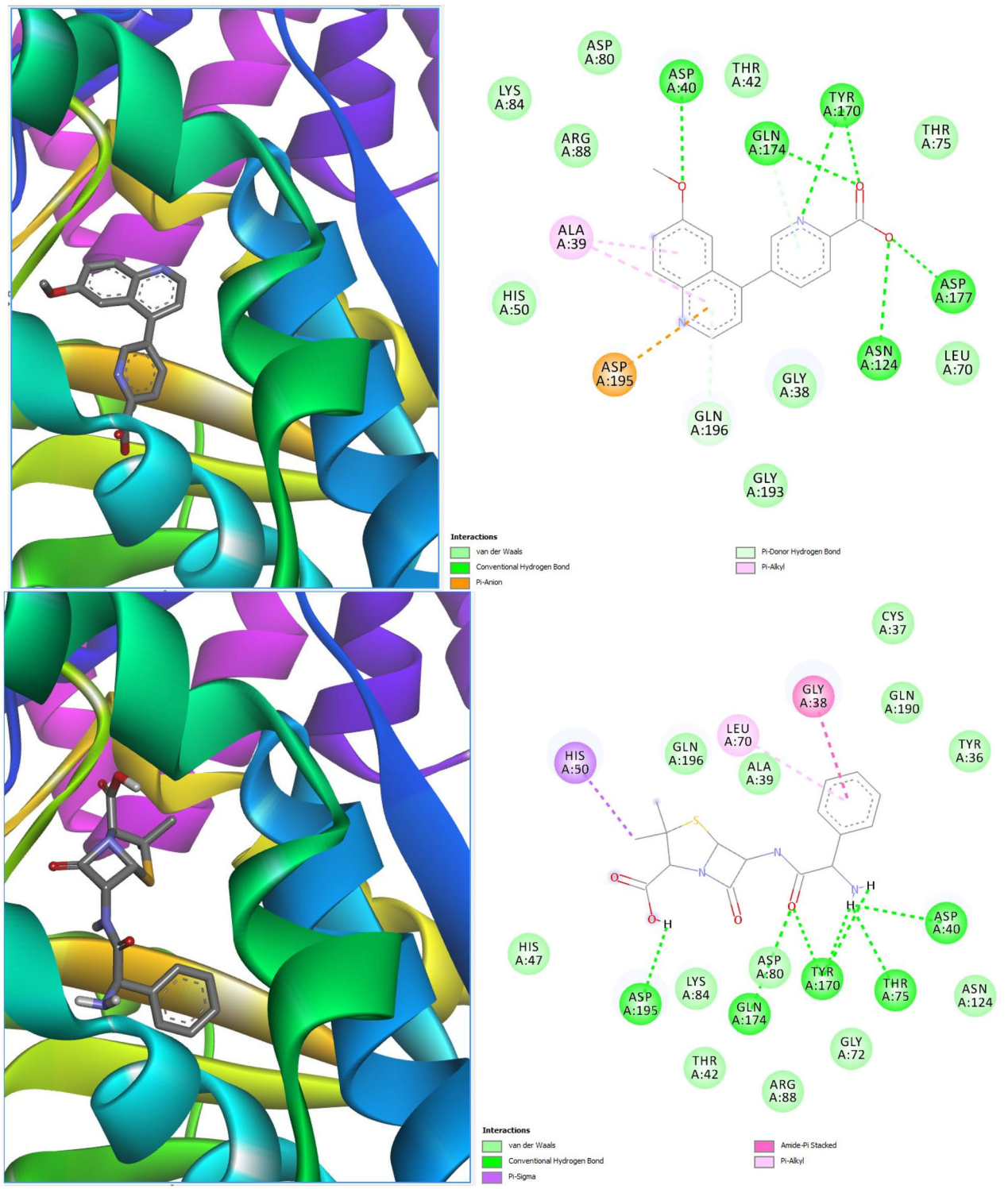


Fig. 3 Prediction of the binding mode of compound **5** and ampicillin toward *S. aureus* tyrosyl-*t*RNA synthetase

faecalis as seen in compounds **2**–**7** and **9**. In *E. Coli*, three compounds **6**, **8**, **9** present a weak growth inhibition with MIC of over 120 µg/mL. Compound **5** shows good growth inhibition with MIC of 68 µg/mL in *S. aureus*. In the concentration tested, no antimicrobial activities were found in *P. aeruginosa* and *C. albicans*. Structure–activity relationship indicated that compounds having an acyclic saturated hydrocarbon moiety are effective for both gram-positive and gram-negative bacteria. Replacing the alkane side chain by an aromatic ring, compounds seem to be

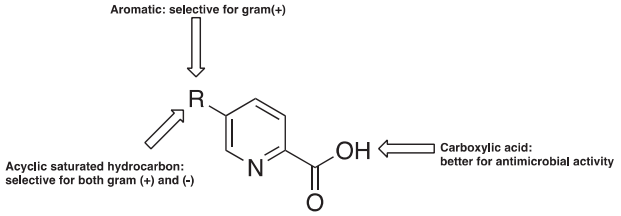


Fig. 4 Simplified structure–activity relationship based on this study

selected for only gram-positive. Interestingly, no hemolysis was observed in hRBCs of library compounds. Docking studies showed that compound **5** and ampicillin sharing the same stable energy. Overall, the results presented in this work are believed to provide essential information for understanding the mechanism of action of FA and for future drug designing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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