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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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 aqueous basic medium to acquire different electrophiles, 3a-m, with good yields. These electrophiles were 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.

KEYWORDS
1-[(E)-3-Phenyl-2-propenyl]piperazine; Bromoacetyl bromide; Amides; Biofilm inhibition; Hemolysis.
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 piperazine ring confer bioactivity to molecules and enhance favorable interaction with macromolecules. Slight change in substitution pattern in piperazine nucleus causes 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 as well as 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 products or prodrugs [8]. The stable and polar amide functionality is an important unit among the organic 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 in continuation of our previous effort on antibacterial evaluation of piperazine-acetamides [10], hereby, we report the bacterial biofilm inhibition of a new series of piperazine-acetamides with the rationale that these molecules might overcome the overwhelming resistance of some microbes and can find consideration 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 were 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$_2$CO$_3$ solution at room temperature. The reactions were accomplished only by vigorous stirring which resulted in the formations 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.

(Scheme 1 and Table 1 here)

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 yield of 91% and melting point of 146-148 °C. Its molecular formula, C$_{22}$H$_{27}$N$_3$O was ascribed by its CHN and EI-MS analysis data. The count of the number of protons in its $^1$H-NMR spectrum and carbon resonances in its $^{13}$C-NMR spectrum was also in agreement with its molecular formula. Presence of different functional group was ascertained by its IR spectral data. The absorption band at $\nu$ 3419 cm$^{-1}$ was characteristic of N-H stretching. The other bands were observed at $\nu$ 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.), 1301 (C-N aromatic str.) cm$^{-1}$. In $^1$H-NMR spectrum (Figure S1a), its aromatic
region (Figure S1b) 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 resonance in aromatic region. These three discrete resonances were observed at δ 7.83 (br.d, J = 7.9 Hz, 1H, H-6''), δ 7.17 (br.t, J = 7.6 Hz, 1H, H-5''''), δ 7.04 (br.t, J = 7.4 Hz, 1H, H-4''') while a singlet at δ 2.24 was characteristic for a methyl substituent (CH₃). The 4-(E)-3-phenyl-2-propenyl moiety was ascertained by two resonances in 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 (CH₂-1') in 2-propenyl unit was merged as multiplet with the signal of acetamidic methylene (CH₂-2) at δ 3.15-3.13 (m, 4H). However, the trans disposition of two methine protons in 2-propenyl unit was clearly indicated by the 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 ¹³C-NMR spectrum (Figure S2), of this molecule also fully corroborated the subsistence of these moieties. Whereby, 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 phenyl ring along with a signal at δ 14.58 for methyl substituent attached at the 2-position (CH₃-2). The acetamidic group was inferred clearly by two peaks at δ 167.81 (C-1) and δ 61.46 (C-2). The phenyl ring in 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 $\delta$ 59.96 (C-1"), 128.46 (C-2") and 126.23 (C-3"). The symmetrical 1,4-piperazinyl heterocycle was attributed with an overlapped signal at $\delta$ 52.76 (C-2', C-3', C-5' & C-6'). So, on account of aforementioned evidences, the structure $5b$ was named as $N$-(2-methylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-pedazinyl}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 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.

Although the observed antibacterial potential is the resultant of a whole molecule, but a limited structure-activity relationship (SAR) was rationalized by analyzing the effect of different aryl part on the bacterial biofilm inhibition. Figure 1 displayed the general structural features of the synthetic compounds.

(Figure 1 here)

Compound $5a$ was having an un-substituted phenyl ring showed least activity against $B. subtilis$ (5.52) and poor activity against $E. coli$ (31.47). The presence of a methyl group on phenyl ring (aryl part) in $5b$ as well as in $5c$ enhanced their antibacterial activity, relative to $5a$, against both strains. However, a better antibacterial potential was observed, when the methyl group was present at 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 2-position. (Figure
2). It means that in compound **5c** when a small sized group was present at \( m \)-position it was behaving as a better antibacterial agent.

(Figure 2 here)

A reverse trend was observed when a medium sized ethyl group was present at ortho position of phenyl ring in **5d**. In fact, this compound was best antibacterial agent (66.77%) among the synthetic series against *B. subtilis* and also exhibited a promising antibacterial potential against *E. coli* (63.15%). The presence of an additional methyl group at 6-position in **5m** enhanced its activity against *E. coli* (66.77%) and it behaved as 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. It was inferred from the results a medium sized group at ortho-position of phenyl ring was going to render a promising antibacterial potential to the molecule relative to other synthetic analogues (Figure 3).

(Figure 3 here)

Among the di-methylated regio-isomers, two compounds **5g** and **5i**, showed very moderate and much resembling antibacterial, (*B. subtilis*; 43.42% & *E. coli*; 43.79%), and (*B. subtilis*; 45.75% & *E. coli*; 41.61%) respectively. The methyl groups were present at 2 and 3-position in **5g** while at 2 and 5-position in **5i**. Compound **5j** with symmetrical di-ortho methyl groups at 2 and 6-position possessed very weak antibacterial potential (*B. subtilis*; 9.24% & *E. coli*; 21.33%) but the isomer **5h** having methyl groups at 2 and 4-position displayed excellent antibacterial potential. Indeed, this compound was most active (71.95%) among the whole series against *E. coli* and was the second most active (63.06%) against *B. subtilis*. It means, when
methyl groups are at *ortho* and *para* position, these impart better antibacterial potential to the molecule (Figure 4).

(Figure 4 here)

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

(Figure 5 here)

So, it was inferred from the structure-activity relationship that two molecules, one with an *ortho*-ethyl group ($5d$) and other with *ortho* & *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 while that of *Escherichia coli* biofilm has been given in Figure S4.

(Table 2 here)

2.3. Hemolytic activity

All the synthesized compounds were subjected to hemolytic assay to find out their cytotoxicity profile. Results of percentage hemolysis are shown in Table 2 which indicated 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 the Triton-X (positive control) having %hemolysis of 89%.
2.4. Conclusion

In conclusion, a series of \(1\-[(E)-3\text{-phenyl-2\text{-propenyl}]piperazine\) derivatives has been synthesized successfully and evaluated for their biofilm inhibition against two pathogenic bacterial strains. SAR studies were carried out to investigate the role of various groups attached to the phenyl ring which exert 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 \textit{ortho}-ethyl group (5d) and other with \textit{ortho} & \textit{para}-methyl groups (5h), were explored as suitable antibacterial agents against both 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 solvent system (20:80). Spots were detected by UV\(_{254}\). Gallonkamp apparatus was used to detect melting points (uncorrected) in capillary tubes. IR spectra (\(\nu\), cm\(^{-1}\)) were recorded by KBr pellet method in the Jasco-320-A spectrophotometer. Elemental analyses were performed on a Foss Heraeus CHN-O-Rapid instrument and were within \(\pm 0.4\%\) of the theoretical values. EI-MS spectra were measured on a JEOL JMS-600H instrument with data processing system. \(^1\)H-NMR spectra (\(\delta\), ppm) were recorded at 600 MHz \((^{13}\text{C-NMR spectra, at 150 MHz})\) 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 \((\delta)\) in ppm. The abbreviations used in 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 mole) of various anilines (1a-m, one in each reaction) were added in round bottom flask with distilled water at room temperature and stirred for 30 minutes. 10% Aqueous Na$_2$CO$_3$ solution was added in the reaction mixture to adjust pH to 9-10. Gradually 0.001 moles of bromoacetyl bromide (2) were added in reaction mixture. The completion of reaction was monitored by TLC. HCl was added drop wise to make pH to 5 till 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 aforementioned 2-bromo-N-(substituted-phenyl)acetamides (3a-m, one in each reaction), was added to the mixture and 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_{3}O, Mol. Mass: 335 g mol^{-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''), 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'''), 126.49 (C-3''), 126.08 (C-2'' & C-6''), 123.25 (C-4'''), 119.28 (C-2''' & 6'''); Anal. Calc. for C\(_{21}\)H\(_{25}\)N\(_3\)O (335.20): C, 75.19; H, 7.51; N, 12.53. Found: C, 74.96; H, 7.40; N, 12.36; El-MS: m/z 335 [M]+, 215 [C\(_{14}\)H\(_{19}\)N\(_2\)]+, 120 [C\(_7\)H\(_6\)NO]+, 117 [C\(_9\)H\(_9\)]+, 77 [C\(_9\)H\(_3\)]+.

**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\(_3\)O, Mol. Mass: 349 g mol^{-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, CH2-2 & CH2-1”), 2.88 (br.s) & 2.73 (br.s, 8H, H-2’, H-3’, H-5’ & H-6’), 2.24 (s, 3H, 2-CH3); 13C-NMR (DMSO-d6, 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’”), 116.30 (C-2’”, C-3’, C-4’ & C-6’), 14.58 (2-CH3); Anal. Calc. for C22H27N3O (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 [C14H19N2]+, 134 [C8H8NO]+, 117 [C9H9]+, 91 [C7H7]+.

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

Light yellow solid, Yield 88%, m.p. 143-144 ºC, C22H27N3O, Mol. Mass: 349 gmol⁻¹; IR (KBr, cm⁻¹): 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.); 1H-NMR (600 MHz, DMSO-d6, 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, CH2-2 & CH2-1”), 2.88 (br.s) & 2.73 (br.s, 8H, H-2’, H-3’, H-5’& H-6’), 2.2 (s, 3H, 3-CH3); 13C-NMR (DMSO-d6, 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-CH2); Anal. Calc. for C22H27N3O (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 [C14H19N2]+, 134 [C8H8NO]+, 117 [C9H9]+, 91 [C7H7]+.
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_{3}O, Mol. Mass: 363 gmol^{-1}; IR (KBr, cm^{-1}) v: 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.); ^1H-NMR (600 MHz, DMSO-d$_6$, δ/ppm): 9.45 (s, 1H, -NHCO), 7.85 (br.d, J = 7.6 Hz, 1H, H-3''), 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-6''), 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); ^13C-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$_3$O (363.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.96; H, 8.01; N, 11.43; El-MS: m/z 363 [M]$^+$, 215 [C$_{14}$H$_{19}$N$_2$]$^+$, 148 [C$_9$H$_{10}$NO]$^+$, 117 [C$_9$H$_9$]$^+$, 105 [C$_9$H$_8$]$^+$.

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$_3$O, Mol. Mass: 363 gmol^{-1}; IR (KBr, cm^{-1}) v: 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.); ^1H-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'').
(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$_2$CH$_3$-4), 1.13 (t, J = 7.5 Hz, 3H, CH$_2$CH$_3$-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-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$_3$O (362.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.84; H, 7.96; N, 11.33; El-MS: m/z 363 [M$^+$], 215 [C$_{14}$H$_{19}$N$_2$]$^+$, 148 [C$_9$H$_{10}$NO]$^+$, 117 [C$_9$H$_9$]$^+$, 105 [C$_8$H$_9$]$^+$.

$N$-(4-Ethoxylphenyl)-2-{4-[(E)-3-phenyl-2-propenyl]-1-pyrazinyl}acetamides (5f)

Light Brown solid, Yield 87%, m.p. 166-168 °C, C$_{23}$H$_{29}$N$_3$O$_2$, 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$_2$O-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$_2$O-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_{3}O_{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_{9}H_{10}NO_{2}]^+, 117 [C_{9}H_{9}]^+, 121 [C_{8}H_{9}O]^+.

_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_{3}O, Mol. Mass: 363 g mol⁻¹; IR (KBr, cm⁻¹) v: 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.), 1343 (C-N aromatic str.); 

1H-NMR (600 MHz, DMSO-d₆, 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 = 7.3 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, CH₂-2), 3.12 (dis.d, J = 6.6 Hz, 2H, CH₂-1'''), 2.59-2.50 (m, 8H, H-2', H-3', H-5' & H-6'), 2.25 (s, 3H, 2-CH₃), 2.10 (s, 3H, 3-CH₃); 13C-NMR (DMSO-d₆, 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-CH₃), 13.33 (2-CH₃); Anal. Calc. for C_{23}H_{29}N_{3}O (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_{9}H_{10}NO]^+, 117 [C_{9}H_{9}]^+, 105 [C_{8}H_{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_{3}O, Mol. Mass: 363 g mol⁻¹; IR (KBr, cm⁻¹) v: 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'''), 7.01 (br., 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'''), 128.00 (C-2''), 130.68 (C-3''''), 128.46 (C-3''' & C-5'''), 128.31 (C-4'''), 128.11 (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$_3$O (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$_9$H$_{10}$NO]$^+$, 117 [C$_9$H$_9$]$^+$, 105 [C$_8$H$_9$]$^+$.

$N$-(2,5-Dimethylphenyl)-2-[(E)-3-phenyl-2-propenyl]-1-perazinylacetamides (5i)

White solid, Yield 85-86%, m.p. 122-124 °C, C$_{23}$H$_{29}$N$_3$O, Mol. Mass: 363 g mol$^{-1}$; 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, $J$ = 7.4 Hz, 2H, H-2'' & H-6''), 7.32 (br.t, $J$ = 7.4 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, $J$ = 7.4 Hz, 1H, H-4''), 6.54 (td, $J$ = 15.9 Hz, 1H, H-5''), 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-CH₃), 16.94 (2-CH₃);


**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, C₂₃H₂₉N₃O, Mol. Mass: 363 g mol⁻¹; IR (KBr, cm⁻¹) \(\nu\): 3441 (N-H, str.), 3065 (C-H, str. of aromatic ring), 2906 (C-H, aliphatic str.), 1632 (C=C, aromatic str.), 1648 (C=C, alkene str.), 1321 (C-N aromatic str.);

\(^1\)H-NMR (600 MHz, DMSO-d₆, \(\delta/\text{ppm}\)): 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, CH₂-1'), 3.11 (s, 2H, CH₂-2'), 2.58-2.50 (m, 8H, H-2', H-3', H-5' & H-6'), 2.13 (s, 6H, 2-CH₃ & 6-CH₃); \(^{13}\)C-NMR (DMSO-d₆, 150 MHz, \(\delta/\text{ppm}\)): 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-CH₃ & 6-CH₃); Anal. Calc. for C₂₃H₂₉N₃O (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 [C₁₄H₁₉N₂]+, 148 [C₉H₁₀NO]+, 117 [C₉H₉]⁺, 105 [C₈H₉]⁺.
**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_{3}O, Mol. Mass: 363 g\text{mol}^{-1};

IR (KBr, cm\(^{-1}\)) \(\nu\): 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\(_6\), \(\delta/\text{ppm}\)):

\(9.49\) (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, \(\delta/\text{ppm}\)):

\(167.84\) (C-1), \(136.63\) (C-1''), \(136.21\) (C-1'''), \(131.84\) (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\(_3\)O (363.23): C, 79.00; H, 8.04; N, 11.56. Found: C, 78.97; H, 7.94; N, 11.42; El-MS: \(m/z\) 363 [M]+, 215 [C\(_{14}\)H\(_{19}\)N\(_2\)]+, 148 [C\(_9\)H\(_{10}\)NO]+, 117 [C\(_9\)H\(_9\)]+, 105 [C\(_8\)H\(_9\)]+.

**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\(_3\)O, Mol. Mass: 363 g\text{mol}^{-1}; IR (KBr, cm\(^{-1}\)) \(\nu\): 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\(_6\), \(\delta/\text{ppm}\)):

\(9.4\) (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, CH2-1''), 3.0 (s, 2H, CH2-2''), 2.88 (br.s) & 2.73 (br.s, 8H, H-2', H-3', H-5' & H-6'), 2.23 (s, 6H, 3-CH3 & 5-CH3); 13C-NMR (DMSO-d6, 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-CH3 & 5-CH3); Anal. Calc. for C23H29N3O (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 [C14H19N2]+, 148 [C9H10NO]+, 117 [C9H9]+, 105 [C8H9]+.

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, C24H31N3O, Mol. Mass: 377 gmol⁻¹; IR (KBr, cm⁻¹) v: 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.); 1H-NMR (600 MHz, DMSO-d6, 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, CH2-2 & CH2-1''), 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, CH2CH3-2), 2.13 (s, 3H, 6-CH3), 1.08 (t, J = 7.5Hz, 5H, CH2CH3-2); 13C-NMR (DMSO-d6, 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 (CH2-
2), 18.19 (CH$_3$-6), 14.52 (CH$_3$-2); Anal. Calc. for C$_{24}$H$_{31}$N$_3$O (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$_9$H$_{11}$]$^+$, 117 [C$_9$H$_9$]$^+$.

3.5. Assessment of bacterial biofilm inhibition

Microtiter-plate method was used for the assessment of the inhibition of bacterial (Bacillus subtilis/Escherichia coli) biofilm formation as described [11,12]. The 24-well flat bottomed plastic tissue culture plate wells of a sterile were filled with 100 $\mu$L of nutrient broth (Oxoid, UK). Concentration, which was 1.0 $\mu$g of the testing sample (dissolved in 1 mL of DMSO), were added in different wells. At last, 20 $\mu$L of the bacterial suspension containing 1$\times$10$^9$ CFU/mL was inoculated. The well of positive control was contained with Ampicillin and nutrient broth (Oxoid, UK) whereas the well of negative control well contained nutrient broth and microbial strain. After that, plates were covered and aerobically incubated for 24 hours at 37 $^\circ$C. Subsequently, using sterile phosphate buffer (pH: 7.2) of 220 $\mu$L the contents of each well were beheld thrice. Plates were vigorously shaken to remove all non-adherent bacteria. Then the bacteria which attached on 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 pates were stained for 5 min. Surplus stain was rinsed of using distilled water. Then plates were re-solubilized with 220 $\mu$L of 33% (v/v) glacial acetic acid per well after air-dried and the bound dye. By using 630 nm microplate reader (Biotek, USA) the optical density (OD) of each well was measured. Against selected bacterial strains all the tests were carried thrice and the results were averaged. The bacterial growth inhibition (Inhibition %) was calculated using the following formula.
Inhibition % = 100 − \( \frac{(OD_{630 \text{ of sample}} × 100)}{OD_{630 \text{ control}}} \)
\( \frac{(OD_{630 \text{ of sample}} × 100)}{OD_{630 \text{ control}}} \)

3.6. Hemolytic activity

Bovine blood samples was collected in EDTA that was diluted with saline (0.9% NaCl), and centrifuge at 1000xg for 10 min. The erythrocytes separated diluted in phosphate buffer saline of pH 7.4 and a suspension was made. Add 20 µL of synthetic compounds solution (10 mg/mL) in 180 µL of RBCs suspension and incubate 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 as by using 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:
References


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

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

**Table 1.** Different groups (-R\(_1\) & -R\(_2\)) in scheme 1.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>-R(_1)</th>
<th>-R(_2)</th>
<th>Compd.</th>
<th>-R(_1)</th>
<th>-R(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a, 3a, 5a</td>
<td>-H</td>
<td>-H</td>
<td>1g, 3g, 5g</td>
<td>2-CH(_3)</td>
<td>3-CH(_3)</td>
</tr>
<tr>
<td>1b, 3b, 5b</td>
<td>2-CH(_3)</td>
<td>-H</td>
<td>1h, 3h, 5h</td>
<td>2-CH(_3)</td>
<td>4-CH(_3)</td>
</tr>
<tr>
<td>1c, 3c, 5c</td>
<td>3-CH(_3)</td>
<td>-H</td>
<td>1i, 3i, 5i</td>
<td>2-CH(_3)</td>
<td>5-CH(_3)</td>
</tr>
<tr>
<td>1d, 3d, 5d</td>
<td>2-CH(_2)CH(_3)</td>
<td>-H</td>
<td>1j, 3j, 5j</td>
<td>2-CH(_3)</td>
<td>6-CH(_3)</td>
</tr>
<tr>
<td>1e, 3e, 5e</td>
<td>4-CH(_2)CH(_3)</td>
<td>-H</td>
<td>1k, 3k, 5k</td>
<td>3-CH(_3)</td>
<td>4-CH(_3)</td>
</tr>
<tr>
<td>1f, 3f, 5f</td>
<td>4-OC(_2)H(_5)</td>
<td>-H</td>
<td>1l, 3l, 5l</td>
<td>3-CH(_3)</td>
<td>5-CH(_3)</td>
</tr>
<tr>
<td>1m, 3m, 5m</td>
<td>2-CH(_2)CH(_3)</td>
<td></td>
<td></td>
<td></td>
<td>6-CH(_3)</td>
</tr>
</tbody>
</table>

**Figure-1:** General structural features of compounds 5a-m.
Figure 2: Structure-activity relationship of compounds 5a, 5b, and 5c.

\[
\begin{align*}
5a & \quad \text{B. subtilis} = 5.52\% \quad \text{E. coli} = 31.47\% \\
5b & \quad \text{B. subtilis} = 14.65\% \quad \text{E. coli} = 58.80\% \\
5c & \quad \text{B. subtilis} = 54.35\% \quad \text{E. coli} = 62.63\%
\end{align*}
\]

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

\[
\begin{align*}
5d & \quad \text{B. subtilis} = 66.77\% \quad \text{E. coli} = 63.15\% \\
5m & \quad \text{B. subtilis} = 56.79\% \quad \text{E. coli} = 66.77\% \\
5e & \quad \text{B. subtilis} = 25.05\% \quad \text{E. coli} = 7.14\% \\
5f & \quad \text{B. subtilis} = 13.69\% \quad \text{E. coli} = 26.29\%
\end{align*}
\]
**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).

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

**Note:** Ampicillin was used as a positive control. Negative control (% Inhibition) = 1.021.
Biographies of authors

Muhammad Athar Abbasi secured his PhD degree in 2005 from International Center for Chemical and Biological Sciences (ICCBS), HEJ, Research Institute of Chemistry, Karachi, Pakistan. He has published more than two hundred research papers in well-reputed journals. His research papers have been acknowledged and cited by various authors. He is working in collaboration with Dr. Aziz-ur-Rehman in Organo-Pharmaceutical Research group.

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Muhammad Shahid is working as Associate Professor at Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan. He has published several research papers in valued journals. In the present study bacterial biofilm inhibition and cytotoxicity was carried out in his laboratory.