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Synthesis, biological evaluation, and molecular docking studies of novel diclofenac derivatives as antibacterial agents

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ABSTRACT

In the recent years, interest in the synthesis of diclofenac derivatives has increased due to their exceptional biological activity. We present here the synthesis of some novel diclofenac derivatives through simple synthetic procedures, where the acylation of carbohydrazide compound **1** with chloroacetyl chloride in dioxane produced the compound **2**. Chloroacetohydrazide compound **2** was further subjected to nucleophilic substitution reactions using different nucleophiles such as: hydrazine hydrate, thiosemicarbazide and *p*-aminobenzenesulfonamide to give the corresponding derivatives **3-5**, respectively. Moreover, the reaction of the hydrazinyl compound **3** with active hydrogen species such as: ethyl acetoacetate and acetyl acetone in refluxed ethanol provided the corresponding pyrazolone derivatives **6** and **7**, respectively. Furthermore, the reaction of previously reported diclofenac ester **8** with 1,2-diaminoethane gave the amino derivative **9**. Finally, condensation reaction of the latter compound with benzaldehyde in dioxan furnished the corresponding Schiff's base compound **10**, while its acylation with chloroacetyl chloride in dioxan produced **11**. Different spectral (IR, NMR and Mass) and elemental analysis techniques were utilized to explore the structure of the synthesized compounds. All the synthesized compounds were tested for their in-vitro antibacterial activity against different strains of bacteria showing satisfactory results, and molecular docking study was performed to investigate the mode of action.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are extensively used to treat almost all forms of swelling, inflammation and relieve pain [1–4]. Along with, recent studies have shown that some of these anti-inflammatory drugs also exhibit anti-microbial activity in addition to their major function [5–9]. Diclofenac drug which has the chemical structure as 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid treated as one of the most (NSAIDs) which used as anti- inflammatory drugs [10]. In humans, Diclofenac metabolized to hydroxyl derivatives, by cytochrome P450 (CYP) enzymes. NSAIDs' mechanism of action is attributed to the inhibition of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme prostaglandin endoperoxidase, as well as pain, fever, and inflammation caused by a high level of prostaglandin, NSAIDs triggers a reduction in excessive production of prostaglandins by inhibiting the enzymes responsible for this secretion [11–15]. Diclofenac derivatives have been shown to be effective against all strains of multi-drug resistant Escherichia coli in addition to its main use as an anti-inflammatory drug [16–20]. As a result, it is possible that diclofenac has a new function in treating UTI (uncomplicated urinary tract infections) caused by *E. coli* [21]. According to the benefits of diclofenac and its analogues, which demonstrated outstanding antimicrobial activity, potential environmental contaminants in addition to their main function as anti-inflammatory agents, they have received much interest from the organic and biochemists in the recent years [22–24]. Diclofenac has demonstrated potent antibacterial activity against both gram-positive and gram-negative organisms, additionally, it has demonstrated synergy with other antibiotics [25–28]. These

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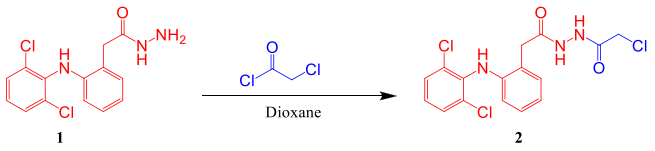


Scheme 1. Reaction of diclofenac carbohydrazide with chloroacetyl chloride.

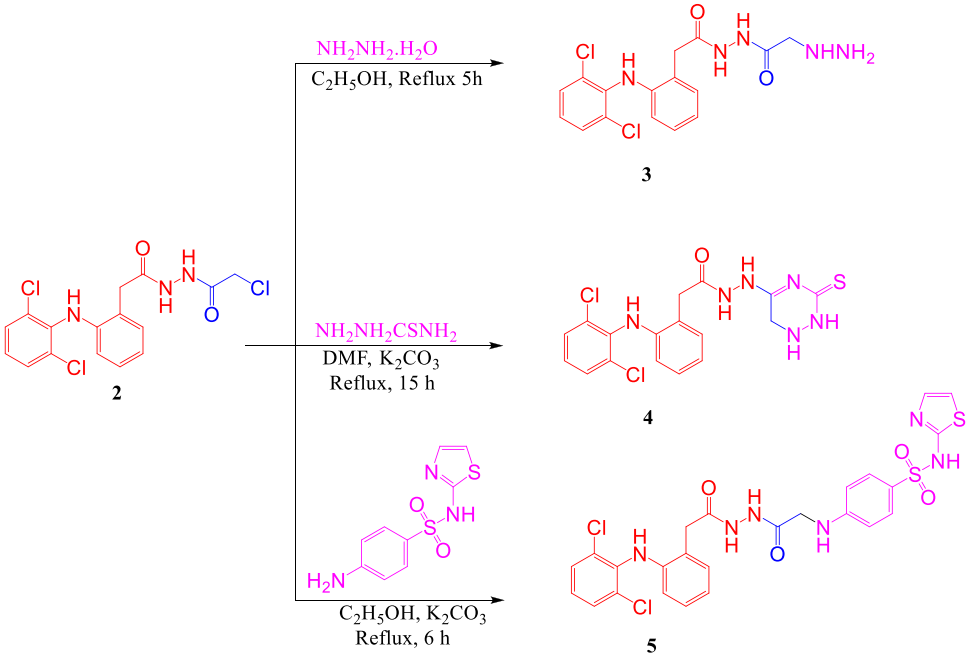
findings demonstrate that diclofenac is an effective non-antibiotic antibacterial. All the mentioned beneficial for diclofenac as potent drug encouraged us to design some new diclofenac derivatives and studying their antimicrobial activity and based on our experience in both organic and medicinal chemistry research in developing new potent drugs with pyrazole, thiazole, indole, and pyrimidine moieties [29–44]. We planned here to synthesize some new heterocyclic diclofenac analogues by substituting the carboxylic acid group of diclofenac acid with a less acidic heterocycle group as effort to find novel, safer and more effective antimicrobial drugs. In addition, the antibacterial activity of the synthesized compounds was examined using different strains of bacteria, and a molecular docking study was conducted to investigate the binding ability of the novel compounds targeted in this study.

2. Results and discussion

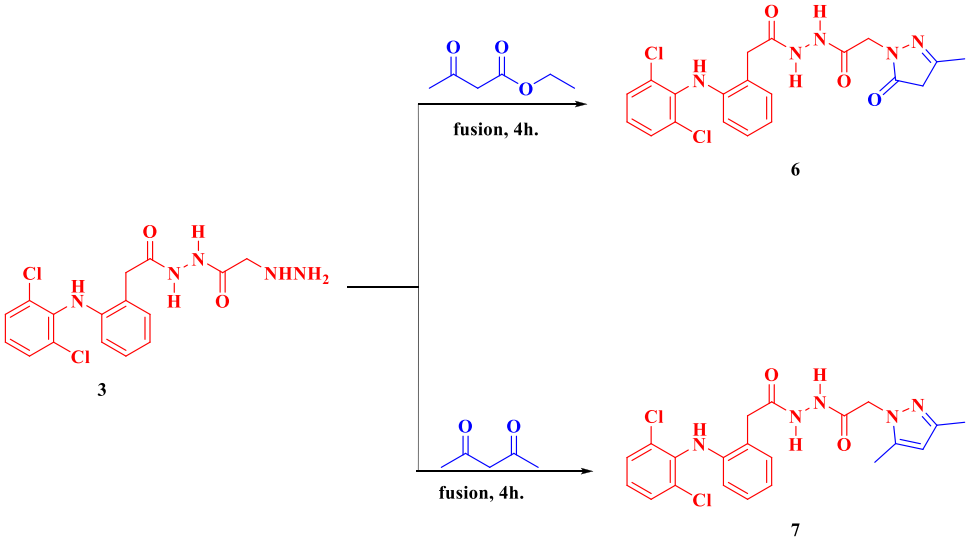
2.1. Chemistry

As shown in Scheme 1, compound **1** was prepared according to the previously reported procedure [45], then it was subjected to acylation using chloroacetyl chloride in dioxane to give corresponding 2-chloro-*N'*-(2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)aceto-hydrazide (**2**). The chemical structure of compound **2** was confirmed based on its elemental and spectral analyses. Where the FT-IR spectrum exhibited the appearance of absorption bands at 3195 and 1659 cm^{-1} characteristic of NH and two amidic carbonyl groups, respectively, also the IR spectrum showed additional peak at 3034 cm^{-1} characteristics of aromatic C–H bonds. Moreover, the spectrum revealed the absence of bands of the NH and NH_2 groups existed in the starting material **1**. While the ^{1}H NMR displayed singlet signals at δ 3.66, 4.16, 6.29, 10.51 and 10.65 ppm which attributed to two CH_2 and three NH groups, respectively, in addition to seven aromatic protons at the range of δ 7.06 to 8.30. Also, the ^{13}C NMR spectrum showed signals at δ 37.04, 45.34, 167.88 and 169.87 ppm which may be belong to two CH_2 and two CO groups, respectively. Finally, the HPLC-MS chromatogram of compound **2** showed a molecular ion peak at m/z 388.10.

Moreover, compound **2** was used as a versatile precursor for the synthesis of other heterocyclic compounds containing the electron donating species (**3-5**) as shown in Scheme 2. Where, the nucleophilic substitution reaction of the chlorine atom in compound **2** with various nucleophiles such as: hydrazine hydrate, thiosemicarbazide and 4-amino-*N*-(thiazol-2-yl)benzene sulfonamide under neat condition for short time followed by refluxing in ethanol afforded the corresponding derivatives (**3-5**). Formation of the new compounds were confirmed by spectral analyses. Where, the FT-IR spectrum of compound **3** exhibited absorption bands at 3327 and 1637 cm^{-1} attributed to NH, NH_2 and CO groups, respectively, additionally the spectrum showed bands at 3025 cm^{-1} for aromatic C–H bonds, and 2925 cm^{-1} for aliphatic C–H bonds. Also, the ^{1}H NMR spectrum in $CDCl_3$ showed the presence of signals at δ 3.28 3.57 ppm features the CH_2 groups, and 6.16, 6.29, 8.45, 10.14 and 11.30 ppm which belongs to NH_2, NH groups, respectively, in addition to signals at 7.09-7.53 ppm confirmed the presence of seven aromatic protons. Moreover, the FT-IR spectrum of the (thioxo-1,2,3,6-tetrahydro-1,2,4-triazin-5-yl)acetohydrazide (**4**) presented absorption bands at 3271, 2924, and 1659 cm^{-1} for NH, aliphatic C–H, and CO groups, respectively. Also, its ^{1}H NMR in $CDCl_3$ showed singlet signals at δ 3.45, 7.93, 8.39, 10.12 and 10.88 ppm which be appropriate with CH_2 triazine and NH groups, respectively, and multiplet signals at 6.92-7.40 ppm for aromatic protons. In comparison with the ^{1}H NMR spectrum of the starting material **3**, the ^{1}H NMR compound **4** showed two additional singlet signals for two NH groups. Whereas the ^{13}C NMR spectrum displayed signals at δ 47.13 and 170.38 for CH_2 triazine and C=S groups, respectively. Compared to the ^{13}C NMR spectrum of compound **3**, ^{13}C NMR spectrum of compound **4** revealed ab-



Scheme 2. Reactions of diclofenac derivative **2** with various amines.

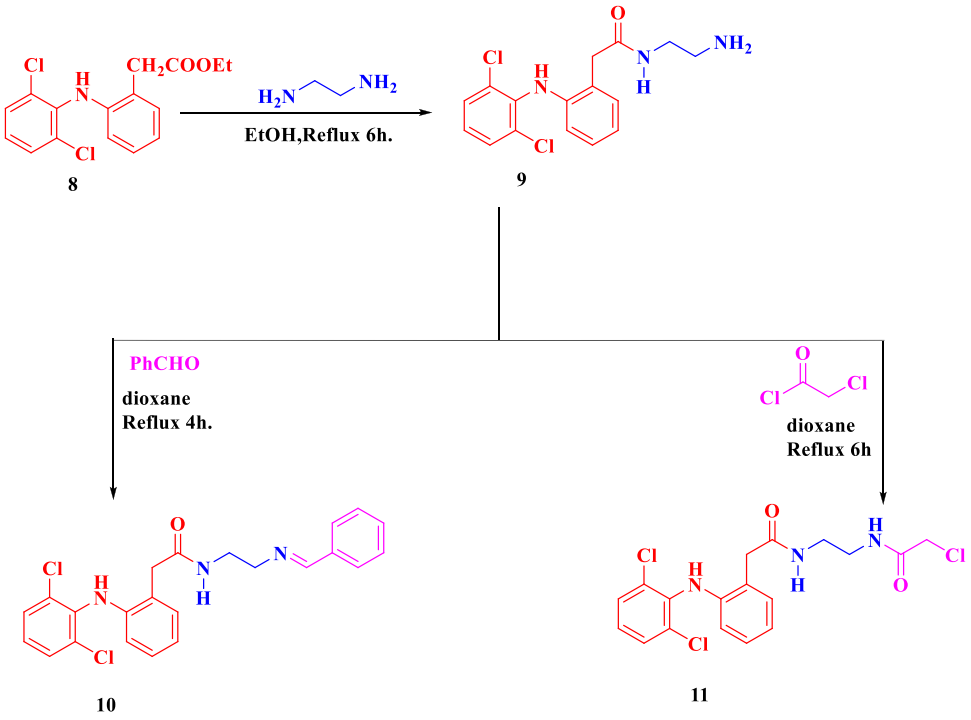


Scheme 3. Reactions of diclofenac derivative **3** with ethylaceto acetate and acetyl acetone.

sence of the carbonyl signal in compound **3** due to water molecule elimination. On the other hand, the FT-IR spectrum of compound **5** demonstrated absorption bands at 3387, 3204, 1728 and 1655 cm^{-1} for 5NH and 2 CO groups, respectively. Though, the ^1H NMR spectrum in DMSO-d_6 showed singlet signals at δ 3.36, 3.92, 6.29, 8.74, 8.98, 10.01, 10.56 and 7.74 ppm for $2CH_2$, 5NH and 2CH thiazolyl groups, respectively. Matching the ^1H NMR spectrum of compound **5** and its starting material compound **4**, it can be easily concluding the increasing in the integration of aromatic protons (11 protons) in the range of 6.54-7.39 ppm. Finally, the ^{13}C NMR spectrum exhibited signals at 38.56, 45.56, 112.97 for $2CH_2$ and 2CH of thiazolyl ring groups, in addition to the presence of signals at 172.78, 173.01 ppm for carbonyl groups.

Furthermore, the reaction of the aminoglycylacetohydrazide **3** with the active hydrogen species such as: ethyl acetoacetate or acetyl acetone in ethanol yielded the corresponding pyrazolone derivatives **6** and **7** as shown in Scheme 3. The chemical structure of compound **6** was assigned by FT-IR, ^1H NMR and ^{13}C NMR spectra. Where, the FT-IR spectrum of compound **6** demonstrated absorption bands at 3355, 3211 and 1687 cm^{-1} that attributed to NH and 2CO groups, respectively, as well as bands at 3034 cm^{-1} for aromatic C–H, 2955, 2921, 2850 cm^{-1} for aliphatic C–H. While the ^1H NMR spectrum showed the presence of singlet signal at δ 1.95 ppm attributed to CH_3 proton and singlet signal at δ 2.98 ppm specific for CH_2 pyrazole. From the ^1H NMR spectra of compounds **3** and **6**, it can be stated that the singlet signal of NH_2 group at 6.16 ppm in compound **3** was disappeared and new signals for CH_2 pyrazole and CH_3 in compound **6** was appeared. On the other hand, the FT-IR spectrum of compound **7** showed absorption bands at 3354,3210 and 1687 cm^{-1} distinct for NH and CO groups, respectively. Whereas the ^{1}HNMR spectrum in DMSO-d6 showed two singlet signals at δ 2.21 and 2.25 ppm for $2CH_3$ groups attached to pyrazole ring and singlet signal at δ 8.02 ppm for CH pyrazole. Matching the ^1H NMR spectra of compounds **3** and **7,** we can conclude the disappearance of singlet signal for NH_2 group in compound **3** and appearance of two singlet signals for two methyl groups in compound **7** at δ 2.21 and 2.25 ppm**.** Finally, the ^{13}C NMR exhibited signals at δ 12.45, 14.96, 107.18, 169.[76] and 172.87 ppm which may be belong to both $2CH_3$, CH pyrazole and 2CO groups, respectively. Equivalent the ^{13}C NMR spectra of compounds **3** and **7,** it can be established the appearance of two new signals for two methyl groups in compound **7** at δ 12.45, 14.96 ppm.

As shown in Scheme 4, the reaction of previously synthesized ethyl 2-(2-((2,6-dichlorophenyl) amino)phenyl) acetate compound [19] (**8**) with ethylene diamine under heat condition on a steam bath produced the corresponding *N*-(2-aminoethyl)-2-(2-((2,6-dichlorophenyl)amino) phenyl)acetamide derivatives (**9**). Compound **9** was characterized by spectral data, where the FT-IR showed appearance of absorption bands at 3353, 3211 and 1642 cm^{-1} for 2NH, NH_2 and CO groups, respectively. From the obtained IR spectrum, the compound **9** can be certainly distinguished from starting compound **8** due to the appearance of new bands for NH_2 group at 3353, 3211 cm^{-1}, in addition to appearance of amidic carbonyl band at lower wave number 1642 cm^{-1}than that of ester in compound **8**. Also, the ^1H NMR spectrum in DMSO-d_6 exhibited two singlet signals at δ 6.23 and 9.02 ppm that can be attributed to 2NH groups and two triplet signals at δ 2.96 and 3.48 for $2CH_2$, in addition to, two singlet signals at δ 3.84 and 6.54 for CH_2 and NH_2 groups respectively, as well as disappearance of the signals characteristic for CH_2 and CH_3 of ester group in compound **8**. Similarly, the ^{13}C NMR out came results proved the presence of three CH_2 and C=O groups at δ 37.89, 44.56, 46.89 and 175.78 ppm, respectively. Comparably, the ^{13}C NMR of compound **9** showed absence of signals related to CH_2 and CH_3 of ester group in compound **8**, and showed appearance of new two signals at 44.56, 46.89 ppm characteristic for the two methylene groups in compound **9**. The condensation of compound **9** with benzaldehyde in dioxane under reflux conditions in the presence of catalytic amount of acetic acid gave the corresponding Schiff's base derivative **10**. The chemical structure of compound **10** was elucidated by elemental and spectral data. Thus, the FT-IR spectrum showed broad absorption band at 3304 cm^{-1} for NH groups and 1640 cm^{-1} for C=O group. And the ^1H NMR in DMSO-d_6 showed multiplet signals at δ 7.08-7.53 ppm for aromatic protons and appearance of singlet signal at δ 8.13 ppm for azomethine CH=N group. By comparing the ^1H NMR spectra of compounds **9** and **10** it can be shown that the integration of aromatic protons in spectrum of compound **10** has increased by 5 protons at the range of at δ 7.08-7.53 ppm, which confirm the formation of the Schiff base **10** as well as the appearance of new signal at 8.13 ppm for the azomethine proton and disappearance of NH_2 group protons of compound **9**. Also, the ^{13}C NMR spectrum displayed signal at δ 144.87 for CH=N proton. Finally, up on the treatment of compound **9** with chloroacetyl chloride in dioxane at reflux temperature, the chloroacylation was occurred to give 2-chloro-*N*-(2-(2-(2-((2,6-



Scheme 4. Synthesis of some diclofenac derivatives **9-11.**

Table 1
The antibacterial activity, inhibition zone (mm), and MIC (μg mL^{-1}) of compounds (**2-11**).

Bacteria Strain / *Compound*	*Staphylococcus aureus (+ve)*	*Bacillus cereus (+ve)*	*Serratia marcescens (-ve)*	*Pseudomonas aeruginosa (-ve)*
2	10[a](9.0)[b]	13(9.0)	15(8.0)	11(9.0)
3	13(8.0)	17(8.0)	17(7.0)	14(7.0)
4	12(8.0)	12(10)	16(8.0)	15(8.0)
5	17(7.0)	13(9.0)	18(7.0)	16(8.0)
6	13(7.0)	15(8.0)	11(9.0)	17(7.0)
7	16(7.0)	19(8.0)	15(8.0)	18(7.0)
9	15(8.0)	19(7.0)	20(7.0)	15(7.0)
10	18(8.0)	16(7.0)	16(7.0)	17(7.0)
11	14(7.0)	18(8.0)	17(8.0)	18(8.0)
Chloramphenicol	22(5.0)	24(5.0)	22(4.0)	20(4.0)

(a) Numbers out parentheses represent the diameter of inhibition zone in (mm) of compounds (**2-11**).
(b) Numbers in parentheses represent the MIC (minimum inhibition concentration) in (μgml^{-1}) of tested compounds.

dichlorophenyl)amino)phenyl)acetamido) ethyl)acetamide (**11**). The structure assignment of compound **11** was carried out by ^{1}H NMR and ^{13}C NMR spectral analyses. The ^{1}H NMR in DMSO-d_6 furnished signals characteristic of four CH_2 and two NH groups at δ 3.12, 3.44, 4.23, 9.65 and 9.97 ppm, respectively. Also, the ^{13}C NMR spectrum displayed signals at δ 38.98, 39.22, 42.35, 169.45 and 172.77 for CH_2 groups and 2C=O groups, respectively. From the ^{13}C NMR spectra of compounds **9** and **11**, we can see that the spectrum of compound **11** showed a new signal for the new formed amidic C=O group.

2.2. Antibacterial evaluation

The initial focus of organic and medicinal chemists at these days is the development of novel pharmaceutical drugs that may be utilized to treat microbial and inflammatory illnesses. Consequently, our research here mainly focuses on the synthesis of novel molecules with significant bioactivity against some bacterial species. Compounds (**2-11**) were evaluated for their antibacterial efficacy against two Gram-positive bacteria (*Bacillus cereus, Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa, Serratia marcescens*). The zones of inhibition (mm) and minimum inhibitory concentration (MIC) (g/mL) of the examined compounds were measured and compared to the antibacterial reference drugs Chloramphenicol. The data in Table 1 represent the antibacterial activity of all synthesized compounds against Gram-positive bacteria (*Bacillus cereus, Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa, Serratia marcescens*). From these data, it can be stated that all the investigated compounds displayed remarkable antibacterial activity against both Gram-positive and Gram-negative bacteria. In addition, from the values of inhibition zones, it can notice that compounds **7, 9,10** and **11** showed the highest antibacterial activity against all strains of bacteria with values close to those of the corresponding antibiotic reference (Chloramphenicol). While compounds **2** and **4** exhibit the lowest antibacterial activity among all the tested compounds. The inhibition of *S. aureus* was caused by compounds **5** and **10** where the MIC for these derivatives is 17 and 18 μg. mL^{-1}, respectively compared to reference drug (22 μg. mL^{-1}) which may be attributed to the existence of sulfonamide

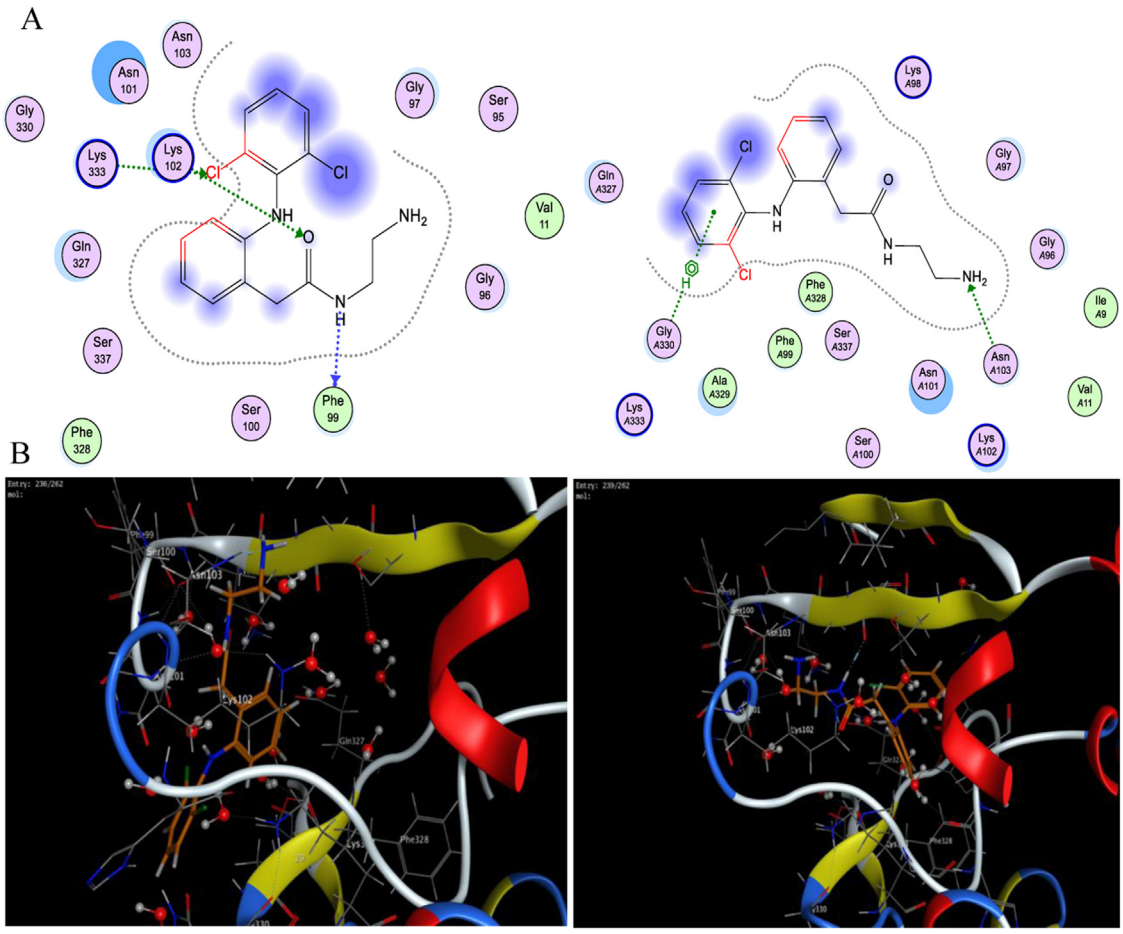


Fig. 1. Potential binding modes of compounds **9** (A) compared to DNA gyrase (yellow) in the polymerase binding site of *E. coli* sliding clamp (PDB ID: 4MJQ). Electrostatic surface representation of protein surface: positive (blue), neutral (white), and negative (red). (B) The corresponding 2D ligand–protein interactions.

moiety in compound **5** and azomethine moiety in compound **10**. While compounds **7, 9** and **11** showed strong activity towards *B. cereus* with MIC 19, 19 and 18 µg. mL^{-1}, respectively, and this also may be ascribed to the insertion of new functional groups to the starting materials such as: pyrazole ring in compound **7** and chlormethyl derivative in compound **11**. Moreover, the inhibition of *S. marcescens was* achieved by compound **5** which contain diclofenac integrated with sulfonamide moiety and compound **9** that include diclofenac tethered with aminoethaneyl moiety with MIC values 18, 20 µg. mL^{-1}, respectively compared to that of chloramphenicol drug (22 µg. mL^{-1}). While compounds **6, 7, 10** and **11** exhibit highest activity against *P. aeruginosa*. In conclusion, the data in Table 1 showed that the compound 2-(2-((2,6-Dichlorophenyl)amino)phenyl)-*N'*-(2-(3,5-dimethyl-1*H*-pyrazol-1-yl)acetyl) acetohydrazide (**7**) which contain pyrazole moiety and Schiff base compound *N*-(2-(Benzylideneamino)ethyl)-2-(2-((2,6-dichlorophenyl)amino)phenyl) acetamide(**10**) exhibited the highest antibacterial activity against all bacteria strains.

2.3. Mode of action and molecular docking studies

It has been established that diclofenac's antibacterial effect is mostly caused by its ability to inhibit the DNA [9]. It was discovered that the sliding clamp, which is a homodimer of the -subunit of DNA polymerase III, is the molecular target of several different nonsteroidal anti-inflammatory drugs (NSAIDs) that display antibacterial properties [46]. The sliding clamp is a protein that looks like a ring and is needed for DNA replication. It works as a mobile platform that wraps around DNA and binds to multiple DNA polymerases through a conserved binding site. This makes DNA synthesis more efficient [46]. Using DNA replication tests and crystallography, it was shown that the NSAIDs carprofen, bromfenac, and vedaprofen target the *E. coli* sliding clamp, preventing the sliding clamp from interacting with DNA polymerase [46]. Inhibition of the bacterial sliding clamp is a likely candidate for the antibacterial actions of our drugs given the structural similarities between diclofenac and DNA gyrase and the fact that both have been shown to have inhibitory effects on DNA synthesis. The crystal structure of the *E. coli* sliding clamp complexed with DNA gyrase was used in a docking study, and the results showed that compounds **9, 10** and **11** bind effectively into subsite I of the polymerase binding site, with binding energies ranging from -7.3 to **-** 5.6 kcal/mol. There are hydrophobic interactions between the two arene rings in the **9** and the Gly330 and Asn103 residues, which serve to enhance the binding of compound **9**. Hydrogen bonds are formed between Lys102, an H-bond donor, and the amide carbonyl groups on the carboxamide moiety in compound **9** (Fig. 1A). Hydrophobic interaction between the dichlorinated aromatic ring and Lys333 also holds compound **9** together. (Fig. 1B). Moreover, there was a hydrogen bonds also are formed between Lys102, an H-bond donor, and the N of the azamethine group of the Schiff base in compound **10** (Fig. 2A). Furthermore, from Fig. 3A, it could be showed that there is a hydrogen bond linked between nitrogen atom of the chloroacetyl moiety in compound **11** and Ser107.

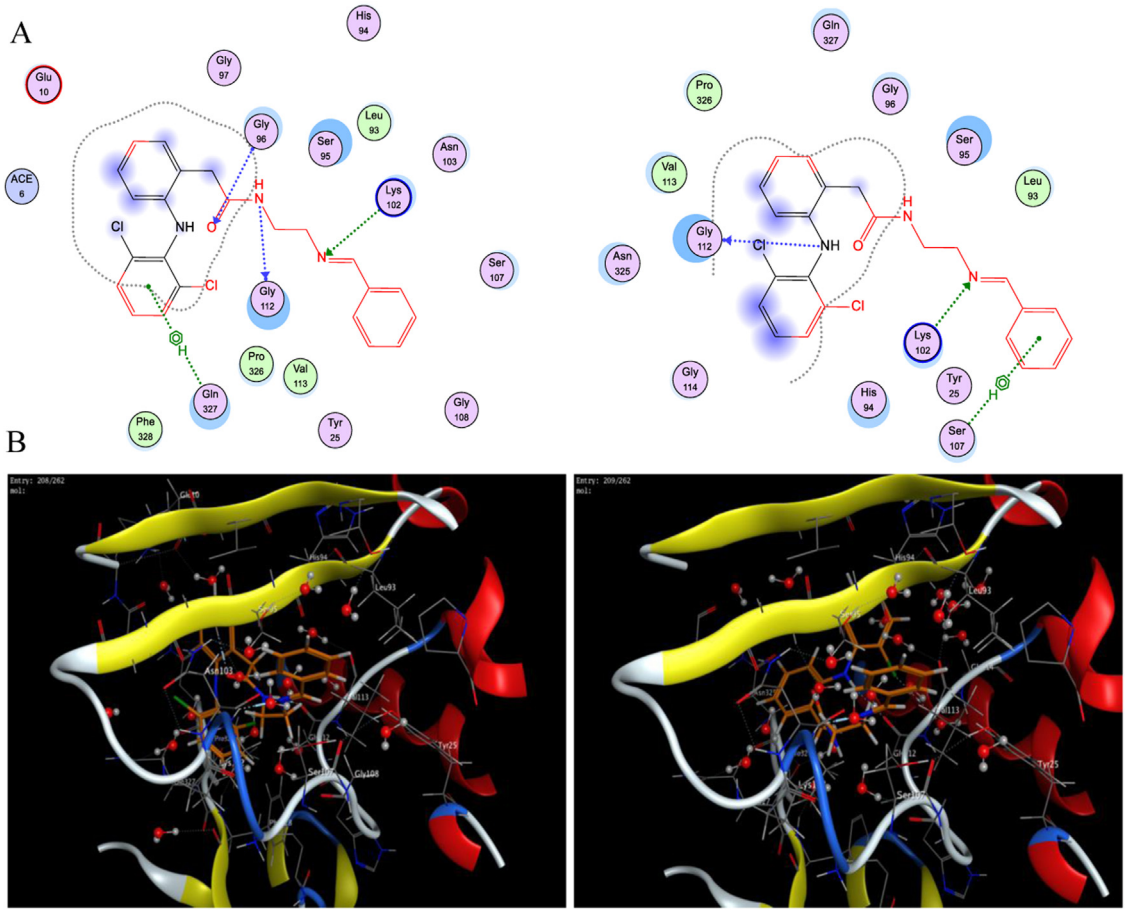


Fig. 2. Potential binding modes of compounds **10** (A) compared to DNA gyrase (yellow) in the polymerase binding site of *E. coli* sliding clamp (PDB ID: 4MJQ). Electrostatic surface representation of protein surface: positive (blue), neutral (white), and negative (red). (B) The corresponding 2D ligand–protein interactions.

3. Experimental

3.1. Chemistry

All solvents and reagents used for the synthesis of the target compounds were of commercial grades used without further purification. Melting points were measured and uncorrected on an electro thermal melting point system [Fisher-John apparatus]. For following the chemical reactions, pre-coated silica gel plates (TLC) (Fluka 70643-50EA. SIGMA-ALDRICH, Germany) were used. FT-IR spectral analyses were recorded using KBr disks on a FT-IR 820/PC Shimadzu. Elemental analyses (C, H, N, Cl, and S) were measured on an Elemental Analyses system GmbH-Vario EL V2.3 micro-analyzer. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker (^{1}H NMR: 400MHz, ^{13}C NMR: 100 MHz) and Bruker Avance Neo 500 MHz with CryoProbe Prodigy system (^{1}H, 500 MHz; ^{13}C, 126 MHz) using TMS as an internal standard. The chemical shifts were recorded in ppm δ scale. All reactions were carried out under an air atmosphere. The starting materials [2-(2,6-dichloroanilino)phenyl]acetic acid hydrazide (**1**) and ethyl- [2-(2,6-dichloroanilino)phenyl]acetate (**8**) were prepared according to previous procedure [45], and used as a precursor for the synthesis of the other compounds with m.p. 158-160 °C and 68-70 °C.

3.1.1. 2-Chloro-N'-(2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl) aceto-hydrazide (2)

To a solution of acetohydrazide derivative **1** (2.2 g, 7 mmol) in dioxane (25 mL), chloroacetyl chloride (2 mL) was added drop wise with stirring, the mixture was refluxed for 6 h. The solid precipitate which formed after pouring the cooled solution on diluted sodium carbonate solution (10%), was collected, and re-crystallized from ethanol as pale-yellow crystals, mp 220-221 °C, yield 2.4 g (85 %). FT-IR (KBr) cm^{-1} showed bands at 3195 (NH groups), 3034 (C–H aromatic), 1659 (2C=O). ^{1}H NMR (500 MHz, DMSO-d_6): 3.66 (s, 2H, CH_2), 4.16 (s, 2H, CH_2), 6.29 (s, 1H, NH), 7.06-8.30 (m, 7H, Ar-H), 10.51 (s, 1H, NH), 10.65 (s, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6): 37.04, 45.34, 116.10, 120.60, 124.08, 124.69, 125.26, 127.47, 129.20, 129.55, 130.42, 134.06, 137.09, 142.85, 143.97, 147.42, 167.88, 169.87. HPLC–MS Chromatograms m/z=388.10 Anal. Calcd. For: $C_{16}H_{14}Cl_3N_3O_2$(386.66): C, 49.70; H, 3.65; Cl, 27.50; N, 10.87% Found: C, 49.75; H, 3.68; Cl, 27.47; N, 10.83%.

3.1.2. N'-(Aminoglycyl)-2-(2-((2,6-dichlorophenyl)amino)phenyl) acetohydrazide (3)

A suspension of the chloroacetylacetohydrazide **2** (0.4 g,1 mmol) and hydrazine hydrate (5 mL) in Absolute ethanol (10 mL) were heated under reflux for 5h., then the reaction mixture was allowed to cool. The solid product was collected and recrystallized from ethanol as ethanol as white crystals; mp 168-170 °C; yield 0.3 g (75 %). FT-IR (KBr) cm^{-1} showed bands at 3327 (NH_2, NH groups), 3025 (C–H aromatic), 2925 (C–H aliphatic), 1637 (2 C=O). ^{1}H NMR (400MHz, $CDCl_3$): 3.28 (s, 2H, CH_2), 3.57 (s, 2H, CH_2), 6.16 (s, 1H, NH_2), 6.29 (s, 1H, NH), 7.09-7.53 (m, 7H, Ar-H), 8.45 (s, 1H, NH), 10.14 (s, 1H, NH), 11.30 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 39.45, 53.65, 120.23, 124.36, 130.15, 133.36, 139.47, 144.78, 145.16, 172.16, 172.37. Anal. Calcd. For: $C_{16}H_{17}Cl_2N_5O_2$ (382.25): C,

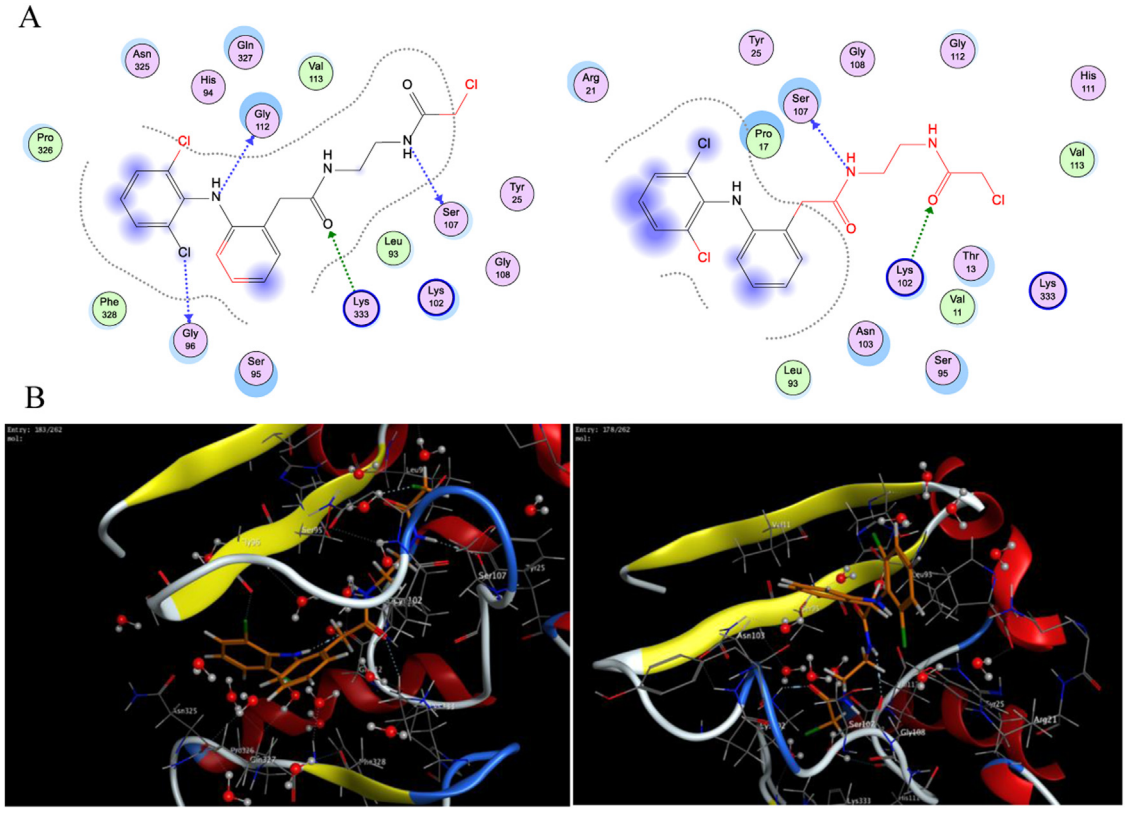


Fig. 3. Potential binding modes of compounds **11** (A) compared to DNA gyrase (yellow) in the polymerase binding site of *E. coli* sliding clamp (PDB ID: 4MJQ). Electrostatic surface representation of protein surface: positive (blue), neutral (white), and negative (red). (B) The corresponding 2D ligand–protein interactions.

50.30; H, 4.46; Cl, 18.55; N, 18.32% Found: C, 50.26; H, 4.41; Cl, 18.50; N, 18.37%.

3.1.3. 2-(2-((2,6-Dichlorophenyl)amino)phenyl)-N'-(3-thioxo-1,2,3,6-tetrahydro-1,2,4-triazin-5-yl)acetohydrazide (4)

To a solution of the chloroacetylacetohydrazide **2** (0.4g, 1 mmol) in DMF (15 mL) and anhydrous potassium carbonate (0.1 g, 1 mmol) thiosemicarbazide (0.1 g, 1 mmol) was added. The reaction mixture was heated under reflux for 15 h. then left to cool, poured with stirring into an ice-cold water mixture (100 ml) and neutralized with diluted HCl. The solid product was collected and recrystallized from [(ethanol: n-hexane) mixture (1:1)] as yellow crystals; mp 250-252 °C; yield 0.3 g (75 %). FT-IR (KBr) cm^{-1} showed bands at 3271 (NH groups), 2924 (C–H aliphatic), 1659 (C=O). ^{1}H NMR (400MHz, $CDCl_3$): 3.40 (s,2H, CH_2), 3.45 (s,2H, CH_2), 6.29 (s,1H, NH),6.92-7.40 (m,7H, Ar-H), 7.93 (s, 1H, NH), 8.39 (s, 1H, NH), 10.12 (s, 1H, NH), 10.88 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 39.45, 47.13, 120.33, 124.36, 127.07, 128.27, 130.15, 132.60, 133.54, 144.61, 147.15, 156.56, 170.38, 171.05. Anal. Calcd. For: $C_{17}H_{16}Cl_2N_6OS$ (423.32): C, 48.24; H, 3.81; Cl, 16.75; N, 19.85; S, 7.57% Found: C, 48.22; H, 3.86; Cl, 16.70; N, 19.89; S, 7.52%.

3.1.4. 4-((2-(2-(2-(2-((2,6-Dichlorophenyl)amino)phenyl)acetyl) hydrazinyl)-2-oxoethyl)amino)-N-(thiazol-2(3H)-ylidene) benzenesulfonamide (5)

The chloroacetamide **2** (0.4 g,1 mmol) and sulfathiazole (0.3 g, 1mmol) in ethanol (10 mL) were refluxed in the presence of anhydrous potassium carbonate (0.1 g, 1 mmol) for 6h., then left to cool, poured into an ice-cold water mixture (100 mL) and neutralized with diluted HCl. The solid precipitate was collected and recrystallized ethanol as yellow crystals; mp 179-181°C; yield 0.4 g (80 %). FT-IR (KBr) cm^{-1} showed bands at 3387 and 3204 (NH groups), 2930 (C–H aliphatic), 1728,1655 (CO groups). ^{1}H NMR (400MHz, DMSO-d_6): 3.36 (s, 2H, CH_2), 3.92 (s, 2H, CH_2), 6.29 (s,1H, NH), 6.54-7.39 (m, 11H, Ar-H), 7.74 (d, 2H, 2CH thiazole), 8.74 (s, 1H, NH), 8.98 (s, 1H, NH), 10.01 (s, 1H, NH), 10.56 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 38.56, 45.56, 112.97, 114.23, 119.87, 123.15, 124.76, 128.34, 128.98, 129.45, 130.29, 130.46, 131.34, 132.17, 134.65, 142.87, 143.76, 153.66, 163.45, 172.78, 173.01. Anal. Calcd. For: $C_{25}H_{22}Cl_2N_6O_4S_2$ (605.51): C, 49.59; H, 3.66; Cl, 11.71; N, 13.88; S, 10.59% Found: C, 49.54; H, 3.63; Cl, 11.76; N, 13.84; S, 10.55%.

3.1.5. 2-(2-((2,6-Dichlorophenyl)amino)phenyl)-N'-(2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)acetyl)acetohydrazide (6)

A mixture of compound **3** (1.1 g, 3 mmol) and ethyl acetoacetate (10 mmol) was refluxed under solvent free conditions for 4 h., then (10 mL) absolute ethanol was added. The reaction mixture was refluxed for additional 1h. The solid product which separated out during reflux, was filtered off and recrystallized ethanol as yellow crystals; mp >300 °C; yield 1.1 g (79%). FT-IR (KBr) cm^{-1} showed bands at 3355, 3211 (NH groups), 3034 (C–H aromatic), 2955, 2921, 2850 (C–H aliphatic), 1687 (C=O groups). ^{1}H NMR (400 MHz, DMSO-d_6): 1.95 (s, 3H, CH_3), 2.98 (s, 2H, CH_2), 3.44 (s, 2H, CH_2), 4.26 (s, 2H, CH_2), 6.35 (s, 1H, NH), 6.79-7.38 (m, 7H, Ar-H), 9.81 (s, 1H, NH), 11.23 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 14.54, 37.98, 50.76, 89.79, 122.37, 130.87, 132.55, 133.15, 135.74, 136.98, 140.19, 144.71, 147.65, 167.87, 170.19, 172.87. Anal. Calcd. For: $C_{20}H_{19}Cl_2N_5O_3$ (448.30): C, 53.58; H, 4.27; Cl, 15.82; N, 15.62% Found: C, 53.53; H, 4.32; Cl, 15.86; N, 15.66%.

3.1.6. 2-(2-((2,6-Dichlorophenyl)amino)phenyl)-N'-(2-(3,5-dimethyl-1H-pyrazol-1-yl) acetyl) acetohydrazide (7)

A mixture of compound 3 (1.1g, 3 mmol) and acetylacetone (10 mmol) was refluxed under solvent free conditions for 4 h.

then (10 ml) Absolute ethanol was added. The reaction mixture was refluxed for additional 1h. The solid product which separated out during reflux, was filtered off and recrystallized ethanol as yellow crystals; mp 295-297 °C; yield 1.1 g (85%). FT-IR (KBr) cm^{-1} showed bands 3354, 3210 (3NH groups), 3036 (C–H aromatic), 2923, 2852 (C–H aliphatic), 1687 (2 C=O), 1597 (C=N stretches). ^{1}H NMR (400MHz, DMSO-d_6): 2.21-2.25 (s, 6H, $2CH_3$), 3.23 (s, 2H, CH_2), 4.76 (s, 2H, CH_2), 6.30 (s, 1H, NH), 6.75-7.20 (m, 7H, Ar-H), 8.02 (s, 1H, CH pyrazole), 10.35 (s, 1H, NH), 10.96 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 12.45, 14.96, 38.35, 47.98, 107.18, 120.55, 124.25, 125.17, 126.22, 131.87, 132.85, 134.65, 136.98, 138.97, 143.09, 145.17, 147.65, 169.76, 172.87. Anal. Calcd. For: $C_{21}H_{21}Cl_2N_5O_2$ (446.33): C, 56.51; H, 4.74; Cl, 15.89; N, 15.69% Found: C, 56.60; H, 4.78; Cl, 15.83; N, 15.64 %.

3.1.7. N-(2-Aminoethyl)-2-(2-((2,6-dichlorophenyl)amino) phenyl)acetamide (9)

The amino ester compound **8** (0.7 g, 2 mmol) and ethylene diamine (1.3 mL, 20 mmol) was refluxed under solvent free conditions for 4 h., then Absolute ethanol (10 mL) was added. Then reflux was continued for additional 2h., then was allowed to cool. The solid precipitate was filtered off, dried, and recrystallized from ethanol as yellow crystals; mp 158-160 °C; yield 0.6 g (85%). FT-IR (KBr) cm^{-1} showed bands at 3353, 3211 (NH_2, NH groups), 3014 (C–H aromatic), 2916 (C–H aliphatic), 1642 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): 2.96 (t,2H, CH_2), 3.48 (t, 2H, CH_2), 3,84 (s, 2H, CH_2), 6.23 (s, 1H, NH), 6.54 (s, 2H, NH_2), 7.15-7.98 (m, 7H, Ar-H), 9.02 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 37.89, 44.56, 46.89, 119.67, 123.17, 126.45, 126.67, 128.67, 129.56, 131.45, 132.31, 134.19, 136.12, 139.24, 143.13, 147.89, 175.78. Anal. Calcd. For: $C_{16}H_{17}Cl_2N_3O$ (338.23):C, 56.82; H, 5.07; Cl, 20.96; N, 12.42 % Found: C, 56.86; H, 5.04; Cl, 20.91; N, 12.47%.

3.1.8. N-(2-(Benzylideneamino)ethyl)-2-(2-((2,6-dichlorophenyl) amino)phenyl) acetamide (10)

A mixture of compound 9 (0. 4g, 1mmol) and benzaldehyde (0.2 ml,2 mmol) in dioxane (10 ml), was heated under reflux for 4 h. then left to cool. And pouring into ice-water mixture, the solid precipitate was filtered off, dried and recrystallized from ethanol as white crystal; mp 260-262 °C; yield 0.3 g (75%). FT-IR (KBr) cm^{-1} showed bands at 3304 (NH groups), 3084 (C–H aromatic), 2955, 2921 and 2850 (C–H aliphatic), 1640 (C=O), 1605 (C=N stretches). ^{1}H NMR (400MHz, DMSO-d_6): 3.02 (s,2H, CH_2), 3.47-3.55 (t,4H, $2CH_2$), 6.40 (s,1H, NH),7.08-7.53 (m,12H, Ar-H), 8.13 (s,1H, CH=N), 8.25 (s,1H, NH).^{13}C NMR (100 MHz, DMSO-d_6): 38.14, 39.01, 43.17, 123.89, 128.15, 129.35, 129.77, 130.71, 131.54, 132.67, 136.49, 144.87, 160.32, 161.55, 172.67.Anal. Calcd. For: $C_{23}H_{21}Cl_2N_3O$ (426.34):C, 64.80; H, 4.97; Cl, 16.63; N, 9.86% Found: C, 64.85; H, 4.93; Cl, 16.66; N, 9.89%.

3.1.9. 2-Chloro-N-(2-(2-(2-((2,6-dichlorophenyl)amino)phenyl) acetamido) ethyl)acetamide (11)

A mixture of compound **9** (0.4 g, 1mmol) and chloroacetyl chloride (0.1 mL, 1.5 mmol) in dioxane (10 mL) was heated under reflux for 6 h., then left to cool. And pouring into ice-water mixture, the solid precipitate was filtered off, dried, and recrystallized from ethanol as white crystals; mp 299-300 °C; yield 0.3 g (60%). ^{1}H NMR (400MHz, DMSO-d_6): 3.12 (s, 2H, CH_2), 3.44 (t, 4H, $2CH_2$), 4.23 (s, 2H, CH_2), 6.24 (s, 1H, NH), 7.17-7.78 (m, 7H, Ar-H), 9.65 (s, 1H, NH), 9.97 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 38.98, 39.22, 42.35, 119.83, 123.90, 124.63.128.17, 129.33, 129.62, 130.78, 131.98, 134.68, 136.46, 142.18, 143.98, 169.45, 172.77. Anal. Calcd. For: $C_{18}H_{18}Cl_3N_3O_2$ (414.71): C, 52.13; H, 4.38; Cl, 25.64; N, 10.13% Found: C, 52.17; H, 4.33; Cl, 25.69; N, 10.18%.

3.2. Antimicrobial activities and minimum inhibitory concentration (MIC)

The species of microorganisms used in the anti-microbial tests were obtained from the Microbiology Department, Faculty of Agriculture, Assiut University. Molecular modeling experiments were performed by the Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt. A Processor Intel (R) Pentium (R) CPU N3510@1.99 GHz and Microsoft Windows 8.1 pro (64 Bit) operating system used both molecular modeling calculations and simulation docking studies. All the new synthesized compounds listed in Table 1 were screened for their in vitro antibacterial activity against model Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacteria (*Serratia marcescens* and *Pseudomonas aeruginosa*). The antibacterial activity and MIC were determined by the agar diffusion assay using the filter paper disc method [47]. The MICs of the synthesized compounds were determined against Gram-positive and Gram-negative bacteria. It was carried out by impregnation of different concentrations of synthesized compounds (50, 100, 150, 200 μg/mL) in DMSO as a solvent and placed on filter paper discs (5 mm). Nutrient agar media was used for the inoculation of bacteria. Standard antibiotic discs (Chloramphenicol 50 mg) and blank discs (impregnated with DMSO) were used as positive and negative control. The plates were then incubated at 37 °C for 24 hr. The zones of inhibition were measured in mm and recorded. The lowest concentration that inhibited the growth of the test organisms was recorded as the MIC. The biological activity as expressed by the growth inhibition zone of the tested microorganism is listed in Table 1. The MICs were recorded.

3.3. Molecular docking

All computational work was accomplished with Molecular Operating Environment (MOE) version 2020.09, Chemical Computing Group ULC, 910–1010 Sherbrooke St. W. Montreal, Quebec, H3A 2A2, Canada. The computational approach was adopted with minor changes from described protocol 50. The complex crystal structure of DNA gyrase with its inhibitor (Clorobiocin) was retrieved from a protein data bank with PDB ID: 1KZN and 5KIR, respectively [48]. All ligands and water molecules were removed, and the PDBQT files were appropriately created. The binding affinity of the synthesised diclofenac derivatives with DNA gyrase was predicted using the average of the lowest energy of docking. Chimera 1.12 software was used to display and evaluate the best-scored conformation of docked models.

4. Conclusion

To sum up, in this work the synthesis of heterocyclic compounds containing diclofenac moiety that possess antibacterial activity was described. The all new synthesized compounds were fully characterized utilizing elemental analysis and different spectral analysis tools such as FT-IR, NMR and Ms spectroscopy. All compounds were in vitro investigated for their antibacterial showing moderate to high activity. The MIC values found for most of the compounds indicate that they are extremely effective as antibacterial agents. Studies using molecular docking indicate that our diclofenac derivatives may target the bacterial sliding clamp and the fungal cyclooxygenase-like enzymes for their antibacterial and antifungal actions. In addition, the drug-like characteristics of the synthesized compounds have the potential to make them orally active candidates.

Declaration of Competing Interest

The authors declared no conflict of interest, including any financial, personal, or other ties with other persons or enterprises.

CRediT authorship contribution statement

Mahmoud M. Hamed: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Mostafa Sayed:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Shawkat A. Abdel-Mohsen:** Data curation, Methodology, Writing – original draft. **Abdelreheem Abdelfatah Saddik:** Formal analysis, Investigation, Methodology, Writing – original draft. **Omneya A. Ibrahim:** Data curation, Formal analysis, Investigation, Methodology, Software. **Adel M. Kamal El-Dean:** Conceptualization, Data curation, Formal analysis, Project administration, Resources, Supervision. **Mahmoud S. Tolba:** Conceptualization, Data curation, Formal analysis, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Data Availability

No data was used for the research described in the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.134371.

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