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Synthesis, antibacterial activity and docking studies of chloroacetamide derivatives

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RESEARCH ARTICLE

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ABSTRACT

Structural modification of lead compounds is a great challenge in organic synthesis. Introduction of different functional groups not only modify the structure of starting material but also improve their biological activeness. Small synthetic molecules are favored in spite of the reality that majority of drug molecules derived from natural sources, are in vogue. In the present work, acetamide derivatives were synthesized using chloroacetyl chloride. After synthesizing targeted series of acetamide derivatives these compounds were further modified using different amines including 2-aminobenzene thiol, benzyl amine, benzene 1,4-diamine, 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one, 4-aminophenol, hydrazine and 4-amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide. All of these synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and X-ray crystallography. The compounds were assessed for their anti-bacterial activity using disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*. The compounds were found to exhibit comparable activity to the standard drug used. This was further supported by molecular docking studies using bacterial *DNA gyrase* and *Topoisomerase II* targets causing bacterial death as they are major bacterial proteins known to be involved in transcription and replication process. Results proved that the compound 2b was the most efficacious antimicrobial compound among the synthesized set of compounds. To tackle the growing drug resistance acetamide based functionalities can be regarded as the active lead compounds to target different drug resistance microorganism.

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1. Introduction

In the process of drug development, synthetic chemistry plays an important role in regards to modification of already known therapeutically active compounds, to make them more safe and clinically active agents [1-5]. Halogenated organic acids and their derivatives are responsible for inhibiting the bacterial development, fungi as well as parasites and viruses [6]. Amides are considered inevitable organic compounds having a number of pertinences. The stability of amide bond derives the synthetic chemists to prepare such compounds based on this functionality. Some derivatives of amides are known to illustrate certain biological properties including their activity as antifungal [7], anticancer [8], antihistamine [9], along with their imminent properties as antibacterial [10]. Many drugs are available having amide linkage (Midodrine [11], Loracarbef [12], cefpiramide [13], procainamide [14]) as shown in Figure 1. The conjugation of chloroacetyl chloride with different amines gives acetamide linkage which acquire biological potential as insecticidal [15], antibacterial [16], antinociceptive [17], and anti-inflammatory [18].

Heterocyclic compounds have been reported previously to exhibit different biological activities [19-21] and thus, sulfonamide functionality have been found to be active against the HIV protease, 5-HTID receptor, carbonic anhydrase, antitumor, glycogen phosphorylase and cholestrolacyl transferase [22,23].

The emerging resistance shown by microorganisms to some of the already known microbial agents, compelled us to continue the synthesis of more anti-microbial substances. Keeping in view the demand and the importance of different medicinally active compound, present work based on the synthesis of acetamide derivatives of biological important moieties and screened them to evaluate their potential against bacteria. The results obtained were further supported by molecular docking studies using enzymes (*Topoisomerase II* and *DNA gyrase)* which are associated with their inhibition by ceasing bacterial replication [24,25].

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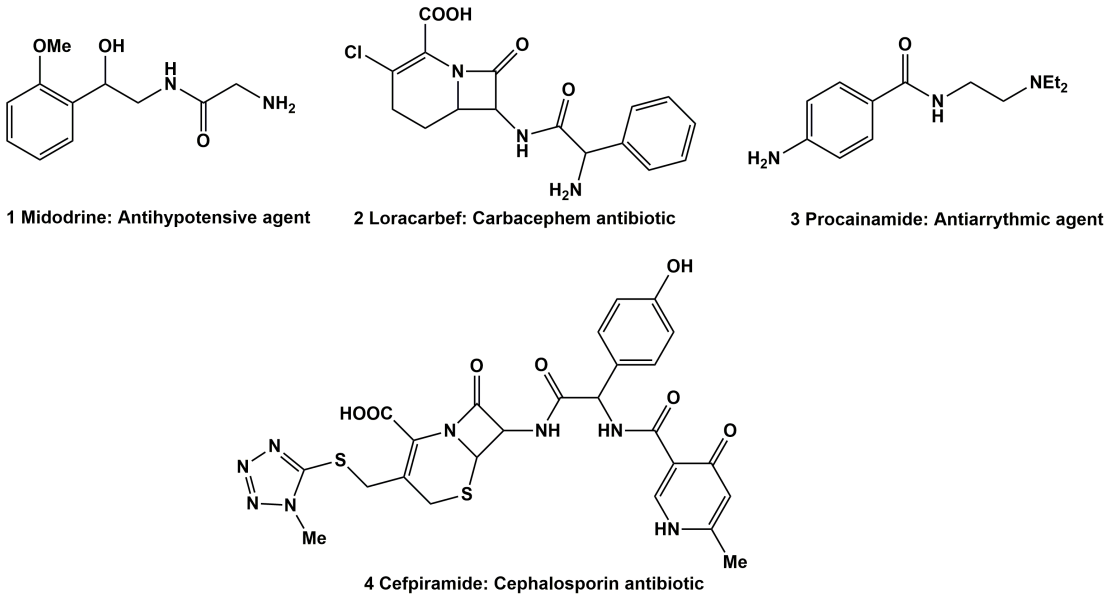


Figure 1. Drugs containing amide moiety.

2. Experimental

All the chemicals used in this research work were obtained from Sigma Aldrich and were of analytical grade. Reaction progress was determined using thin layer chromatography technique (TLC, 0.25 mm Merck silica gel plates (60 F_{254}) and visualized the spots under UV light (254 nm). Stuart melting point gadget was used to ascertain the melting points of products formed and were uncorrected. FT-IR (KBr disc) spectra of synthesized series of compounds were recorded in transmittance mode (4000-400 cm^{-1}). Bruker AVANCE NMR DMX 400 was used to determine ^{1}H- and ^{13}C-NMR spectra in DMSO-d_6. Structural analyses were performed with automatic diffractometer along with the reflection intensities collected by using Bruker kappa APEX CCD diffractrometer.

2.1. Chemistry

Chloroacetyl chloride was allowed to react with different amines as a result of which different acetamide derivatives were obtained (**1**-**8**). The corresponding acetamide derivatives were treated with a number of amines to produce respective targeted derivatives having acetamide functionality in common (**1a**-**1b**, **2a**-**2b** and **3a**-**3g**). The synthetic pathway is represented in Scheme 1 and 2 [23].

2.1.1. Syntheses of chloroacetamide derivatives (1-8)

Chloroacetamide derivatives were synthesized as shown in Scheme 1. Amines were dissolved in chloroform followed by the addition of pyridine and cooled at 0-5 °C temperature using ice-water bath. A separately prepared solution of chloroacetyl chloride in chloroform was added drop wise to above mixtures. The mixtures were stirred for 3 hours in water ice mixture and for further three hours at ambient temperature. The solvents were evaporated on rotary evaporator to obtain the products. Products were washed with water and were further crystallized in methanol.

2.1.2. Syntheses of acetamide derivatives (1a-1b, 2a-2b, 3a-3g)

The obtained chloroacetamide derivatives were dissolved in pyridine. The separately prepared solutions of amines in THF were added drop wise to these mixtures of chloroacetamide derivatives and stirred the reaction mixtures for 6-8 hours at ambient temperature. The progression of reactions was observed using TLC. Rotary evaporator was used for solvent evaporation in order to obtain the products. Products were washed and crystallized in methanol (Scheme 2).

2-Chloro-N-(2-nitrophenyl)acetamide (**1**): Yield: 81.3%. M.p.: 92-93 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3238 (NH, stretch), 1656 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.29 (2H, s, CH_2Cl), 7.20 (1H, s, NHCO), 7.73-8.40 (4H, m, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 42.1 (CH_2Cl), 125.3, 125.6, 125.9, 130.1, 131.3, 142.8 (benzene ring), 166.1 (NHCO).

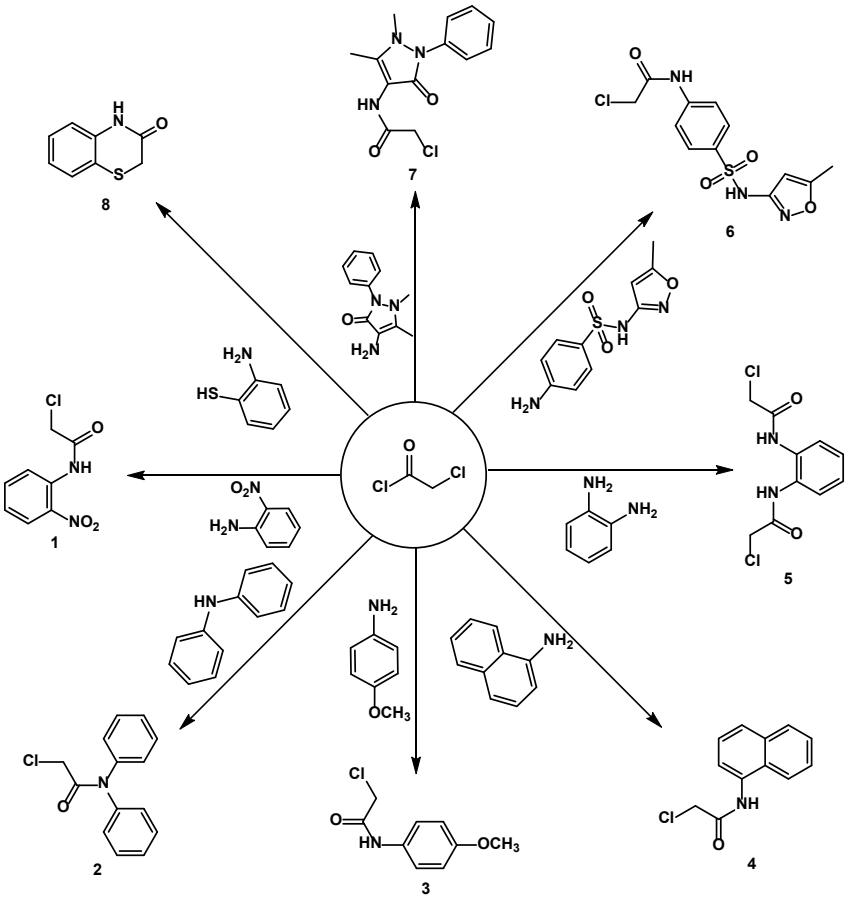
2-Chloro-N,N-diphenylacetamide (**2**): Yield: 80.4%. M.p.: 124-125 °C. FT-IR (KBr pellet, ν, cm^{-1}): 1682 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.32 (2H, s, CH_2Cl), 7.21 (2H, t, p-Aromatic CH) 7.45 (4H, m, m-Aromatic CH), 7.50 (4H, m, o-Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 40.8 (CH_2Cl), 122, 127.6, 130.1, 140.3 (benzene rings), 158.7 (NHCO).

2-Chloro-N-(4-methoxyphenyl)acetamide (**3**): Yield: 85%. M.p.: 120-121 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3234 (NH stretch), 1686 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.10 (3H, s, OCH_3), 4.25 (2H, s, CH_2Cl), 7.12 (1H, s, NHCO), 6.97-7.51 (4H, m, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 42.9 (CH_2Cl), 56.1 (OCH_3), 114.9, 121.9, 131.1, 159.6 (benzene ring), 164.8 (NHCO).

2-Chloro-N-(naphthalene-1-yl)acetamide (**4**): Yield: 60.1%. M.p.: 163-164 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3139 (NH stretch), 1733 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.40 (2H, s, CH_2Cl), 7.24 (1H, s, NHCO), 6.97-8.12 (7H, m, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 42.7 (CH_2Cl), 105.6, 118.9, 121.2, 124.9, 125.7, 126.8, 127.3, 129.1, 134.6, 135.8 (naphthalene ring), 165.1 (NHCO).

N, N-(1, 2-Phenylene)bis(2-chloroacetamide) (**5**): Yield: 25%. M.p.: 198-200 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3339 (NH stretch), 1657 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.10 (4H, s, CH_2Cl), 7.39 (2H, s, NHCO), 7.10 (2H, d, Aromatic), 8.20 (2H, t, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 42.2 (CH_2Cl), 124.3, 124.5, 131.1 (benzene ring), 165.7 (NHCO).

2-Chloro-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl) acetamide (**6**): Yield: 81%. M.p.: Dec. at 300 °C.



Scheme 1. Syntheses of chloroacetyl chloride derivatives (**1**-**8**).

FT-IR (KBr pellet, ν, cm^{-1}): 3233 (SO_2NH, stretch), 3320 (NH stretch), 1728 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.21 (3H, s, CH_3), 3.97 (1H, s, $NHSO_2$), 4.29 (2H, s, CH_2Cl), 7.11 (1H, s, NHCO), 7.51-7.94 (4H, m, Aromatic CH), 6.12 (1H, s, CH, Azol-ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 12.9 (CH_3), 42.8 (CH_2Cl), 164.2 (NHCO), 118.5, 130.4, 136.1, 140.4 (benzene ring), 95.4, 150.7, 171.1 (azol ring).

2-Chloro-N-(1, 5-dimethyl-3-oxo-2-phenyl-2, 3-dihydro-1H-pyrazol-4-yl)acetamide (**7**): Yield: 76%. M.p.: 180-181 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3286 (NH stretch), 1293 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.23 (3H, s, C-CH_3), 3.14 (3H, s, N-CH_3), 4.20 (2H, s, CH_2Cl), 7.80 (1H, broad peak, NHCO), 6.81-7.42 (5H, m, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 13.1 (C-CH_3), 35.1 (N-CH_3), 46.4 (CH_2Cl), 165.4 (NHCO), 117.7, 130.0, 136.6, 142.4, (benzene ring), 104.2, 135.2 (pyrazol ring), 159.9 (CO, pyrazol ring).

2H-Benzo[b][1,4]thiazin-3-(4H)-one (**8**): Yield: 30%. M.p.: 177-179 °C. FT-IR (KBr pellet, ν, cm^{-1}): 1760 (CO). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.78 (2H, s, CH_2, ring), 7.10-7.34 (4H, m, Aromatic CH), 8.21 (1H, s, NHCO). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 43.4 (CH_2S, ring), 123.5, 124.1, 126.0, 130.3, 133.2, 134.2 (benzene ring), 169.4 (NHCO).

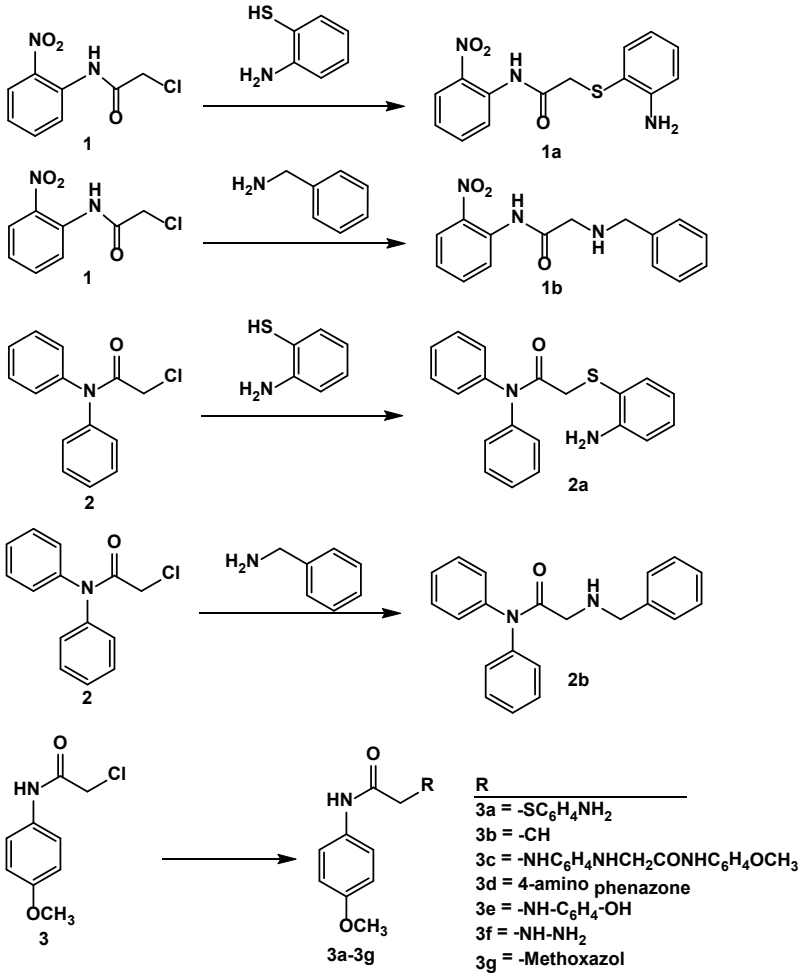
2-((2-Aminophenyl)thio)-N-(2-nitrophenyl)acetamide (**1a**): Yield: 30.7%. M.p.: 84-85 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3233 (NH stretch), 1566 (C=O), 1650 (NH_2). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.10 (2H, s, CH_2S), 7.31 (1H, s, NHCO), 6.37 (2H, s, NH_2), 6.81-8.45 (8H, m, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.9 (CH_2S), 115.1, 118.3, 118.9, 125.3, 125.4, 125.7, 128.1, 131.4, 131.6, 135.9, 142.3, 148.0 (benzene rings), 168.1 (NHCO).

2-(Benzylamino)-N-(2-nitrophenyl)acetamide (**1b**): Yield: 81.3%. M.p.: 103-104 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3337 (NHCO stretch), 3288 (NH), 1609 (CO). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.65 (1H, p, CH_2NH), 3.62 (2H, d, $COCH_2NH$), 3.66 (2H, d, CH_2NH), 7.239 (1H, s, NHCO), 7.21-7.81 (9H, m, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 53.4 ($COCH_2NH$), 54.1 (CH_2NH), 124.9, 125.1, 125.5, 127.3, 127.1, 128.5, 131.3, 131.6, 140.5, 141.6 (benzene rings), 168.9 (NHCO).

2-[(2-Aminophenyl)sulfanyl]-N, N-diphenylacetamide (**2a**): Yield: 30.4%. M.p.: 217-219 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3424 (NH_2), 3240 (N-CO stretch), 1596 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.72 (2H, s, CH_2S), 6.28 (2H, s, NH_2) 6.87-7.47 (14H, m, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 36.9 (CH_2S), 114.9, 118.8, 119.3, 121.9, 126.0, 128.0, 128.1, 128.9, 129.6, 140.3, 148.1 (benzene rings), 164.1 (NHCO).

2-(Benzylamino)-N, N-diphenylacetamide (**2b**): Yield: 79.3%. M.p.: 95-97 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3338 (NH stretch), 1731 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.39 (1H, s, CH_2NHCH_2), 3.37 (2H, s, $COCH_2NH$), 3.66 (2H, s, $PhCH_2NH$), 7.25-7.68 (15H, m, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.0 ($COCH_2NH$), 55.0 (CH_2NH), 122.9, 126.0, 127.9, 128.5, 129.6, 141.2, 140.3 (benzene rings), 164.1 ($COCH_2NH$).

2-((2-Aminophenyl)thio)-N-(4-methoxyphenyl)acetamide (**3a**): Yield: 28%. M.p.: 127-128 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3444 (NH_2), 3230 (N-CO stretch), 1696 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.73 (3H, s, OCH_3), 3.41 (2H, s, $COCH_2S$), 6.17 (2H, s, NH_2), 7.29 (1H, s, NHCO), 6.49-7.86 (8H, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 ($COCH_2S$), 55.8 (OCH_3), 113.5, 115.1, 118.9, 120.4, 122.6, 128.9, 131.8, 134.6, 148.5, 156.9 (benzene rings), 168.8 (NHCO).



Scheme 2. Syntheses of other acetamide derivatives (**1a**-**1b**, **2a**-2**b** and **3a**-**3g**).

N-(4-Methoxyphenyl)propionamide (**3b**): Yield: 25%. M.p.: 117-120 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3151 (NH stretch), 1657 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.83 (3H, s, OCH_3), 1.02 (3H, t, $COCH_2CH_3$), 2.5 (2H, q, $COCH_2CH_3$), 7.23 (1H, s, NHCO), 6.97, 7.51 (4H, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 55.8 (OCH_3), 10.02 ($COCH_2CH_3$), 30.9 ($COCH_2CH_3$), 114.5, 122.6, 130.8, 158.9 (benzene ring), 173.1 (NHCO).

2,2'-(1,4-Phenylene-bis(azanediyl))bis(N-(4-methoxypenyl) acetamide) (**3c**): Yield: 22%. M.p.: 172-178 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3322 (NH stretch), 1757 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.89 (2H, s, NHPhNH), 3.89 (6H, s, OCH_3), 3.78 (4H, s, $COCH_2NH$), 7.21 (2H, s, NHCO), 6.35 (4H, s, HNC_6H_4NH), 6.91 (4H, d, $CH_3OC_6H_4NH$), 7.69 (4H, d, $CH_3OC_6H_4NH$). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 55.5 ($COCH_2NH$), 56.7 (OCH_3), 103.5, 114.6, 117.7, 123.6, 131.8, 134.1 158.7, 169.1 (benzene ring), 169.6 (NHCO).

2-((1, 5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)-N-(4-methoxyphenyl)acetamide (**3d**): Yield: 28%. M.p: Dec. at 297 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3238 (NH stretch), 1726 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.87 (1H, s, CH_2NH) 2.36 (3H, s, CH_3), 3.21 (3H, s, N-CH_3), 3.49 (3H, s, OCH_3), 3.67 (2H, s, $COCH_2NH$), 7.29 (1H, broad peak, NHCO), 6.90-7.51 (9H, m, Aromatic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 13.1 (CH_3), 33.6 (N-CH_3), 47.3 ($COCH_2NH$), 54.7 (OCH_3), 115.5, 116.6, 122.6, 122.7, 123.1, 124.7, 129.1, 130.8, 132.3, 133.8, 158.9, 160.7 (aromatic rings), 169.5 (NHCO).

2-((4-Hydroxyphenyl)amino)-N-(4-methoxyphenyl) acetamide (**3e**): Yield: 30%. M.p: Dec. at 211 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3333 (NH stretch), 1667 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.53 (3H, s, OCH_3), 3.82 (2H, s, $COCH_2NH$), 4.34 (1H, s, CH_2NH), 7.13 (1H, s, NHCO), 6.87-7.91 (8H, Aromatic CH), 5.35 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 55.7 (OCH_3), 55.0 ($COCH_2NH$), 114.0, 116.1, 117.8, 122.6, 130.9, 139.1, 146.7, 157.7 (aromatic rings), 169.5 (NHCO).

2-Hydrazinyl-N-(4-methoxyphenyl)acetamide (**3f**): Yield: 22%. M.p.: Dec. at 275 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3499 (NH_2, stretch), 3322 (NH stretch) 1687 (NH_2, bending), 1596 (CO). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.49 (2H, s, CH_2NHNH_2), 3.78 (3H, s, OCH_3), 2.2 (1H, s, $NHNH_2$), 2.4 (2H, s, $NHNH_2$) 6.71(2H, d, Aromatic CH), 7.59 (2H, d, Aromatic CH), 7.19 (1H, s NHCO). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 56.8 (OCH_3), 57.9 ($COCH_2NH$), 115.7, 123.5, 132.9, 157.8 (aromatic ring), 168.5 (NHCO).

N-(4-Methoxyphenyl)-2-((4-(N-(5-methylisoxazol-3-yl) sulfa moyl)phenyl)amino)acetamide (**3g**): Yield: 18%, M.p.: Dec. at 300 °C. FT-IR (KBr pellet, ν, cm^{-1}): 3138 (NH stretch), 1856 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.39 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 3.81 (2H, s, $COCH_2NH$), 4.63 (1H, s, SO_2NH), 4.78 (1H, s, CH_2NH), 7.37 (1H, s, NHCO), 6.80-7.70 (8H, s, Aromatic CH), 6.61 (1H, s CH, methoxazol ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 12.0 (CH_3), 56.2 ($COCH_2NH$), 57.6 (OCH_3), 110.1, 113.5, 121.6, 125.1, 1280.0, 133.9, 1551,166.0 (benzene ring), 95.0, 153.1, 171.3 (azol ring), 169.9 (NHCO).

2.2. X-ray crystallography

Crystal structures of the synthesized compounds were analyzed by X-ray crystallography technique. Structures of these compounds were elucidated by SHELXL program [26]. Single crystal data was compiled using Bruker KAPPA APEX CCD diffractometer. To measure intensity graphite monochromated Mo-Kα radiation was used. Ultimate refining on F^2 was executed by SHELXL-97 full-matrix least squares techniques. Moreover, packing of the crystal structure was analyzed by using the programs as PLUTO and PLATON [27].

2.3. Antibacterial activity

The synthesized compounds were evaluated for their potential antibacterial activity against *Staphylococcus aureus* as Gram positive bacteria and *Escherichia coli* as Gram negative bacteria by disc diffusion method [28] using concentration of 10 µg/1.0 µL where cefixime a standard drug was taken as reference. According to this method the bacterial strain was inoculated on the solid agar media and the sample disks soaked with sample solution was placed on the already inoculated bacterial agar petri plates. The petri plates were incubated at 37 °C for 24 h. After incubation at 37 °C for 24 h the zone of inhibition was measured by using a scale in triplicate and the average measurement was then recorded in millimeters.

2.4. Molecular docking studies

To discern the possible coupling mode and to explore the least binding determination energy against *DNA gyrase* subunit B and *Topoisomerase II*, molecular docking of the synthesized series of compounds was performed using AutoDock v4.2 and MGL tools v1.5.6 [29]. The composition of ligands was sketched and their energies were minimized using ACD/ChemSketch [30]. The *DNA gyrase* subunit B (PDB ID 1KZN) and *Topoisomerase II* (PDB ID 1JIJ) was obtained from RCSB protein data bank [31]. Prior to dock the ligands the protein composition was first prepared with the cleaning of co-crystallized ligands as well as water molecules included in protein structure. Furthermore, hydrogen atoms were added before the addition of charges to the enzyme. To carry out molecular docking, binding site was first identified by selecting a lattice around the co-crystallized specific inhibitors clorobiocin and [2-amino-3-(4-hydroxyphenyl)propionyl-amino](1,3,4,5-tetrahydroxy-4-hydroxymethylpiperidin-2-yl)acetic acid, respectively. A total of 100 different orientations were created by using Algorithm as Lamarckian Genetic Algorithm (LGA) with a grid box having 80×80×80 dimension. Binding free energies for all of the compounds were collected and by carefully visualizing the 3D poses most befitting binding mode was selected. Powerful molecular graphics viewer was used such as Discovery Studio Visualizer v4.0 to figure out the most probable binding mode [32].

3. Results and discussion

Spectroscopic techniques such as (IR, ^{1}H NMR, ^{13}C NMR and XRD) were used to determine the structure of the series synthesized. Antibacterial activity of the compounds was determined by using disc diffusion method and it was further confirmed by molecular docking study.

3.1. Chemistry

Different acetamide derivatives were synthesized by fusion of chloroacetyl chloride with variation of different amines such as 2-aminobenzene thiol, benzylamine, benzene 1,4-diamine, 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one, 4-aminophenol, hydrazine and 4-amino-*N*-(5-methyl isoxazol-3-yl)benzenesulfonamide (Scheme 1 and 2). Spectroscopic techniques such as IR, ^{1}H NMR, ^{13}C NMR and XRD were used to govern the structure of the series synthesized.

In view of FT-IR spectra, the absence of two peaks corresponding to -NH_2 (N-H stretching absorptions) and appearance of single absorption band in the range 3500-3300 cm^{-1} indicated that the conversion of reactants (amines) into products (amides). The presence of absorption peak in the range 600-800 cm^{-1} indicated that product contains (C-Cl) linkage for the compounds **1**-**7**. Compound **8** was a cyclic compound having no (C-Cl) linkage, so there is no such absorption peak in this range. The compounds **1a**-**1b**, **2a**-**2b**, **3a**-**3g** did not include the absorption band in this region; this was a clear indication of the formation of new acetamide moieties from chloroacetyl chloride derivatives.

All of the synthetic compounds were also characterized by NMR spectroscopy. The presence of singlet appeared in the region of δ 4.10-4.33 ppm correspond to the protons of CH_2 group confirmed the formation of chloroacetamide derivatives (**1**-**7**) while the compound **8** have a clear singlet below this region due to the cyclized product where CH_2 group was shielded due to the negative inductive effect of sulphur and electron withdrawing effect of carbonyl group present in its neighborhood. Furthermore, the shifting of the singlet from δ 4.10-4.33 to 3.61-3.87 ppm confirmed the formation of acetamide compounds **1a**-**1b**, **2a**-**2b** and 3**a**-**3g**. Similar pattern was observed for CH_2 in the ^{13}C NMR, the signal which appeared in the range of δ 40.1-43.9 ppm confirmed the formation of chloroacetamide derivatives. Furthermore, the signals in range of δ 164.0-170.1 ppm confirmed the presence of carbonyl (C=O) in the products due to the formation of amide.

3.2. XRD analysis

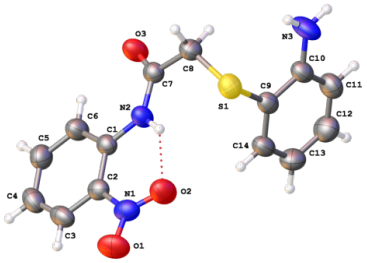
Crystal analysis confirmed the molecular structure of the synthesized compounds. The crystal structures of some of the compounds are shown in Figure 2. Our group has published the crystal structure of the compounds **5** and **3a** [33,34]. Moreover crystal structure data for the compounds **1a**, **2b** and **6** is shown in Table 1 and Table 2.

3.3. Antibacterial activity

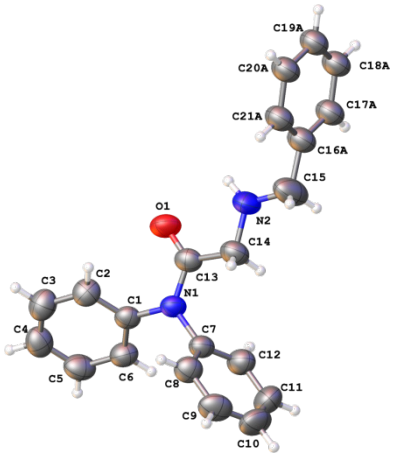
Nearly all tested compounds showed significant to good activities against *Staphylococcus aureus* and *Escherichia coli*. Among the synthesized series, compound **2b** was found to have highest antibacterial potential with inhibition zone diameter of 28 and 29 mm against the above mentioned bacterial strains, respectively. Other compounds of the series also showed momentous antibacterial potential, detail of which is included in Table 3.

3.4. Molecular docking

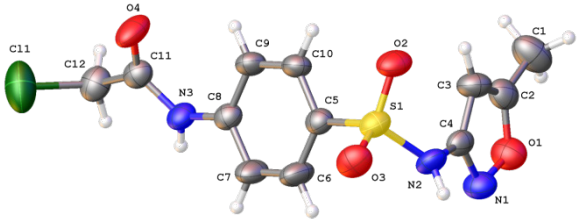
Molecular docking of the compounds was performed inside the *DNA gyrase subunit B* and *Topoisomerase II*. Before docking the compounds, the reference co-crystallized ligand clorobiocin and [2-amino-3-(4-hydroxy-phenyl)-propionyl amino]-(1,3,4,5-tetrahydroxy-4-hydroxymethyl-piperidin-2-yl)- acetic acid was docked. For re-docking of the reference ligands, 1.5 - 2.0 Å RMSD values were used. The probable minimum binding free energy of the compounds was calculated ranging from -4.35 to -8.75 KCal/mol in case of *DNA gyrase* and in case of *Topoisomerase II* value were in the range of -4.92 to -8.60 KCal/mol (Table 4). The compound **2b** was observed to bind with highest binding affinity of -8.75 KCal/mol inside *DNA gyrase* and with -8.60 KCal/mol inside *Topoisomerase II*.



Compound **1a**



Compound **2b**



Compound **6**

Figure 2. Crystal structure of compounds **1a**, **2b** and **6**.

Table 1. Crystal data and details of the structure refinement for compounds **1a**, **2b** and **6**.

Identification code	Compound **1a**	Compound **2b**	Compound **6**
Empirical formula	$C_{14}H_{13}N_3O_3S$	$C_{21}H_{20}N_2O$	$C_{12}H_{12}ClN_3O_4S$
Formula weight	303.33	316.39	329.76
Temperature (K)	296(2)	296(2)	296(2)
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/n$	$P2_1/c$
a (Å)	12.0457(9)	5.8785(6)	12.6434(19)
b (Å)	5.1341(3)	17.660(2)	14.5593(17)
c (Å)	22.8505(19)	16.991(2)	7.9999(11)
α (°)	90	90	90
β (°)	101.118(2)	95.448(6)	93.845(6)
γ (°)	90	90	90
Volume (Å^3)	1386.64(18)	1755.9(4)	1469.3(3)
Z	4	4	4
ρ_{calc} (g/cm^3)	1.453	1.197	1.491
μ (mm^{-1})	0.247	0.074	0.420
F(000)	632.0	672.0	680.0
Crystal size (mm^3)	0.43 × 0.28 × 0.26	0.36 × 0.24 × 0.22	0.30 × 0.25 × 0.10
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection (°)	3.446 to 54.468	4.614 to 52	3.228 to 54.586
Index ranges	$-15 \le h \le 15$	$-7 \le h \le 7$	$-15 \le h \le 16$
	$-6 \le k \le 5$	$-21 \le k \le 20$	$-17 \le k \le 18$
	$-29 \le l \le 29$	$-20 \le l \le 20$	$-10 \le l \le 9$
Reflections collected	12126	13575	12725
Independent reflections	3096 [R_{int} = 0.0276, R_{sigma} = 0.0277]	3457 [R_{int} = 0.0503, R_{sigma} = 0.0627]	3271 [R_{int} = 0.0665, R_{sigma} = 0.0841]
Data/restraints/parameters	3096/0/190	3457/0/182	3271/0/191
Goodness-of-fit on F^2	1.021	1.018	1.006
Final R indexes [I≥2σ (I)]	R_1 = 0.0377, wR_2 = 0.0886	R_1 = 0.0629, wR_2 = 0.1484	R_1 = 0.0559, wR_2 = 0.1225
Final R indexes [all data]	R_1 = 0.0572, wR_2 = 0.0994	R_1 = 0.1442, wR_2 = 0.1886	R_1 = 0.1314, wR_2 = 0.1500
Largest diff. peak/hole (e.Å^{-3})	0.21/-0.21	0.29/-0.23	0.34/-0.33
CCDC no	1832001	1832002	1832000

Table 2. Selected bond lengths [Å] and angles [°] for compounds **1a**, **2b** and **6**.

Compound	Bond lengths			
1a	S(1)-C(9)	1.7746(19)	N(3)-C(10)	1.375(2)
	S(1)-C(8)	1.8043(19)	C(1)-C(6)	1.393(2)
	O(1)-N(1)	1.2201(18)	C(1)-C(2)	1.410(2)
	O(2)-N(1)	1.2262(19)	C(2)-C(3)	1.382(2)
	O(3)-C(7)	1.218(2)	C(3)-C(4)	1.365(3)
	N(1)-C(2)	1.461(2)	C(4)-C(5)	1.371(3)
	N(2)-C(7)	1.352(2)	C(5)-C(6)	1.381(3)
	N(2)-C(1)	1.397(2)	C(7)-C(8)	1.514(2)
	Bond angles			
	C(9)-S(1)-C(8)	99.55(9)	O(3)-C(7)-N(2)	124.91(16)
	O(1)-N(1)-O(2)	122.06(17)	O(3)-C(7)-C(8)	119.02(15)
	O(1)-N(1)-C(2)	118.49(16)	N(2)-C(7)-C(8)	116.03(15)
	O(2)-N(1)-C(2)	119.44(14)	C(7)-C(8)-S(1)	118.66(12)
	C(7)-N(2)-C(1)	129.09(15)	N(3)-C(10)-C(11)	120.96(17)
	C(6)-C(1)-N(2)	121.93(15)	N(3)-C(10)-C(9)	120.95(18)
	C(6)-C(1)-C(2)	116.27(16)	C(11)-C(10)-C(9)	118.06(17)
2b	**Bond lengths**			
	O(1)-C(13)	1.221(3)	N(1)-C(7)	1.447(3)
	N(1)-C(13)	1.366(3)	N(2)-C(15)	1.405(4)
	N(1)-C(1)	1.442(4)	N(2)-C(14)	1.435(4)
	Bond angles			
	C(1)-N(1)-C(7)	116.8(2)	O(1)-C(13)-C(14)	121.0(3)
	C(6)-C(1)-N(1)	120.1(3)	N(1)-C(13)-C(14)	116.7(2)
	C(2)-C(1)-N(1)	120.3(3)	N(2)-C(14)-C(13)	110.4(2)
6	**Bond lengths**			
	Cl(1)-C(12)	1.751(4)	N(1)-C(4)	1.308(4)
	S(1)-O(2)	1.428(2)	N(2)-C(4)	1.390(4)
	S(1)-O(3)	1.430(2)	N(3)-C(11)	1.346(4)
	Bond angles			
	O(2)-S(1)-N(2)	108.79(15)	O(3)-S(1)-N(2)	104.40(14)
	O(3)-S(1)-C(5)	109.16(17)	N(2)-S(1)-C(5)	105.92(15)
	C(4)-N(1)-O(1)	104.5(3)	C(4)-N(2)-S(1)	122.3(2)

Table 3. Inhibition zone diameter values (mm) against *Staphylococcus aureus* and *Escherichia coli*.

Compounds	Inhibition zone diameter (mm)	
	Staphylococcus aureus	***Escherichia coli***
1	17	16
2	19	18
3	16	18
4	18	16
5	17	17
6	20	19
7	21	20
8	17	18
1a	21	19
1b	17	16
2a	24	23
2b	28	29
3a	19	19
3b	19	17
3c	22	20
3d	26	25
3e	25	19
3f	19	18
3g	24	24
Cefixime	29	30

Table 4. Least binding energies of the top ranked poses (orientations) against bacterial *DNA gyrase subunit B* and *Topoisomerase II*.

Compounds	Lowest Binding Energies (Kcal/mol)	
	DNA Gyrase	***Topoisomerase II***
1	-4.79	-4.92
2	-5.47	-6.81
3	-5.35	-5.04
4	-6.47	-6.58
5	-5.91	-6.31
6	-6.62	-8.02
7	-6.54	-6.88
8	-5.78	-6.13
1a	-7.44	-7.24
1b	-5.60	-5.76
2a	-7.28	-7.19
2b	-8.75	-8.60
3a	-6.48	-6.68
3b	-5.27	-5.09
3c	-4.35	-6.51
3d	-7.65	-8.20
3e	-5.82	-6.12
3f	-4.70	-5.46
3g	-5.74	-8.08
Reference	-6.95	-7.57

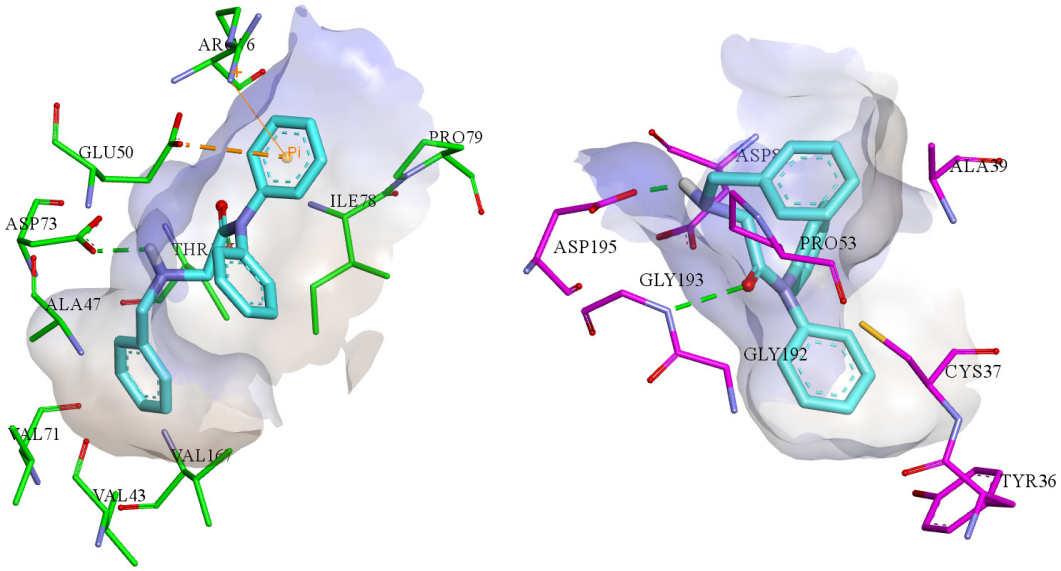


Figure 3. Possible binding mode of compound **2b** with lowest binding energy (colored cyan) inside active pocket of *DNA gyrase subunit B* and *Topoisomerase II*.

This supports the results of wet lab as inhibition zone for compound **2b** was found to be 28 and 29 mm, respectively. Inside *DNA gyrase*, it forms hydrogen bonding interactions with residue Asp73 and inside *Topoisomerase II*, it was found to form hydrogen bonding interaction with residue Gly193 and Asp195. This is due to the presence of -NH and C=O functional group. Several other hydrophobic interactions also stabilize the compound **2b** pose (Figure 3).

4. Conclusion

A series of nineteen acetamide based compounds were synthesized including derivatives of chloroacetyl chloride. Structural elucidation was carried out using different physiochemical techniques such as FT-IR, NMR spectroscopy and X-ray Crystallography. The respective techniques confirmed the molecular structure of the compounds synthesized. All the compounds were assessed to exhibit antibacterial activity also supported by 3D interactions using docking studies. This study will be useful for further evaluation of acetamide functionality.

Supporting information

CCDC-1832001 (**1a**), CCDC-1832002 (**2b**), and CCDC-1832000 (**6**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallo-graphic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

Disclosure statement

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