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Synthesis and structure-activity relationship of 1-[(*E*)-3-phenyl-2-propenyl] piperazine derivatives as suitable antibacterial agents with mild hemolysis

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Bromoacetyl bromide;
Amides;
Biofilm inhibition;
Hemolysis.

Abstract. A new series of 1-[(*E*)-3-phenyl-2-propenyl]piperazine derivatives (**5a-m**) as antibacterial agents was designed and synthesized. The synthetic strategy was initiated by coupling different anilines (**1a-m**) with bromoacetyl bromide (**2**) in an aqueous basic medium to acquire different electrophiles, **3a-m**, with good yields. These electrophiles further reacted with 1-[(*E*)-3-phenyl-2-propenyl]piperazine (**4**) to yield the desired compounds, *N*-(substituted)-2-{4-[(*E*)-3-phenyl-2-propenyl]-1-perazinyl} acetamides (**5a-m**). The structures of these compounds were established from their IR, ^{1}H-NMR, ^{13}C-NMR, EI-MS, and CHN analysis data. The bacterial biofilm inhibitory potential of these piperazine derivatives was tested against two pathogenic strains, *Bacillus subtilus*, and *Escherichia coli*. Two compounds, **5d** and **5h**, were identified as suitable antibacterial agents. The cytotoxicity of these molecules was profiled through hemolytic assay, and it was inferred that all the compounds were nearly harmless for membrane of red blood cells.

1. Introduction

Piperazine is a six-membered heterocyclic ring containing two nitrogen atoms in it and is a constituent part of several bioactive molecules. The polar nitrogen atoms in a piperazine ring assign bioactivity to molecules and ensure a favorable interaction with macromolecules. Slight changes in the substitution pattern in piperazine nucleus cause a distinguishable difference in their pharmacological activities [1-3]. Piperazine derivatives are classified to have privileged structure and are frequently found in biologically active compounds across a number of different therapeutic uses such as antimicrobial, anti-tubercular, anticonvulsant, antidepressant, anti-inflammatory, antimalarial, antiarrhythmic, antioxidant, and antiviral [4-6]. Piperazine derivatives have been reported as promising enzyme inhibitors, and some molecules containing this moiety have also found applications in the field of engineering and polymers [7].

The polar and stable amide functionality is the key unit amongst organic molecules and, also, in naturally occurring materials, e.g., peptides and proteins. It has a wide range of applications where it is used as intermediates or as an active pharmaceutical product or prodrug [8]. The stable and polar amide functionality is an important unit among the organic

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molecules of natural occurrence (e.g., peptides and proteins). It is also found in many synthetic substances of therapeutic interests [9].

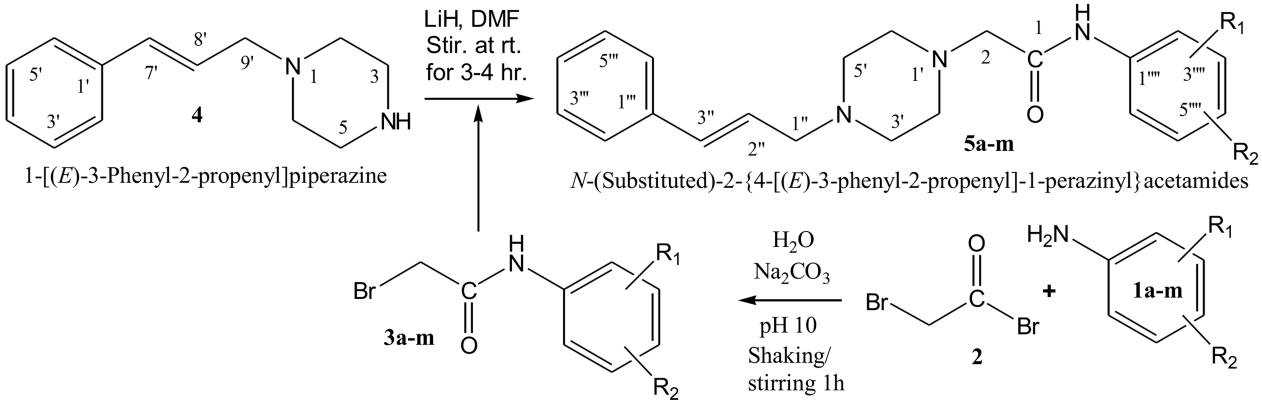
Based on these considerations and further to our previous effort for the antibacterial evaluation of piperazine-acetamides [10], hereby, this study reports the bacterial biofilm inhibition of a new series of piperazine-acetamides with a rationale that these molecules might overcome the overwhelming resistance of some microbes and can find application in antibiotic therapy.

2. Results and discussion

2.1. Chemistry

The synthetic route to new 1-[(*E*)-3-phenyl-2-propenyl] piperazine derivatives (**5a-m**) is outlined in Scheme 1, and varying groups are listed in Table 1. The procedures and conditions of the reactions are discussed in the experimental section. The synthesized compounds are subjected to structural analysis using IR, EI-MS, ^{1}H-NMR, ^{13}C-NMR, and CHN techniques. Initially, various electrophiles, 2-bromo-*N*-(substituted-phenyl)acetamides (**3a-m**), were synthesized by the reaction of bromoacetyl bromide (**2**) with various anilines (**1a-m**) in 10% aqueous Na_2CO_3 solution at room temperature. The reactions were accomplished only by vigorous stirring, which resulted in the formation of desired products in excellent yields. These electrophiles were then coupled with 1-[(*E*)-3-phenyl-2-propenyl]piperazine (**4**) to achieve a series of new *N*-(substituted)-2-{4-[(*E*)-3-phenyl-2-propenyl]-1-perazinyl} acetamides (**5a-m**), and their structures were corroborated with spectral analysis.

One of the compounds is discussed hereby in detail for the expediency of the readers. For example, compound **5b** was obtained as light grey solid with a yield of 91% and melting points of 146-148°C. Its molecular formula, $C_{22}H_{27}N_3O$, was ascribed by its CHN and EI-MS analysis data. The number of protons in its ^{1}H-NMR spectrum and carbon resonances in its ^{13}C-NMR spectrum was in agreement with its molecular formula. The presence of different functional groups was ascertained by its IR spectral data. The absorption band at ν 3419 cm^{-1} was characteristic of N-H stretching. The other bands were observed at ν 3057 (C-H, str. of aromatic ring), 2893 (C-H, aliphatic str.), 1604 (aromatic C=C stretching), 1654 (C=O str.), 1640 (C=C, alkene str.), and 1301 (C-N aromatic str.) cm^{-1}. In ^{1}H-NMR spectrum (Figure S1a in Supplementary Information), its aromatic region (Figure S1b in Supplementary Information) showed the highly deshielded singlet at δ 9.37, which was assigned to −NH proton of acetamide group (−NHCO). The *N*-(2-methylphenyl) moiety attached to acetamido group was demonstrated by three discrete and one merged resonances in the aromatic region. These three discrete resonances were observed at δ 7.83 (br.d, J = 7.9 Hz,



Scheme 1. Synthesis of various *N*-(substituted)-2-{4-[(*E*)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (**5a-m**).

Table 1. Different groups (-R_1 and -R_2) in Scheme 1.

Compd.	-R_1	-R_2	Compd.	-R_1	-R_2
1a, 3a, 5a	-H	-H	**1g, 3g, 5g**	2-CH_3	3-CH_3
1b, 3b, 5b	2-CH_3	-H	**1h, 3h, 5h**	2-CH_3	4-CH_3
1c, 3c, 5c	3-CH_3	-H	**1i, 3i, 5i**	2-CH_3	5-CH_3
1d, 3d, 5d	2-CH_2CH_3	-H	**1j, 3j, 5j**	2-CH_3	6-CH_3
1e, 3e, 5e	4-CH_2CH_3	-H	**1k, 3k, 5k**	3-CH_3	4-CH_3
1f, 3f, 5f	4-OC_2H_5	-H	**1l, 3l, 5l**	3-CH_3	5-CH_3
1m, 3m, 5m	2-CH_2CH_3			6-CH_3	

1H, H-6′′′′), δ 7.17 (br.t, J = 7.6 Hz, 1H, H-5′′′′), and δ 7.04 (br.t, J = 7.4 Hz, 1H, H-4′′′′), while a singlet at δ 2.24 was characteristic of a methyl substituent (CH_3-2). The 4-(*E*)-3-phenyl-2-propenyl moiety was ascertained by two resonances in the aromatic region at δ 7.44 (br.d, J = 7.3 Hz, 2H, H-2′′′ & H-6′′′) and δ 7.32 (br.t, J = 7.5 Hz, 2H, H-3′′′ & H-5′′′) for two *ortho* and two *meta* protons of phenyl group. While the signal of *para* proton (H-4′′′) of this phenyl ring was merged as multiplet with the signal of *meta* proton (H-3′′′′) of *N*-(2-methylphenyl) group at δ 7.23-7.21 (m, 2H). Similarly, the signal of methylene (CH_2-1′′) in the 2-propenyl unit was merged as multiplet with the signal of acetamidic methylene (CH_2-2) at δ 3.15-3.13 (m, 4H). However, the *trans* disposition of two methine protons in the 2-propenyl unit was clearly indicated by larger coupling constants in their respective signals at δ 6.50 (d, J = 15.9 Hz, 1H, H-3′′) and δ 6.30 (td, J = 6.6, 15.8 Hz, 1H, H-2′′). The symmetric 1,4-piperazinyl ring in the molecule was represented by two signals at δ 2.88 (br. s, 4H, H-2′ & H-6′) and δ 2.73 (br. s, 4H, H-3′ & H-5′). The ^{13}C-NMR spectrum (Figure S2 in Supplementary Information) of this molecule also fully corroborated the subsistence of these moieties. Then, the *N*-(2-methylphenyl) group attached to an acetamido group was verified by typical six resonances at δ 136.03 (C-1′′′′), 126.03 (C-2′′′′), 127.41 (C-3′′′′), 124.19 (C-4′′′′), 130.21 (C-5′′′′), and δ 121.61 (C-6′′′′) for the phenyl ring along with a signal at δ 14.58 for the methyl substituent attached at the 2-position (CH_3-2). The acetamido group was inferred clearly by two peaks at δ 167.81 (C-1) and δ 61.46 (C-2). The phenyl ring in the 4-(*E*)-3-phenyl-2-propenyl unit was ascertained by four signals at δ 136.56 (C-1′′′), 126.18 (C-2′′′ & C-6′′′), 128.53 (C-3′′′ & C-5′′′), and δ 127.41 (C-4′′′), while the propenyl part was evident with three signals at δ 59.96 (C-1′′), 128.46 (C-2′′), and 126.23 (C-3′′). The symmetrical 1,4-piperazinyl heterocycle was characterized by an overlapped signal at δ 52.76 (C-2′, C-3′, C-5′ & C-6′). Moreover, on account of the aforementioned evidence, the structure **5b** is named as *N*-(2-methylphenyl)-2-{4-[(*E*)-3-phenyl-2-propenyl]-1-perazinyl}acetamide. A similar pattern was adopted for the structural characterization of other derivatives in the series.

2.2. Bacterial biofilm inhibition and structure-activity relationship

The antibacterial activity of synthetic derivatives, **5a-m**, was checked by the biofilm inhibition method using two bacterial pathogenic strains, i.e., *Bacillus subtilis* and *Escherichia coli*. Some of the compounds exhibited considerable antibacterial potential (Table 2) against these strains, relative to ampicillin (*B. subtilis*: 77.49% & *E. coli*: 78.88%), a standard drug to measure the extent of antibacterial activity.

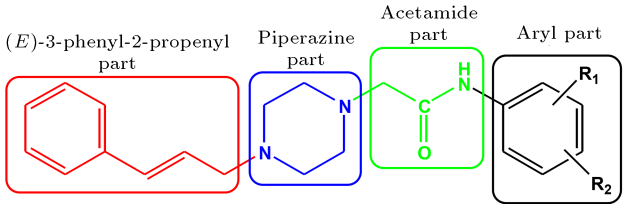


Figure 1. General structural features of compounds **5a-m**.

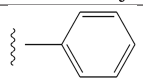
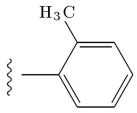
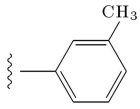
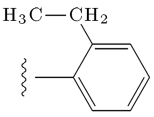
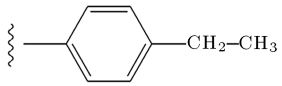
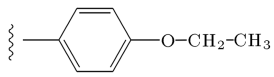
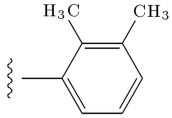
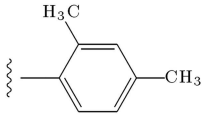
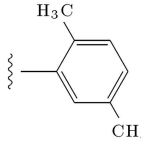
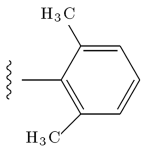
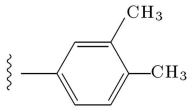
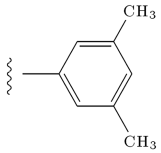
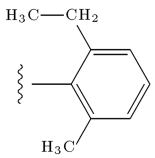
Although the observed antibacterial potential results from the whole molecule, a limited Structure-Activity Relationship (SAR) was rationalized by analyzing the effect of different aryl parts on the bacterial biofilm inhibition. Figure 1 displays the general structural features of the synthetic compounds.

Compound **5a** featuring an unsubstituted phenyl ring showed the least activity against *B. subtilis* (5.52) and poor activity against *E. coli* (31.47). The presence of a methyl group on the phenyl ring (aryl part) in **5b** and **5c** enhanced their antibacterial activity, relative to **5a**, against both strains. However, better antibacterial potential was observed when the methyl group was present at the 3-position in **5c** (*B. subtilis: 54.35%* & *E. coli: 62.63%*), as compared to that of **5b** (*B. subtilis*: 14.65% & *E. coli*: 58.80%), in which it was present at the 2-position (Figure 2). It means that in compound **5c** when a small-sized group was present at the m-position, it behaved as a better antibacterial agent.

A reverse trend was observed when a medium-sized ethyl group was present at *ortho* position of the phenyl ring in **5d**. In fact, this compound is the best antibacterial agent (66.77%) among the synthetic series against *B. subtilis* and, also, exhibits a promising antibacterial potential against *E. coli* (63.15%). The presence of an additional methyl group at the 6-position in **5m** enhanced its activity against *E. coli* (66.77%), and it behaved as the second most active compound among the synthetic derivatives. However, relative to **5d**, a slight decrease in antibacterial activity was observed in **5m** against *B. subtilis* (56.79%). Both *para*-group bearing molecules **5e** (*B. subtilis*: 25.05% & *E. coli*: 7.14%) and **5f** (*B. subtilis*: 13.69% & *E. coli*: 26.29%) displayed considerably weak antibacterial potential. According to the results, a medium-sized group at *ortho*-position of the phenyl ring was going to render a promising antibacterial potential to the molecule relative to other synthetic analogues (Figure 3).

Among the di-methylated *region*-isomers, two compounds, **5g** and **5i**, showed very moderate and much resembling antibacterials (*B. subtilis*: 43.42% & *E. coli*: 43.79%) and (*B. subtilis*: 45.75% & *E. coli*: 41.61%), respectively. The methyl groups were present at the 2- and 3-position in **5g**, while they were present at the 2- and 5-position in **5i**. Compound **5j** with sym-

Table 2. Percentage (%) of biofilm inhibition against *Bacillus subtilis/Escherichia coli* and hemolytic activity of 1-[(*E*)-3-phenyl-2-propenyl]piperazine derivatives (**5a-m**).

Compound	Aryl part	*B. subtilis*	*E. coli*	Hemolysis
5a		5.52	31.47	9.26
5b		14.65	58.80	9.47
5c		54.35	62.63	15.26
5d		66.77	63.15	7.79
5e		25.05	7.14	19.68
5f		13.69	26.29	9.79
5g		43.42	43.79	6.00
5h		63.06	71.95	13.79
5i		45.75	41.61	9.79
5j		9.24	21.33	5.05
5k		39.49	50.93	7.89
5l		27.60	61.59	12.32
5m		56.79	66.77	15.58
	Ampicillin	77.49	78.88	–
	Triton-X-100	Positive control		89.00
	PBS	Negative control		0.54

Note: Ampicillin was used as a positive control. Negative control (% inhibition) = 1.021.

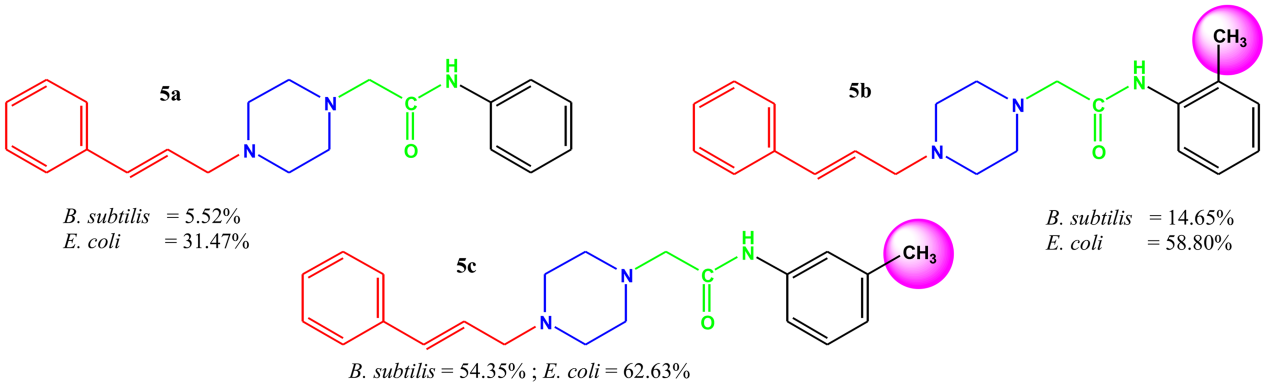


Figure 2. Structure-activity relationship of compounds **5a**, **5b**, and **5c**.

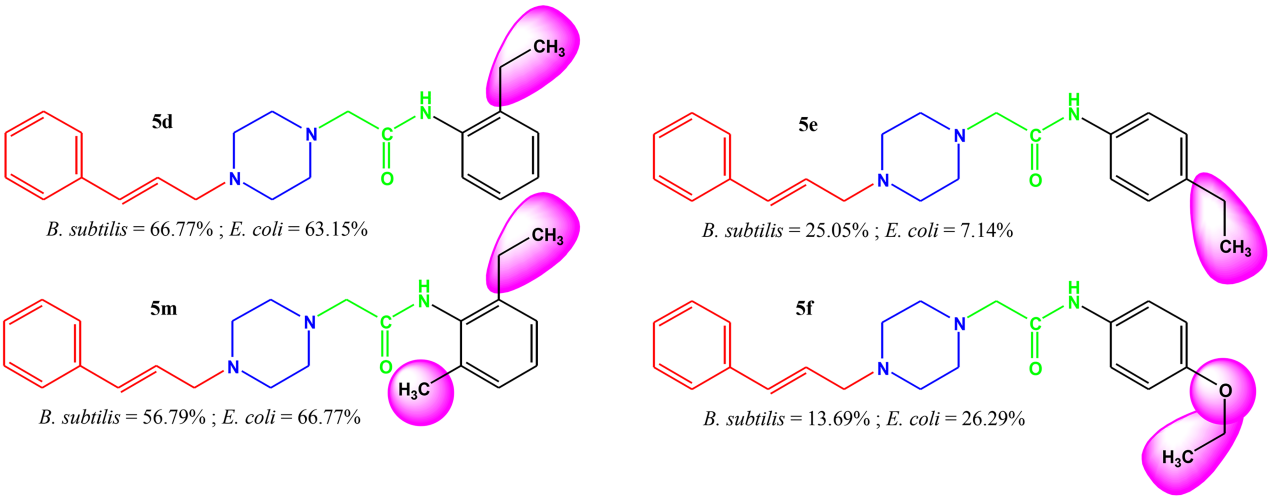


Figure 3. Structure-activity relationship of **5d**, **5e**, **5m**, and **5f**.

metrical di-*ortho* methyl groups at the 2- and 6-position possessed very weak antibacterial potential (*B. subtilis*: 9.24% & *E. coli*: 21.33%); however, the isomer **5h** with methyl groups at the 2- and 4-position displayed excellent antibacterial potential. Indeed, this compound is the most active (71.95%) among the whole series against *E. coli* and is the second most active (63.06%) against *B. subtilis*. It means that when methyl groups are at *ortho* and *para* positions, these impart better antibacterial potential to the molecule (Figure 4).

The regioisomers, **5k** and **5l**, exhibited moderately weak antibacterial potential against *B. subtilis* (39.49% and 27.60%, respectively) and considerably good potential against *E. coli* (50.93% and 61.59%, respectively). A closer look at the comparative percentage biofilm inhibition data exposed that the former with 3,4-dimethyl groups was a better antibacterial agent against *B. subtilis*, while the latter with symmetrical 2,6-dimethyl groups displayed better antibacterial potential against *E. coli* (Figure 5).

Therefore, it was inferred from the structure-activity relationship that two molecules, one with an *ortho*-ethyl group (**5d**) and the other with *ortho* and *para*-methyl groups (**5h**), generally behaved as suitable antibacterial agents against both strains. The phase-contrast microscopic view of inhibition of *Bacillus subtilis* biofilm is given in Figure S3 (in Supplementary Information), while that of *Escherichia coli* biofilm is given in Figure S4 (in Supplementary Information).

2.3. Hemolytic activity

All the synthesized compounds were subjected to hemolytic assay to determine their cytotoxicity profile. Results of percentage hemolysis are shown in Table 2, indicating that all the compounds were nearly nontoxic for membrane of red blood cells, and their hemolysis values ranged from 5.05% to 19.68%, which were much lower than Triton-X (positive control) having %hemolysis of 89%.

2.4. Conclusion

In conclusion, a series of 1-[(*E*)-3-phenyl-2-propenyl] piperazine derivatives was synthesized successfully and evaluated for their biofilm inhibition against two

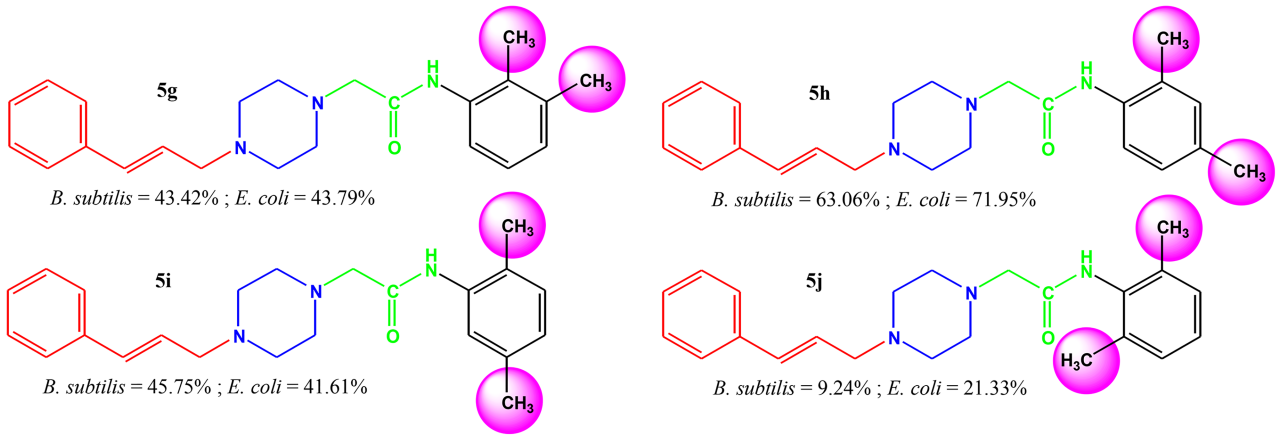


Figure 4. Structure-activity relationship of **5g**, **5h**, **5i**, and **5j**.

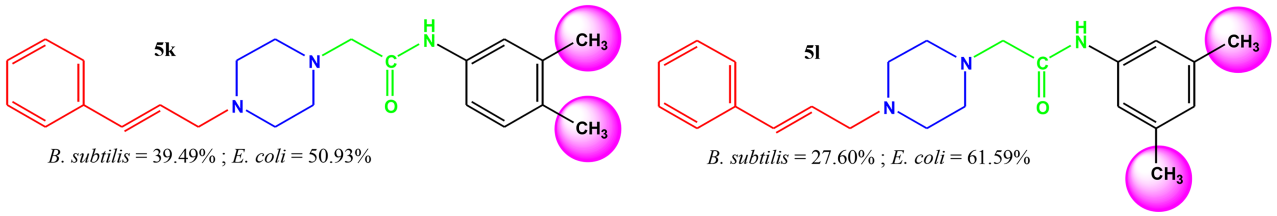


Figure 5. Structure-activity relationship of **5k** and **5l**.

pathogenic bacterial strains. SAR studies were carried out to investigate the role of various groups attached to the phenyl ring, exerting imperative influence on the antibacterial potential of these molecules. From the hemolytic activity, it was ascertained that these molecules were nearly nontoxic for membrane of red blood cells. Particularly, two molecules, one with an *ortho*-ethyl group (**5d**) and the other with *ortho* and *para*-methyl groups (**5h**), were explored as suitable antibacterial agents against both of the studied strains.

3. Experimental

3.1. General

All the chemicals, along with analytical grade solvents, were purchased from Sigma Aldrich, Alfa Aesar (Germany), or Merck through local suppliers. Pre-coated silica gel Al-plates were used for TLC with ethyl acetate and *n*-hexane as the solvent system (20:80). Spots were detected by UV_{254}. Gallonkamp apparatus was used to detect melting points (uncorrected) in capillary tubes. IR spectra (ν, cm^{-1}) were recorded by the KBr pellet method in the Jasco-320-A spectrophotometer. Elemental analyses were performed by a Foss Heraeus CHN-O-Rapid instrument and were within $\pm 0.4\%$ of the theoretical values. EI-MS spectra were measured by a JEOL JMS-600H instrument with a data-processing system. ^{1}H-NMR spectra (δ, ppm) were recorded at 600 MHz (^{13}C-NMR spectra, at 150 MHz) in DMSO-d_6 using the Bruker Advance III 600 Ascend spectrometer using BBO probe. The coupling constant (J) is given in Hz and chemical shift (δ) in ppm. The abbreviations used in the interpretation of ^{1}H NMR spectra are as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br.t, broad triplet; q, quartet; quint, quintet; sex, sextet; sep, septet; m, multiplet; dist., distorted.

3.2. Preparation of 2-bromo-N-(substituted-phenyl) acetamides (3a-m)

Equimolar amounts (0.001 moles) of various anilines (**1a-m**, one in each reaction) were added to the round bottom flask with distilled water at room temperature and stirred for 30 minutes. Herein, 10% aqueous Na_2CO_3 solution was added into the reaction mixture to adjust pH to 9-10. Gradually, 0.001 moles of bromoacetyl bromide (**2**) were added into the reaction mixture. The completion of reaction was monitored by TLC. HCl was added drop-wise to set pH to 5 until precipitates were formed. The product was filtered, washed with distilled water, and dried to obtain 2-bromo-*N*-(substituted-phenyl) acetamides (**3a-m**) as electrophiles.

3.3. General procedure for the synthesis of N-(substituted)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5a-m)

The calculated amount of 1-[(*E*)-3-phenyl-2-propenyl] piperazine (**4**; 0.1 mmol) was taken in a round bottomed flask (50 mL); then, dimethyl formamide DMF

(10.0 mL) was added to dissolve it, followed by the addition of lithium hydride (0.1 mmol) to the mixture. The mixture was stirred for 30 minutes at room temperature and, then, slowly an electrophile from the aforementioned 2-bromo-*N*-(substituted-phenyl) acetamides (**3a-m**, one in each reaction) was added to the mixture; next, the solution was further stirred for three hours. The progress of reaction was monitored via TLC till single spot. The product was precipitated by adding water. It was filtered, washed with distilled water, and crystallized from aqueous methanol.

3.4. Structural characterization

N-phenyl-2-{4-[(E)-3-phenyl-2-propenyl] -1-perazinyl}acetamides (5a)

Light brown solid, Yield 72%, m.p. 138-139°C, $C_{21}H_{25}N_3O$, Mol. Mass: 335 gmol^{-1}; IR (KBr, cm^{-1}) ν: 3410 (N-H, str.), 3053 (C-H, str. of aromatic ring), 2887 (C-H, aliphatic str.), 1590 (C=C, aromatic str.), 1654 (C=O str.), 1634 (C=C, alkene str.), 1295 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.67 (s, 1H, -NHCO), 7.62 (br.d, J = 7.6 Hz, 2H, H-2‴ & H-6‴), 7.44 (br.d, J = 7.3 Hz, 2H, H-2‴ & H-6‴), 7.33-7.28 (m, 4H, H-3‴, H-5‴, H-3‴ & H-5‴), 7.23 (br.t, J = 7.3 Hz, 1H, H-4‴), 7.05 (br.t, J = 7.3 Hz, 1H, H-4‴), 6.54 (d, J = 15.9 Hz, 1H, H-3″), 6.30 (td, J = 6.8, 15.8 Hz, 1H, H-2″), 3.12-3.11 (m, 4H, CH_2-2 & CH_2-1″), 2.88 (br.s) & 2.73 (br.s, 8H, H-2′, H-3′, H-5′ & H-6′); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 168.09 (C-1), 138.45 (C-1‴), 136.54 (C-1‴), 131.93 (C-2″), 128.54 (C-3‴ & C-5‴), 128.45 (C-3‴ & C-5‴), 127.28 (C-4‴), 126.94 (C-3″), 126.08 (C-2‴ & C-6‴), 123.25 (C-4‴), 119.28 (C-2‴ & 6‴), 61.69 (C-2), 60.01 (C-1″), 52.69 & 52.41 (C-2′, C-3′, C-5′ & C-6′); Anal. Calc. for $C_{21}H_{25}N_3O$ (335.20): C, 75.19; H, 7.51; N, 12.53. Found: C, 74.96; H, 7.40; N, 12.36; EI-MS: m/z 335 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 120 $[C_7H_6NO]^+$, 117 $[C_9H_9]^+$, 77 $[C_6H_5]^+$.

N-(2-methylphenyl)-2{-4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides(5b)

Light grey solid, Yield 91%, m.p. 146-148°C, $C_{22}H_{27}N_3O$, Mol. Mass: 349 gmol^{-1}; IR (KBr, cm^{-1}) ν: 3419 (N-H, str.), 3057 (C-H, str. of aromatic ring), 2893 (C-H, aliphatic str.), 1604 (C=C, aromatic str.), 1654 (C=O str.), 1640 (C=C, alkene str.), 1301 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.37 (s, 1H, -NHCO), 7.83 (br.d, J = 7.9 Hz, 1H, H-6‴), 7.44 (br. d, J = 7.3 Hz, 2H, H-2‴ & H-6‴), 7.32 (br.t, J = 7.5 Hz, 2H, H-3‴ & H-5‴), 7.23-7.21 (m, 2H, H-4‴ & H-3‴) 7.17 (br.t, J = 7.6 Hz, 1H, H-5‴), 7.04 (br.t, J = 7.4 Hz, 1H, H-4‴), 6.5 (d, J = 15.9 Hz, 1H, H-3″), 6.30 (td, J = 6.6 & 15.8 Hz, 1H, H-2″), 3.15-3.13 (m, 4H, CH_2-2 & CH_2-1″), 2.88 (br.s) & 2.73 (br.s, 8H, H-2′, H-3′, H-5′ & H-6′), 2.24 (s, 3H, 2-CH_3); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 167.81 (C-1), 136.56 (C-1‴), 136.03 (C-1‴), 130.21 (C-5‴), 128.53 (C-3‴ & C-5‴), 128.46 (C-2″), 127.41 (C-4‴ & C-3‴), 126.23 (C-3″), 126.18 (C-2‴ & C-6‴), 126.03 (C-2‴) 124.19 (C-4‴), 121.69 (C-6‴), 61.46 (C-2), 59.96 (C-1″), 52.76 (C-2′, C-3′, C-4′ & C-6′), 14.58 (2-CH_3); Anal. Calc. for $C_{22}H_{27}N_3O$ (349.22): C, 75.61; H, 7.79.; N, 12.02. Found: C, 75.51; H, 7.63; N, 11.96; EI-MS: m/z 349 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 134 $[C_8H_8NO]^+$, 117 $[C_9H_9]^+$, 91 $[C_7H_7]^+$.

N-(3-methylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5c)

Light yellow solid, Yield 88%, m.p. 143-144°C, $C_{22}H_{27}N_3O$, Mol. Mass: 349 gmol^{-1}; IR (KBr, cm^{-1}) ν: 3423 (N-H, str.), 3061 (C-H, str. of aromatic ring), 2899 (C-H, aliphatic str.), 1621 (C=C, aromatic str.), 1654 (C=O str.), 1647 (C=C, alkene str.), 1312 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 8.32 (s, 1H, -NHCO), 7.46-7.40 (m, 5H, H-2‴, H-6‴, H-2‴ & H-4‴ & H-6‴), 7.34 (br.t, J = 7.5 Hz, 2H, H-3‴ & H-5‴), 7.23 (br.t, J = 7.5 Hz, 1H, H-4‴), 7.18 (br.t, J = 7.8 Hz, 1H, H-5‴), 6.61 (d, J = 16.3 Hz, 1H, H-3″), 6.32 (td, J = 6.7 & 15.9 Hz, 1H, H-2″), 3.16 (m, 4H, CH_2-2 & CH_2-1″), 2.88 (br.s) & 2.73 (br.s, 8H, H-2′, H-3′, H-5′& H-6′), 2.2 (s, 3H, 3-CH_3); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 167.78 (C-1), 141.62 (C-1‴), 138.09 (C-3‴), 137.39 (C-4‴), 136.24 (C-1‴), 128.93 (C-2″), 128.50 (C-3‴ & C-5‴), 127.59 (C-4‴), 127.19 (C-3″), 126.24 (C-2‴ & C-6‴), 124.01 (C-5‴), 119.83 (C-6‴), 116.30 (C-2‴), 61.17 (C-2), 59.59 (C-1″), 59.33 & 58.62 (C-2′, C-3′, C-5′ & C-6′), 20.03 (3-CH_2); Anal. Calc. for $C_{22}H_{27}N_3O$ (349.22): C, 75.61; H, 7.79.; N, 12.02. Found: C, 79.49; H, 7.61; N, 11.88; EI-MS: m/z 349 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 134 $[C_8H_8NO]^+$, 117 $[C_9H_9]^+$, 91 $[C_7H_7]^+$.

N-(2-ethylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5d)

Light pink solid, Yield 92%, m.p. 149-151°C, $C_{23}H_{29}N_3O$, Mol.Mass: 363 gmol^{-1}; IR (KBr, cm^{-1}) ν: 3436 (N-H, str.), 3062 (C-H, str. of aromatic ring), 2903 (C-H, aliphatic str.), 1627 (C=C, aromatic str.), 1654 (C=O str.), 1650 (C=C, alkene str.), 1319 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.45 (s, 1H, -NHCO), 7.85 (br.d, J = 7.8 Hz, 1H, H-6‴), 7.44 (br.d, J = 7.3 Hz, 2H, H-2‴ & H-6‴), 7.38 (d, J = 7.6 Hz, 1H, H-3‴), 7.32 (br.t, J = 7.5 Hz, 2H, H-3‴ & H-5‴), 7.24 (br.t, J = 7.3 Hz, 1H, H-4‴), 7.19 (t, J = 7.6 Hz, 1H, H-5‴), 7.08 (t, J = 7.4 Hz, 1H, H-4‴), 6.57 (d, J = 15.9 Hz, 1H, H-3″), 6.32 (td, J = 6.7 & 15.8 Hz, 1H, H-2″), 3.18 (dis.d, J = 6.36 Hz, 2H, H-1″), 3.15 (s, m, 2 H, CH_2-2), 2.88 (br.s) & 2.73 (br. s, 8H, H-2′, H-3′, H-5′ & H-6′), 2.61 (q, J = 7.44 Hz, 2H, CH_2-2), 2.56 (dis.t, J = 7.56 Hz, 3H, CH_3-2); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 167.94 (C-1), 136.49 (C-1‴), 135.33 (C-

2″), 134.27 (C-1‴′), 128.65 (C-5‴′), 128.54 (C-3‴ & C-5‴), 127.48 (C-2‴′), 127.28 (C-4‴), 126.48 (C-3″), 126.21 (C-2‴ & C-6‴), 126.03 (C-3‴′), 124.51 (C-4‴′), 122.11 (C-6‴′), 61.29 (C-2), 59.79 (C-1″), 52.61 (C-2′, C-3′, C-4′ & C-6′), 23.87 (CH_2-2), 14.27 (CH_3-2); Anal. Calc. for $C_{23}H_{29}N_3O$ (363.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.96; H, 8.01; N, 11.43; EI-MS: m/z 363 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 148 $[C_9H_{10}NO]^+$, 117 $[C_9H_9]^+$, 105 $[C_9H_8]^+$.

N-(4-ethylphenyl)-2-{4-[(E)-3-phenyl -2-propenyl]-1-perazinyl}acetamides (5e)

White solid, Yield 77%, m.p. 160-161°C, $C_{23}H_{29}N_3O$, Mol. Mass: 363 $gmol^{-1}$; IR (KBr, cm^{-1}) ν: 3441 (N-H, str.), 3065 (C-H, str. of aromatic ring), 2907 (C-H, aliphatic str.), 1629 (C=C, aromatic str.), 1654 (C=O str.), 1653 (C=C, alkene str.), 1322 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.59 (s, 1H, -NHCO), 7.53 (br.d, J = 8.4 Hz, 2H, H-3‴′ & H-5‴′), 7.44 (d, J = 7.3 Hz, 2H, H-2‴ & H-6‴), 7.32 (br.t, J = 7.5 Hz, 2H, H-3‴ & H-5‴), 7.23 (br.t, J = 7.3 Hz, 1H, H-4‴), 7.13 (d, J = 8.4 Hz, 2H, H-2‴′ & H-6‴′), 6.53 (d, J = 15.9 Hz, 1H, H-3″), 6.30 (td, J = 6.6 & 15.9 Hz, 1H, H-2″), 3.12 (d, J = 6.4 Hz, 2H, CH_2-1″), 3.10 (s, 2H, CH_2-2), 2.88 (br.s) & 2.73 (br. s, 8H, H-2′, H-3′, H-5′ & H-6′), 2.55 (q, J = 7.5 Hz, 2H, CH_2CH_3-4), 1.13 (t, J = 7.5 Hz, 3H, CH_2CH_3-4); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 167.94 (C-1), 138.75 (C-4‴′), 136.59 (C-1‴), 136.21 (C-1‴′), 132.11 (C-2″), 128.51 (C-3‴ & C-5‴), 127.79 (C-3‴′ & 5‴′), 127.36 (C-4‴), 126.84 (C-3″), 126.16 (C-2‴ & C-6‴), 119.45 (C-2‴′ & C-6‴′), 61.72 (C-2), 60.04 (C-1″), 52.71-52.45 (C-2′, C-3′, C-5′ & C-6′), 27.57 (CH_2-4), 15.68 (CH_3-4); Anal. Calc. for $C_{23}H_{29}N_3O$ (362.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.84; H, 7.96; N, 11.33; EI-MS: m/z 363 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 148 $[C_9H_{10}NO]^+$, 117 $[C_9H_9]^+$, 105 $[C_8H_9]^+$.

N-(4-ethoxylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5f)

Light Brown solid, Yield 87%, m.p. 166-168°C, $C_{23}H_{29}N_3O_2$, Mol. Mass: 379 $gmol^{-1}$; IR (KBr, cm^{-1}) ν: 3443 (N-H, str.), 3067 (C-H, str. of aromatic ring), 2909 (C-H, aliphatic str.), 1633 (C=C, aromatic str.), 1654 (C=O str.), 1652 (C=C, alkene str.), 1323 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.58 (s, 1H, -NHCO), 7.52 (d, J = 9.0 Hz, 2H, H-3‴′ & 5‴′), 7.43 (br.d, J = 7.4 Hz, 2H, H-2‴ & H-6‴), 7.32 (t, J = 7.4 Hz, 2H, H-3‴ & H-5‴), 7.23 (br.t, J = 7.3 Hz, 1H, H-4‴), 6.85(d, J = 9.0 Hz, 2H, H-2‴′ & 6‴′), 6.54 (d, J = 15.9 Hz, 1H, H-3″), 6.30 (td, J = 6.6 &15.9 Hz, 1H, H-2″), 3.10 (d, J = 6.42 Hz, 2H, CH_2-1″), 3.08 (s, 2H, CH_2-2), 2.53-2.50 (m, 8H, H-2′, H-3′, H-5′ & H-6'), 3.97 (q, J = 6.9 Hz, 2H, CH_2O-4),1.30 (t, J = 6.9, 3H, CH_3-4); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ in ppm): 167.58 (C-1), 154.50 (C-4‴′), 136.58 (C-1‴), 131.88 (C-2″), 131.54 (C-1‴′), 128.45 (C-3‴ & C-5‴), 127.27 (C-4‴), 127.03 (C-3″), 126.09 (C-2‴ & C-6‴), 120.92 (C-3‴′ & C-5‴′), 114.22 (C-2‴′ & C-6‴′), 63.00 (CH_2O-4), 61.70 (C-2), 60.06 (C-1″), 52.78 & 52.44 (C-2′, C-3′, C-5′ & C-6′), 14.58 (CH_3-4); Anal. Calc. for $C_{23}H_{29}N_3O_2$ (379.23): C, 72.79; H, 7.70; N, 11.07. Found: C, 72.68; H, 7.61; N, 11.01; EI-MS: m/z 379 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 164 $[C_9H_{10}NO_2]^+$, 117 $[C_9H_9]^+$, 121 $[C_8H_9O]^+$.

N-(2,3-dimethylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5g)

Light yellow solid, Yield 69-70%, m.p. 157-159°C, $C_{23}H_{29}N_3O$, Mol. Mass: 363 $gmol^{-1}$; IR (KBr, cm^{-1}) ν: 3454 (N-H, str.), 3077 (C-H, str. of aromatic ring), 2945 (C-H, aliphatic str.), 1643 (C=C, aromatic str.), 1654 (C=O str.), 1672 (C=C, alkene str.), 1343 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.37 (s, 1H, -NHCO), 7.53 (br.d, J = 7.9 Hz, 1H, H-6‴′), 7.44 (d, J = 7.3 Hz, 2H, H-2‴ & H-6‴), 7.32 (br.t, J = 7.32 Hz, 2H, H-3‴ & H-5‴), 7.23 (br.t, J = 7.3 Hz, 1H, H-4‴), 7.05 (br.t, J = 7.8 Hz, 1H, H-5‴′), 6.97 (br.d, J = 7.4 Hz, 1H, H-4‴′), 6.54 (d, J = 15.9 Hz, 1H, H-3″), 6.30 (td, J = 6.6 & 15.8 Hz, 1H, H-2″), 3.33 (br.s, 2H, CH_2-2), 3.12 (dis.d, J = 6.6 Hz, 2H, CH_2-1″), 2.59-2.50 (m, 8H, H-2′, H-3′, H-5′ & H-6′), 2.25 (s, 3H, 2-CH_3), 2.10 (s, 3H, 3-CH_3); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 167.80 (C-1), 136.60 (C-1‴′), 136.53 (C-1‴), 135.75 (C-3‴′), 131.94 (C-2″), 128.45 (C-3‴ & C-5‴), 127.93 (C-2‴′), 127.28 (C-4‴), 126.95 (C-3″), 126.08 (C-2‴ & C-6‴), 126.00 (C-4‴′), 125.25 (C-6‴′) 120.51 (C-5‴′), 61.44 (C-2), 60.02 (C-1″), 52.82 & 52.71 (C-2′, C-3′, C-5′ & C-6′), 20.10 (3-CH_3), 13.33 (2-CH_3); Anal. Calc. for $C_{23}H_{29}N_3O$ (363.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.79; H, 7.94; N, 11.46; EI-MS: m/z 363 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 148 $[C_9H_{10}NO]^+$, 117 $[C_9H_9]^+$, 105 $[C_8H_9]^+$.

N-(2,4-dimethylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5h)

Light yellow solid, Yield 81-82%, m.p. 127-129°C, $C_{23}H_{29}N_3O$, Mol. Mass: 363 $gmol^{-1}$; IR (KBr, cm^{-1}) ν: 3441 (N-H, str.), 3069 (C-H, str. of aromatic ring), 2940 (C-H, aliphatic str.), 1643 (C=C, aromatic str.), 1654 (C=O str.), 1648 (C=C, alkene str.), 1330 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.28 (s, 1H, -NHCO), 7.71 (br.d, J = 8.1 Hz, 1H, H-6‴′), 7.43 (br.d, J = 7.4 Hz, 2H, H-2‴& H-6‴), 7.31 (br.t, J = 7.5 Hz, 2H, H-3‴ & H-5‴), 7.22 (br.t, J = 7.3 Hz, 1H, H-4‴), 7.01 (br.s, 1H, H-3‴′), 6.97 (br.d, J = 8.1 Hz, 1H, H-5‴′), 6.53 (d, J = 15.9 Hz, 1H, H-3″), 6.29 (td, J = 6.8 & 15.9 Hz, 1H, H-2″), 3.11-3.10 (m, 4H, CH_2-2 & CH_2-1″), 2.87 (br.s) & 2.73 (br.s, 8H, H-2′, H-3′, H-5′ & H-6′), 2.23 (s, 3H, 4-CH_3), 2.19 (s, 3H, 2-CH_3); ^{13}C-NMR (DMSO-d_6, 150 MHz,

δ/ppm): 167.60 (C-1), 136.58 (C-1‴), 134.49 (C-1⁗), 133.08 (C-2⁗), 132.00 (C-2″), 130.68 (C-3⁗), 128.46 (C-3‴ & C-5‴), 128.31 (C-4⁗), 127.93 (C-4‴), 126.90 (C-3″), 126.59 (C-5⁗) 126.11 (C-2‴& C-6‴), 121.69 (C-6⁗), 61.44 (C-2), 60.05 (C-1″), 52.86 & 52.81 (C-2′, C-3′, C-5′ & C-6′), 20.35 (4-CH_3), 17.34 (2-CH_3); Anal. Calc. for $C_{23}H_{29}N_3O$ (363.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.81; H, 7.91; N, 11.41; EI-MS: m/z 363 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 148 $[C_9H_{10}NO]^+$, 117 $[C_9H_9]^+$, 105 $[C_8H_9]^+$.

N-(2,5-dimethylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5i)

White solid, Yield 85-86%, m.p. 122-124°C, $C_{23}H_{29}N_3O$, Mol. Mass: 363 $gmol^{-1}$; IR (KBr, cm^{-1}) ν: 3453 (N-H, str.), 3057 (C-H, str. of aromatic ring), 2907 (C-H, aliphatic str.), 1637 (C=C, aromatic str.), 1654 (C=O str.), 1657 (C=C, alkene str.), 1321 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.31 (s, 1H, -NHCO), 7.68 (s, 1H, H-6⁗), 7.44 (d, J = 7.4Hz, 2H, H-2‴& H-6‴), 7.32 (br.t, J = 7.5 Hz, 2H, H-3‴ & H-5‴), 7.23 (br.t, J = 7.3 Hz, 1H, H-4‴), 7.08 (d, J = 7.6 Hz, 1H, H-3⁗), 6.84 (br.d, J = 7.44 Hz, 1H, H-4⁗), 6.54 (td, J = 15.9 Hz, 1H, H-3″), 6.31 (td, J = 6.6 &15.9 Hz, 1H, H-2″), 3.11 (d, 2H, CH_2-1″), 3.10 (s, 2H, CH_2-2), 2.59-2.50 (m, 8H, H-2′, H-3′, H-5′ & H-6′), 2.24 (s, 3H, 5-CH_3), 2.18 (s, 3H, 2-CH_3); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 167.66 (C-1), 136.54 (C-1‴), 135.77 (C-5⁗), 135.19 (C-1⁗), 131.94 (C-2″), 129.93 (C-4⁗), 128.45 (C-3‴ & C-5‴), 127.28 (C-4‴), 126.95 (C-3″), 126.08 (C-2‴ & C-6‴), 125.12 (C-2⁗), 124.70 (C-3⁗) 122.03 (C-6⁗), 61.44 (C-2), 60.01 (C-1″), 52.81 & 52.79 (C-2′, C-3′, C-5′ & C-6′), 20.69 (5-CH_3), 16.94 (2-CH_3); Anal. Calc. for $C_{23}H_{29}N_3O$ (363.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.76; H, 7.99; N, 11.51; EI-MS: m/z 363 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 148 $[C_9H_{10}NO]^+$, 117 $[C_9H_9]^+$, 105 $[C_8H_9]^+$.

N-(2,6-dimethylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5j)

White solid, Yield 75-76%, m.p. 159-160°C, $C_{23}H_{29}N_3O$, Mol. Mass: 363 $gmol^{-1}$; IR (KBr, cm^{-1}) ν: 3441 (N-H, str.), 3065 (C-H, str. of aromatic ring), 2906 (C-H, aliphatic str.), 1632 (C=C, aromatic str.), 1652 (C=O str.), 1648 (C=C, alkene str.), 1321 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.15 (s, 1H, -NHCO), 7.43 (d, J = 7.0 Hz, 2H, H-2‴& H-6‴), 7.32 (br.t, J = 6.9 Hz, 2H, H-3‴ & H-5‴), 7.23 (br.t, J = 6.9 Hz, 1H, H-4‴), 7.06 (br.s, 3H, H-3⁗, H-4⁗ & H-5⁗), 6.54 (br.d, J = 15.9 Hz, 1H, H-3″), 6.31 (td, J = 6.6 & 15.8 Hz, 1H, H-2″), 3.12 (d, 2H, CH_2-1″), 3.11 (s, 2H, CH_2-2), 2.58-2.50 (m, 8H, H-2′, H-3′, H-5′ & H-6′), 2.13 (s, 6H, 2-CH_3 & 6-CH_3); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 167.84 (C-1), 136.56 (C-1‴), 134.98 (C-1⁗), 134.89 (C-2⁗ & C-6⁗), 131.87 (C-2″), 128.44 (C-3‴ & C-5‴), 127.50 (C-3⁗ & C-5⁗), 127.26 (C-4‴), 127.03 (C-3″), 127.26 (C-2‴& C-6‴), 126.22 (C-4⁗), 61.35 (C-2), 60.05 (C-1″), 53.01 & 52.38 (C-2′, C-3′, C-5′ & C-6′), 18.13 (2-CH_3 & 6-CH_3); Anal. Calc. for $C_{23}H_{29}N_3O$ (363.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.98; H, 7.91; N, 11.47; EI-MS: m/z 363 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 148 $[C_9H_{10}NO]^+$, 117 $[C_9H_9]^+$, 105 $[C_8H_9]^+$.

N-(3,4-dimethylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5k)

Light yellow solid, Yield 75-77%, m.p. 133-135°C, $C_{23}H_{29}N_3O$, Mol. Mass: 363 $gmol^{-1}$; IR (KBr, cm^{-1}) ν: 3444 (N-H, str.), 3064 (C-H, str. of aromatic ring), 2904 (C-H, aliphatic str.), 1629 (C=C, aromatic str.), 1652 (C=O str.), 1656 (C=C, alkene str.), 1321 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.49 (s, 1H, -NHCO), 7.44 (br. d, J = 7.4 Hz, 2H, H-2‴& H-6‴), 7.36 (br.s, 1H, H-2⁗), 7.33 (dis.dd, J = 1.0 & 8.0 Hz, 1H, H-6⁗), 7.30 (br.t, J = 7.5 Hz, 2H, H-3"' & H-5‴), 7.22 (br.t, J = 7.3 Hz, 1H, H-4‴), 7.03 (br.d, J = 8.0 Hz, 1H, H-5⁗), 6.52 (d, J = 15.9 Hz, 1H, H-3″), 6.30 (td, J = 6.5 & 15.7 Hz, 1H, H-2″), 3.11 (br.d, J = 7.0 Hz, 2H, CH_2-1''), 3.08 (s, 2H, CH_2-2), 2.88 (br.s) & 2.73 (br.s, 8H, H-2′, H-3', H-5′ & H-6′), 2.19 (s, 3H, 4-CH_3), 2.15 (s, 3H, 3-CH_3); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 167.84 (C-1), 136.63 (C-1‴), 136.21 (C-1⁗), 132.28 (C-3⁗), 131.84 (C-2⁗), 131.12 (C-2″), 129.74 (C-4⁗), 128.51 (C-3‴ & C-5‴), 127.33 (C-4‴), 127.09 (C-3″), 126.15 (C-2‴& C-6‴), 120.56 (C-5⁗), 116.85 (C-6⁗), 61.76 (C-2), 60.12 (C-1″), 52.81 & 52.62 (C-2′, C-3′, C-5′ & C-6′), 19.55 (CH_2-3), 18.72 (CH_3-4); Anal. Calc. for $C_{23}H_{29}N_3O$ (363.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.97; H, 7.94; N, 11.42; EI-MS: m/z 363 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 148 $[C_9H_{10}NO]^+$, 117 $[C_9H_9]^+$, 105 $[C_8H_9]^+$.

N-(3,5-dimethylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5l)

Light yellow solid, Yield 83%, m.p. 151-153°C, $C_{23}H_{29}N_3O$, Mol. Mass: 363 $gmol^{-1}$; IR (KBr, cm^{-1}) ν: 3358 (N-H, str.), 3187 (C-H, str. of aromatic ring), 2947 (C-H, aliphatic str.), 1648 (C=C, aromatic str.), 1654 (C=O str.), 1677(C=C, alkene str.), 1348 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.4 (s, 1H, -NHCO), 7.44 (br.d, J = 7.3 Hz, 2H, H-2‴& H-6‴), 7.32 (br.t, J = 7.5 Hz, 2H, H-3‴ & H-5‴), 7.25 (br.s, 1H, H-2⁗), 7.23 (br.t, J = 7.3 Hz, 1H, H-4‴), 6.91 (br.s, 1H, H-6⁗), 6.69 (br.s, 1H, H-4⁗), 6.54 (d, J = 15.9 Hz, 1H, H-3″), 6.30 (td, J = 6.6 & 15.9 Hz, 1H, H-2″), 3.12 (br.d, J = 6.42 Hz, 2H, CH_2-1″), 3.0 (s, 2H, CH_2-2), 2.88 (br.s) & 2.73 (br.s, 8H, H-2′, H-3′, H-5′ & H-6′), 2.23 (s, 6H, 3-CH_3 & 5-CH_3); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 167.98

(C-1), 138.31 (C-1′′′′), 137.62 (C-3′′′′ & C-5′′′′), 136.62 (C-1′′′), 132.02 (C-2′′), 128.51 (C-3′′′ & C-5′′′), 127.34 (C-4′′′), 126.97 (C-3′′), 126.51 (C-2′′′& C-6′′′), 124.87 (C-4′′′′), 117.06 (C-2′′′′ & C-6′′′′) 61.73 (C-2), 60.06 (C-1′′), 52.74 & 52.51 (C-2′, C-3′, C-5' & C-6′), 21.00 (3-CH_3 & 5-CH_3); Anal. Calc. for $C_{23}H_{29}N_3O$ (363.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.87; H, 7.95; N, 11.42; EI-MS: m/z 363 $[M]^+$, 215 $[C_{14}H_{19}N_2]^+$, 148 $[C_9H_{10}NO]^+$, 117 $[C_9H_9]^+$, 105 $[C_8H_9]^+$.

N-(2-ethyl-6-methylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-perazinyl}acetamides (5m)

Light pink solid, Yield 92-93%, m.p. 136-138°C, $C_{24}H_{31}N_3O$, Mol. Mass: 377 $gmol^{-1}$; IR (KBr, cm^{-1}) ν: 3448 (N-H, str.), 3008 (C-H, str. of aromatic ring), 2955 (C-H, aliphatic str.), 1633 (C=C, aromatic str.), 1684 (C=O str.), 1657 (C=C, alkene str.), 1323 (C-N aromatic str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 9.28 (s, 1H, -NHCO), 7.44 (br.d, J = 7.3 Hz, 2H, H-2′′′& H-6′′′), 7.32 (br.t, J = 7.5 Hz, 2H, H-3′′′ & H-5′′′), 7.23 (t, J = 7.3 Hz, 1H, H-4′′′), 7.12 (dd, J = 8.2 & 8.2 Hz, 1H, H-4′′′′), 7.07 (dis.d, J = 7.5 Hz, 2H, H-3′′′′ & H-5′′′′), 6.6 (d, J = 15.9 Hz, 1H, H-3′′), 6.31 (td, J = 6.6 & 15.9 Hz, 1H, H-2′′), 3.15-3.13 (m, 2H, CH_2-2 & CH_2-1′′), 2.88 (br.s) & 2.73 (br.s, 8H, H-2′, H-3′, H-5′ & H-6′), 2.50 (q, J = 7.5 Hz, 2H, CH_2CH_3-2), 2.13 (s, 3H, 6-CH_3), 1.08 (t, J = 7.5Hz, 5H, CH_2CH_3-2); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): 168.23 (C-1), 140.82 (C-1′′′′), 136.52 (C-1′′′), 135.45 (C-2′′′′), 134.29 (C-4′′′′), 132.17 (C-2′′), 128.45 (C-3′′′ & C-5′′′), 127.84 (C-3′′′′), 127.54 (C-6′′′′), 127.32 (C-4′′′), 126.59 (C-3′′), 126.12 (C-2′′′& C-6′′′), 125.84 (C-5′′′′), 61.23 (C-2), 59.94 (C-1′′), 52.84 & 52.34 (C-2′, C-3′, C-5′ & C-6′), 24.39 (CH_2-2), 18.19 (CH_3-6), 14.52 (CH_3-2); Anal. Calc. for $C_{24}H_{31}N_3O$ (377.25): C, 76.35; H, 8.28; N, 11.13. Found: C, 76.27; H, 8.21; N, 11.09; EI-MS: m/z 377 $[M]^+$, 215 $[C_{14}H_{19}N_2]$+, 162 $[C_{10}H_{12}NO]^+$, 119 $[C_9H_{11}]^+$, 117 $[C_9H_9]^+$.

3.5. Assessment of bacterial biofilm inhibition

The microtiter-plate method was used for the assessment of the inhibition of bacterial (*Bacillus subtilis/Escherichia coli*) biofilm formation, as described in [11,12]. The 24-well flat-bottomed plastic tissue culture plates of a sterile were filled with 100 μL of nutrient broth (Oxoid, UK). Concentration, which was 1.0 μg of the testing sample (dissolved in 1 mL of DMSO), was added in different wells. At last, 20 μL of the bacterial suspension containing 1×10^9 CFU/mL was inoculated. The well of positive control contained ampicillin and nutrient broth (Oxoid, UK), whereas the well of negative control contained nutrient broth and microbial strain. Afterwards, plates were covered and aerobically incubated for 24 hours at 37°C. Subsequently, by applying sterile phosphate buffer (pH: 7.2) of 220 μL, the contents of each well were beheld thrice. Plates were vigorously shaken to remove all non-adherent bacteria. Then, the bacteria attached to plates were fixed with 220 mL of 99% methanol per well. After every 15 min, the plates were emptied and left to dry. Then, by using 220 mL of 50% crystal violet per well, the plates were stained for 5 min. Surplus stain was rinsed with distilled water. Then, plates were re-solubilized with 220 μL of 33% (v/v) glacial acetic acid per well after air-dried and the bound dye. By using a 630 nm microplate reader (Biotek, USA), the Optical Density (OD) of each well was measured. Against selected bacterial strains, all the tests were carried out thrice, and the results were averaged. The bacterial growth inhibition (inhibition%) was calculated through the following formula:

$$\text{Inhibition}\% = 100 - \frac{(OD_{630 \text{ of sample}} \times 100)}{OD_{630 \text{ control}}}.$$

3.6. Hemolytic activity

Bovine blood samples were collected in EDTA, diluted with saline (0.9% NaCl), and centrifuged at 1000xg for 10 min. The separated erythrocytes were diluted in phosphate buffer saline of pH 7.4, and a suspension was made. Then, 20 μL of the synthetic compound solution (10 mg/mL) in 180 μL of RBCs suspension was added and incubated for 30 min at room temperature. PBS was used as negative control and Triton 100-X was taken as positive control [13,14]. The %age of hemolysis was taken through the formula:

$$(\%)\text{of hemolysis} = \frac{\text{absorbance of sample} - \text{absorbance of negative control}}{\text{absorbance of positive control}} \times 100.$$

Supplementary Information

Supplementary Information is available at:
http://scientiairanica.sharif.edu/jufile?ar_sfile=123581

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