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Antibacterial and Urease Inhibitory activity of New Piperazinyl *N*-4 Functionalized Ciprofloxacin-oxadiazoles

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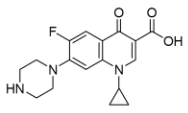
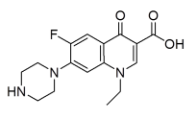
Abstract

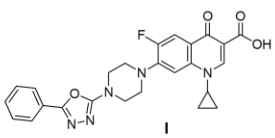
This research includes design of new ciprofloxacin bearing oxadiazole at the *N*-4 piperazinyl for the purpose of having urease inhibitory activity as well as antibacterial activity. Hence, a group of new *N*-4 piperazinyl oxdiazole derivatives of ciprofloxacin was prepared and characterized using different spectroscopic and analytical techniques including ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis. Compounds **5b** and **5c** experienced moderate activity against the urease producing *Klebsiella pneumoniae* strains better than the standard drug used chloramphenicol and less than the parent drug ciprofloxacin. On the other hand, most of the tested compounds showed a urease inhibitory activity more than the parent drug, ciprofloxacin and comparable to standard, thiourea. The docked compounds exhibited better binding to urease enzyme of *H. pylori* than standard, AHA with binding scores for correlate to the anti-urease assay results. Compound **5b** was the most potent anti-*Klebsiella pneumoniae* urease inhibitor with activity higher the standard (IC_{50} = 67.8 and 78.89 µM, respectively).

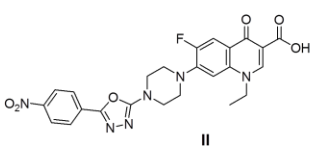
1. Introduction

Quinolones had emerged as interesting synthetic compounds with diverse chemical structures and biological activities. [1-3] They primary appeared as antibacterial agents with considerable impact in management of different infectious diseases. [4,5] Therefore, quinolones attracted researchers to design more derivatives, leading to various members of this class are widely used as drugs nowadays. [4,6-8] Moreover, many derivatives with promising activity were prepared, including candidates that reached clinical investigations. [9-12] Chemical modifications at C3 and C7 of typical quinolone scaffold have the greatest influence on their activity [13], where position 7 largely affects activity, access and direction toward different molecular targets. [14-18] Moreover, introducing bulky groups at C7 was reported to broaden the spectrum of antibacterial activity and reduce the likelihood of developing resistance. [19-21] Currently, used quinolones drugs became also subjects for structural variation and derivatization. Among modifications with positive results, oxadiazole hybrids of ciprofloxacin and norfloxacin, such as compounds **I** and **II** which revealed antibacterial activity better than their parent drugs. [22] Beside antibacterial activity, fluoroquinolones appeared as multifaceted bioactive compounds with numerous biological activities including urease inhibitory potential. [13,23,24] Urease is a nickel metalloenzyme that acts by converting urea to ammonia, leading to elevation of pH of the medium. [25,26] Urease is produced by different microorganisms and has a crucial rule for bacterial survival and pathogenesis. [27] Clear examples are *Proteus mirabilis* that utilized urease activity for urine basification to a favored level and *Helicobacter pylori* which have to alkalinize acidic gastric content for their survival and pathogenesis. [28,29] Urease plays a key role in developing urinary lithiasis and pyelonephritis during infection with urease positive bacteria, as well as peptic ulcer and gastric tumors due to *H. pylori* infection. [28,30,31] Additionally, urease was found to be entangled in different other acute and chronic diseases. [32,33] Concomitantly targeting bacteria and urease was proved to be beneficial for control of some diseases. [34] These findings lead to the emergence of urease as a valuable molecular target. [35] Guided by these aspects, this study was directed toward preparation of ciprofloxacin analogues as hybrids with oxadiazole derivatives, and evaluation of their activity against representatives for urease positive bacteria in addition to evaluation of urease inhibitory activity of the target compounds.







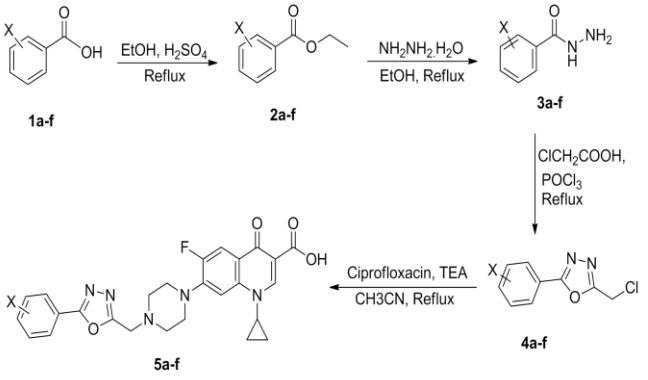


2. Results and discussion

2.1. Chemistry

Protocol for synthesis of the target compounds is illustrated by **(Scheme 1).** Benzoic acid derivatives **1a**–**f** were converted to the corresponding ethyl esters **2a**–**f** which in turn were reacted with hydrazine hydrate to afford hydrazides **3a**–**f** as reported. [36-38] Benzoic acid hydrazides were further cyclized to give 2-chloromethyl oxadiazole intermediates **4a**–**f** as in literatures. [37,38]. The later intermediates were linked to the piperazinyl *N*-4 of ciprofloxacin by alkylation reaction to yield the target compounds **5a**–**f.**

Different spectroscopic and analytical tools were applied for identification of the synthesized compounds including ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis. Compounds **5a**–**f** were characterized by a singlet signal (2H) at δ ~3.97 ppm representing CH_2 of the bridge between ciprofloxacin and oxadiazole moiety in ^{1}H-NMR-spectra. Additionally, compounds **5c** was characterized by a singlet signal (3H) at δ 2.41 ppm assigned to *p*-CH_3 group, while compound **5d** revealed a similar singlet signal at δ 3.84 ppm due to *p*-OCH_3 protons, ^{13}C-NMR Spectra of compounds **5a-f** were charecterized by a signal at δ ~52.50 assigned to CH_2 of the bridge between piperazine ring and oxadiazole scaffold. Compounds **5c** was also characterized by an additional aliphatic signal at δ 21.77 ppm related to *p*-$\underline{C}H_3$, however, compound **5d** showed a *p*-O$\underline{C}H_3$ signal at δ 55.58 ppm. Elemental analysis and MS furtherly confirmed the assigned structures of the synthesized compounds.



5a, X=H; **5b**, X=4-Cl; **5c**, X=4-CH_3; **5d**, X=4-OCH_3; **5e**, X=4-NO_2, **5f**, X=2-Cl

Scheme 1: Synthesis of target compounds **5a-f**

2.2. Biology

2.2.1. Antibacterial activity

Standard agar cup diffusion method was applied for antibacterial screening using two Gram-negative and urease producing bacterial strains, *Klebsiella pneumonia and Proteus mirabilis*. Compounds **5b** revealed activity against *Klebsiella pneumonia* more than the reference drug, chloramphenicol (MIC = 135.23 and 217.08 µM, respectively). However, antibacterial activity of this derivatives was less than the parent drug, ciprofloxacin (MIC = 40.16 µM). Compound 5e experienced IC_{50} of 458.27 µM, meanwhile, compounds **5a**, **5d** and **5f** showed anti *Klebsiella pneumonia* activity only at the highest concentration used, 2 mM. On the other hand, the test compounds were not active against *Proteus mirabilis* **(Tables 1)**.

2.2.2. Urease inhibitory activity

Indophenol test for detection of ammonia (Weatherburn method) [39] was applied for evaluation of urease inhibitory activity of the test compounds along with ciprofloxacin using thiourea as a reference enzyme inhibitor. [40] The assay depends on colored indophenol produced due to reaction of phenolic compounds and chlorine with ammonia liberated as a result of the enzyme catalytic activity on urea. Reduction in color intensity of samples treated with test compounds and standard at different concentrations was measured in comparison with untreated control one. Percentages of enzyme inhibition were calculated using the formula: 100-(optical $density_{test}$/optical $density_{control}$) X 100. [41]

Assay results revealed that most of the tested compounds have a urease inhibitory activity more than the parent drug, ciprofloxacin and comparable to standard, thiourea **(Figure 1)**. Compound **5b** was the most potent urease inhibitor with activity better than thiourea (IC_{50} = 67.80 and 78.89 µM, respectively).

2.2.3. Molecular docking

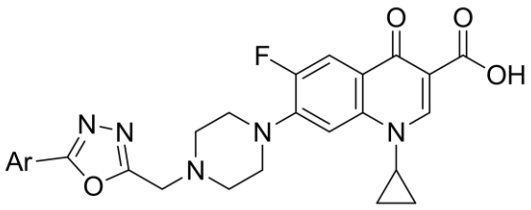
MOE Program was used to study docking of test compounds on active site of urease enzyme. Docking reliability was validated using the known X ray structure of *Helicobacter pylori* urease in complex with AHA (PDB: 1E9Y). [42] Molecular docking studies for the most active compounds **5b** and **5e** showed that all these derivatives interact with the bi-nickel center of the urease enzyme. The tested ligands were found to bind strongly to urease as indicated by binding energy values. Compounds **5b** and **5e** revealed binding scores of -41.17 and -39.36 kcal/mol, respectively that were better than that of acetohydroxamic acid (binding energy -33.44 kcal/mol). **(Figure 2)** shows binding mode of acetohydroxamic acid with *H. pylori* urease in a 2D structure, revealing coordination with the bi-nickel center in urease binding site and formation of a hydrogen bond with His221.

Docking results for compound **5b,** showed that the carbonyl of quinolone carboxylate could coordinate with Ni3002 and form a hydrogen bond with His136, His138 and Asp362. Hydroxyl group of the carboxylate group coordinates also with Ni3001 and involved in a hydrogen bonding with Asp362. Carbonyl oxygen at C4 of the quinolone ring core makes a hydrogen bond with His248 and His274. Additionally, benzene ring of quinolone interacts with Arg338 through arene-

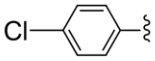
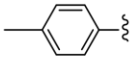
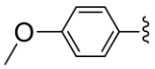
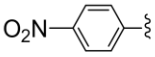
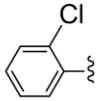
cation interaction and the *N*4 piperazinyl nitrogen forms a hydrogen bond with His328 (**Figure 3**). Binding mode of compound **5e** shows that the two oxygens of nitro group coordinate with the two Ni atoms, and hydroxyl group of the carboxylate moiety forms a hydrogen bond with Asp165 (**Figure 4**).

By comparing binding mode for each of the docked compounds **5b** and **5e** to the bi-Ni of urease enzyme center, we can note a variable binding mode according to nature of substituent at the phenyl moiety. Compound **5b** binds through carbonyl and hydroxyl of quinoline-3-carboxylate group. On the other hand, the nitrophenyl derivative (compound **5e**) can bind additionally through its nitro group with urease pocket. All the docked compounds exhibited better binding to urease enzyme of *H. pylori* than standard, AHA, and binding scores for docked derivatives correlate to the anti-urease assay results.

Table 1: Antibacterial activity of the tested compounds



5a-f

Compoud No.	Ar	*Klebsiella pneumonia*		*Proteus mirabilis*	
		Inhibition zone (mm) at 2mM	MIC (µM)	Inhibition zone (mm) at 2mM	MIC (µM)
5a		10	ND[a]	0	ND[a]
5b		15	135.23	0	ND[a]
5c		12	458.27	0	ND[a]
5d		8	ND[a]	0	ND[a]
5e		0	ND[a]	0	ND[a]
5f		10	ND[a]	0	ND[a]
Ciprofloxacin		26	40.16	15	113.19
Chloramphenicol		14	217.08	19	261.45

[a] **Not determined**

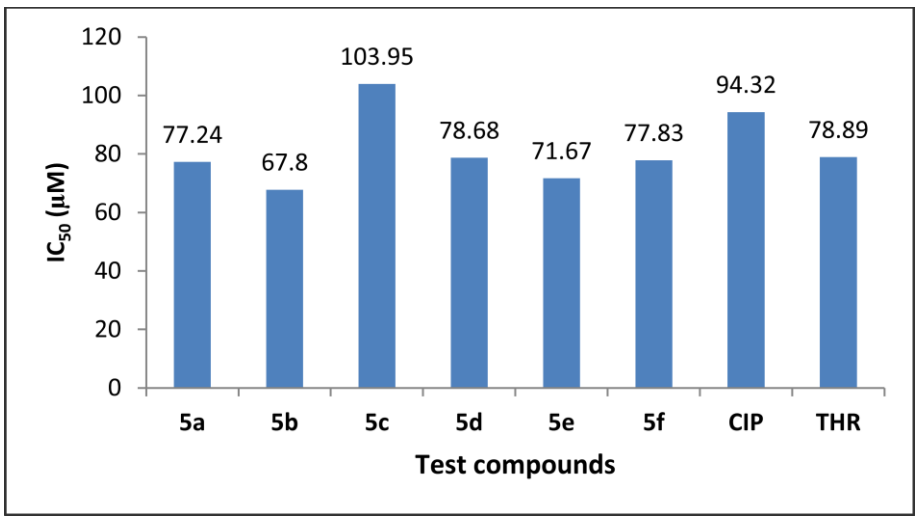


Figure 1: Urease inhibitory activity of the test compounds expressed as IC_{50} in µM.

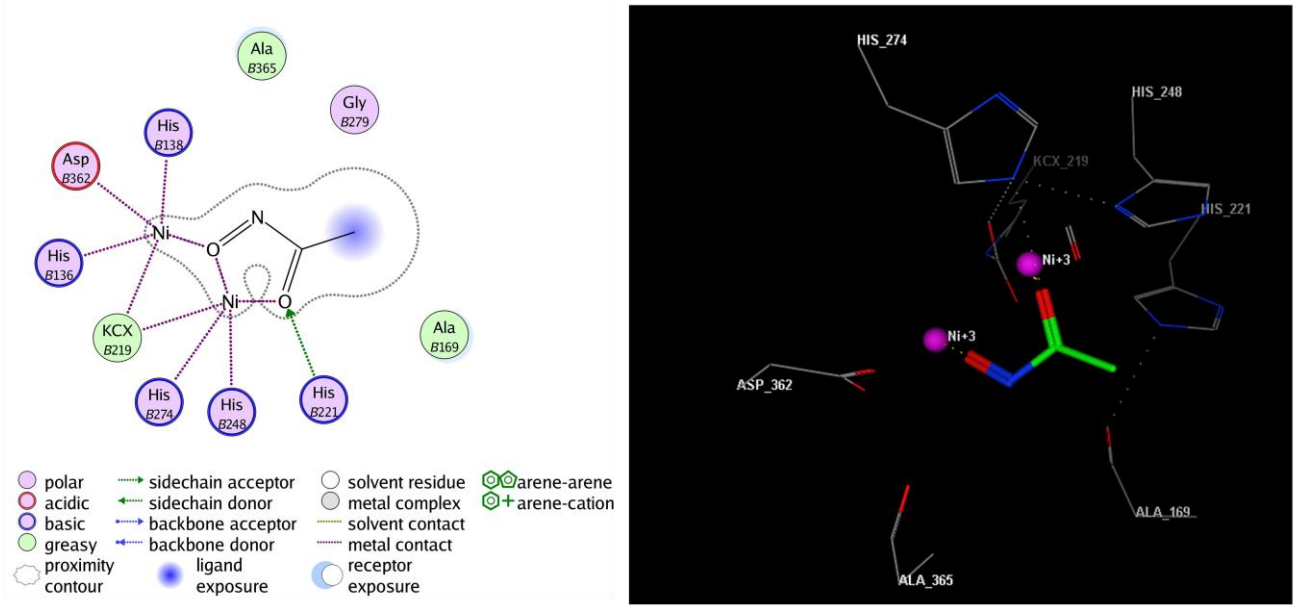


Figure 2: 2D And 3D docking of acetohydroxamic acid with *H. pylori* urease.

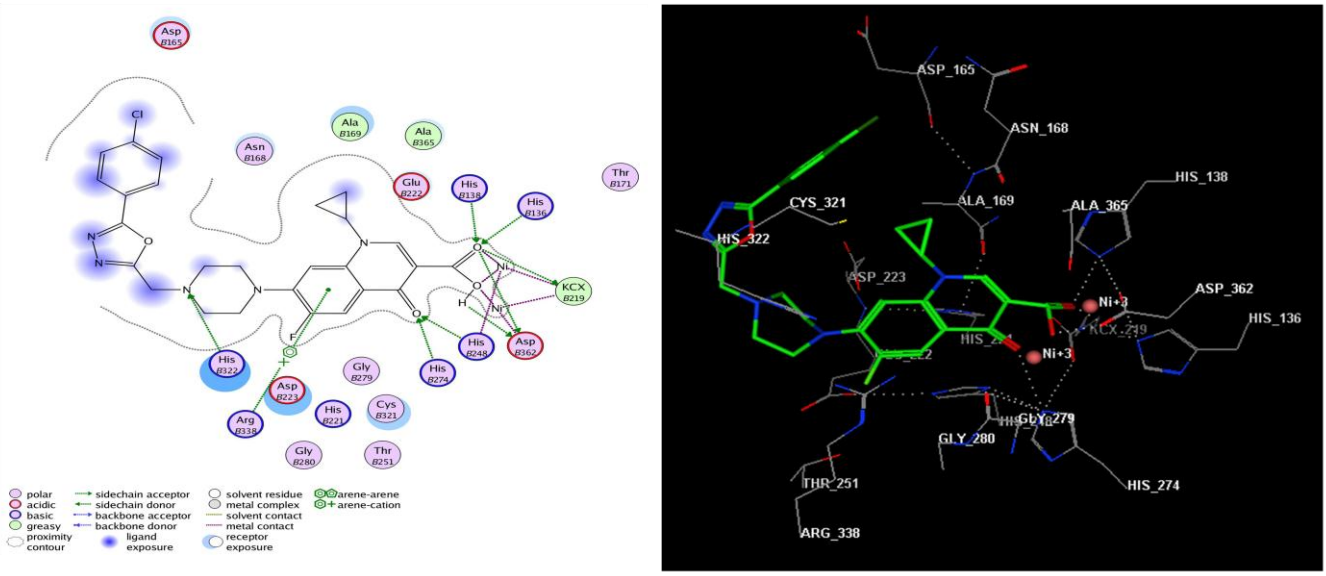


Figure 3: 2D And 3D docking of compound **5b** with *H. pylori* urease.

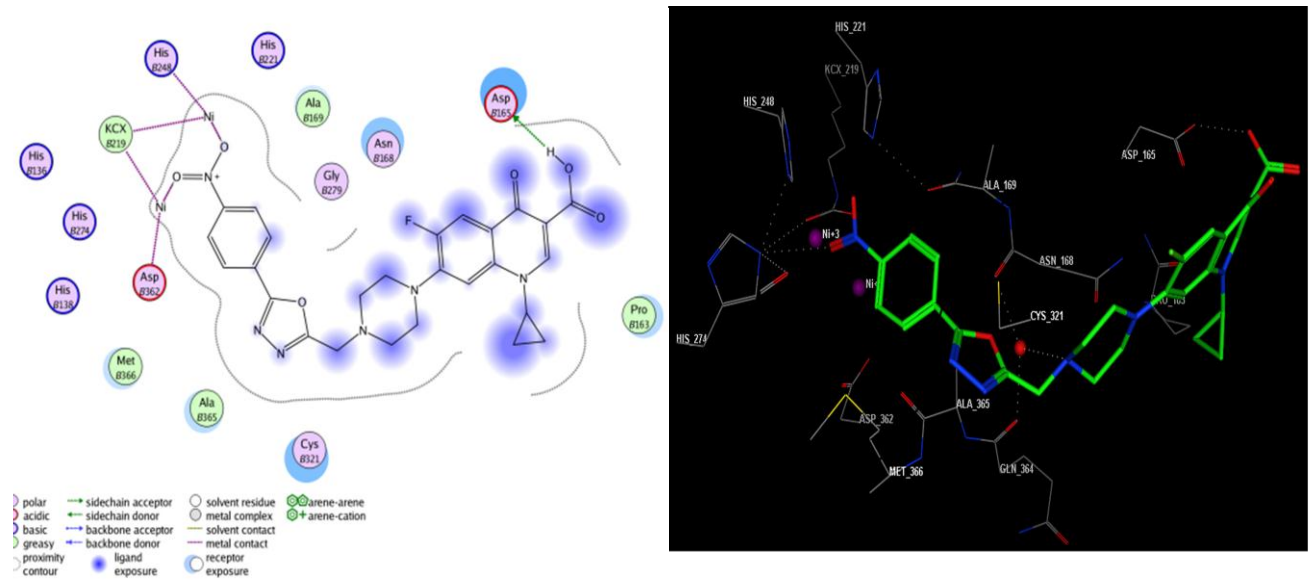


Figure 4: 2D and 3D docking of compound **5e** with *H. pylori* urease.

3. Conclusion

Analogues of ciprofloxacin, by functionalization at the piperazinyl *N*-4 with oxadiazole derivatives were synthesized and biologically investigated. The prepared compounds showed reduced antibacterial activity than their parent drug ciprofloxacin, however, compounds **5b** revealed activity against *Klebsiella pneumoniae* better than the standard drug used chloramphenicol (MIC =135.23 and 217.08 µM, respectively). These results indicated that presence of *p*-chloro substitution on phenyl moiety of oxadiazole hybrids was superior for antibacterial activity than other modifications in this series. On the other hand, the majority of the synthesized compounds experienced urease inhibitory activity better than the parent drug, ciprofloxacin and comparable to the standard urease inhibitor thiourea. Compound **5b** was the most potent urease inhibitor with activity higher the standard (IC_{50} = 67.8 and 78.89 µM, respectively).

4. Experimental section

4.1. Chemistry

All chemicals used for preparation of the target compounds were of the commercially available analytical grade quality. Reaction progress as well as product purity were monitored using TLC (Kieselgel 60 G F254 precoated plates, E. Merck, Dermastadt, Germany). Spots were detected by exposure to UV lamp (Spectroline CM-10, Seattle, USA) at λ 254 and 365 nm using. Melting points were determined on Stuart SMP1 electrothermal melting point apparatus (Stuart Scientific, Staffordshire, UK) and were uncorrected. ^{1}H-NMR And ^{13}C-NMR spectra were recorded on JEOL JNM ECX 500 MHz (JEOL Ltd, Musashino, Akishima, Tokyo, Japan) or BRUKER Avance III400 MHz spectrophotometer (Bruker AG, Switzerland) at 500 and 125 or 400 and 100 for ^{1}H and ^{13}C, respectively. TMS was used as an internal standard and $CDCl_3$ or DMSO-d_6 as a solvent. Chemical shift (δ) values are expressed in parts per million (ppm) and coupling constants (J) in Hertz (Hz). Signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet. Elemental analysis was carried out at the regional center for mycology and biotechnology, Al-Azhar University, Cairo, Egypt. Mass spectroscopy was performed using DI-50 unit of Shimadzu GC/MS-QP 5050A at the Regional Center for Mycology and Biotechnology, Al-Azhar University Cairo, Egypt.

4.1.1. General procedure for synthesis of 1-cyclopropyl-6-fluoro-4-oxo-7-(4-((5-(substituted)phenyl-1,3,4-oxadiazol-2-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 5a-f

Organic acids **1a**–**f** were esterified using ethanol and conc. sulfuric to obtain esters **2a-e** which in turn reacted with hydrazine hydrate to give hydrazides **3a**–**f**. The later were reacted with chloroacetic acid to prepare 2-(chloromethyl)-5-(substituted)phenyl-1,3,4-oxadiazole intermediates **4a**–**f** according to reported procedures. [37,38,43,44] The appropriate intermediate **4a**–**e** (0.001 mol) in a mixture with ciprofloxacin (0.331 g, 0.001 mol) and triethyamine (0.202 g, 0.002 mol) in acetonitrile (10 mL) were heated at reflux for 7–8 h. The formed precipitate was filtered off while hot, washed with acetonitrile and dried. [17]

4.1.1.1. 1-Cyclopropyl-6-fluoro-4-oxo-7-{4-[(5-phenyl-1,3,4-oxadiazol-2-yl)methyl]piperazin-1-yl}-1,4-dihydroquinoline-3-carboxylic acid 5a

Yield 0.279 g (57%); faint yellow powder, mp: 212–14℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H, C2-H), 8.06–8.03 (m, 2H, Ar-H), 7.89 (d, J = 13.0 Hz, 1H, C5-H), 7.56–7.47 (m, 3H, Ar-H), 7.33 (d, J = 7.1 Hz, 1H, C8-H), 3.98 (s, 2H, CH_2), 3.57–3.49 (m, 1H, cyclopropyl-H), 3.41–3.35 (m, 4H, piperazine-4H), 2.90–2.84 (m, 4H, piperazine-4H), 1.36 (q, J = 6.9 Hz, 2H, cyclopropyl-H), 1.21–1.12 (m, 2H, cyclopropyl-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 13C NMR (125 MHz,) δ 177.06, 167.01, 165.56, 163.12, 153.93, 147.46, 145.74, 139.10, 132.03, 129.18, 127.06, 123.72, 119.89, 112.35, 108.03, 105.08, 52.50, 51.98, 49.66, 35.41, 8.29; MS m/z calcd for $C_{26}H_{24}FN_5O_4$ [M^+]: 489.18, found: 489.42; Anal. calcd for $C_{26}H_{24}FN_5O_4$: C, 63.80; H, 4.94; N, 14.31; found: C, 63.92; H, 4.98; N, 14.24.

4.1.1.2. 7-{4-[(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl]piperaz-in-1-yl}-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 5b

Yield 0.288 g (55%); faint yellow powder, mp: 230–32℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H, C2-H), 8.02–7.95 (m, 2H, Ar-H), 7.86 (d, J = 10.9 Hz, 1H, C5-H), 7.50–7.43 (m, 2H, Ar-H), 7.30 (d, J = 7.7 Hz, 1H, C8-H), 3.97 (s, 2H, CH_2), 3.57–3.52 (m, 1H, cyclopropyl-H), 3.38–3.37 (m, 4H, piperazine-4H), 2.87–2.85 (m, 4H, piperazine-4H), 1.35 (m, 2H, cyclopropyl-H), 1.14 (m, 2H, cyclopropyl-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 177.04, 166.97, 163.26, 154.46, 147.50, 145.70, 139.11, 138.35, 129.67, 128.35, 121.83, 119.66, 115.56, 112.26, 108.05, 105.06, 52.55, 51.97, 49.63, 35.40, 8.28; MS m/z calcd for $C_{26}H_{23}ClFN_5O_4$ [M^+]: 523.14, found: 523.65; Anal. calcd for $C_{26}H_{23}ClFN_5O_4$: C, 59.60; H, 4.42; N, 13.37; found: C, 59.54; H, 4.38; N, 13.44.

4.1.1.3. 1-Cyclopropyl-6-fluoro-4-oxo-7-{4-[(5-(p-tolyl)-1,3,4-oxadiazol-2-yl)methyl]piperazin-1-yl}-1,4-dihydroquinoline-3-carboxylic acid 5c

Yield 0.342 g (68%); yellow powder, mp: 214–18℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H, C2-H), 7.96–7.87 (m, 3H, C5-H & 2Ar-H), 7.36–7.26 (m, 3H, C8-H & 2Ar-H), 3.97 (s, 2H, CH_2), 3.57–3.49 (m, 1H, cyclopropyl-H), 3.43–3.33 (m, 4H, piperazine-4H), 2.92–2.79 (m, 4H, piperazine-4H), 2.41 (s, 3H, Ph-CH_3), 1.40–1.30 (m, 2H, cyclopropyl-H), 1.20–1.12 (m, 2H, cyclopropyl-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 177.10, 167.06,

165.72, 162.81, 153.68, 147.43, 145.77, 142.53, 139.06, 129.84, 127.01, 120.89, 119.86, 112.40, 107.96, 104.96, 52.49, 51.94, 49.65, 35.39, 21.77, 8.31; MS m/z calcd for $C_{27}H_{26}FN_5O_4$ [M^+]: 503.20, found: 503.41; Anal. calcd for $C_{27}H_{26}FN_5O_4$: C, 64.40; H, 5.20; N, 13.91; found: C, 64.29; H, 5.17; N, 13.97.

4.1.1.4. 1-Cyclopropyl-6-fluoro-7-{4-[(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carbox-ylic acid 5d

Yield 0.280 g (40%); faint yellow powder, mp: 206–08℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H, C2-H, C2-H), 7.95 (d, J = 9.2 Hz, 2H, Ar-H), 7.85 (d, J = 13.6 Hz, 1H, C5-H), 7.32 (d, J = 7.2, 1H, C8-H), (d, J = 9.2 Hz, 2H, Ar-H), 3.94 (s, 2H, CH_2), 3.84 (s, 3H, Ph-OCH_3), 3.59–3.45 (m, 1H, cyclopropyl-H), 3.42–3.31 (m, 4H, piperazine-4H), 2.90–2.79 (m, 4H, piperazine-4H), 1.38–1.30 (m, 2H, cyclopropyl-H), 1.17–1.12 (m, 2H, cyclopropyl-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 177.02, 167.07, 165.46, 162.58, 152.63, 147.42, 145.69, 142.16, 139.09, 128.82, 119.78, 116.13, 114.56, 112.27, 107.93, 105.11, 55.58, 52.47, 51.95, 49.63, 35.46, 8.27; MS m/z calcd for $C_{27}H_{26}FN_5O_5$ [M^+]: 519.19, found: 519.34; Anal. calcd for $C_{27}H_{26}FN_5O_5$: C, 62.42; H, 5.04; N, 13.48; found: C, 62.56; H, 5.09; N, 13.39.

4.1.1.5. 1-Cyclopropyl-6-fluoro-7-{4-[(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carbox-ylic acid 5e

Yield 0.326 g (61%); faint yellow powder, mp: 256–60℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 14.80 (s, 1H, COOH), 8.70 (s, 1H. C2-H), 8.32 (d, J = 8.6 Hz, 2H, Ar-H), 8.21 (d, J = 8.6 Hz, 2H, Ar-H), 7.97 (d, J = 13.0 Hz, 1H, C5-H), 7.29 (d, J = 6.9 Hz, 1H, C8-H), 3.98 (s, 2H, CH_2), 3.51–3.41 (m, 1H, cyclopropyl-H), 3.40–3.29 (m, 4H, piperazine-4H), 2.92–2.82 (m, 4H, piperazine-4H), 1.39–1.28 (m, 2H, cyclopropyl-H), 1.21–1.10 (m, 3H, cyclopropyl-H); 13C NMR (100 MHz, $CDCl_3$) δ 177.30, 166.84, 164.39, 162.00, 152.13, 147.50, 144.61, 139.05, 137.59, 131.70, 128.23, 124.40, 122.34, 112.55, 108.41, 105.05, 52.53, 51.90, 49.63, 35.20, 8.22; MS m/z calcd for $C_{26}H_{23}FN_6O_6$ [M^+]: 534.17, found: 534.41; Anal. calcd for $C_{26}H_{23}ClFN_5O_4$: C, 58.42; H, 4.34; N, 15.72; found: C, 58.28; H, 4.29; N, 15.81.

4.1.1.6. 7-{4-[(5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl]piperaz-in-1-yl}-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 5f

Yield 0.183 g (35%); yellow powder, mp: 114–16℃; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 1H, C2-H), 7.95 (d, J = 7.4 Hz, 1H, Ar-H), 7.87 (d, J = 13.0 Hz, 1H, C5-H), 7.52 (d, J = 8.0 Hz, 1H, Ar-H), 7.46 (t, J = 7.2 Hz, 1H, Ar-H), 7.39 (t, J = 7.5 Hz, 1H, Ar-H), 7.35–7.29 (m, 1H, C8-H), 4.03 (s, 2H, CH_2), 3.59–3.47 (m, 1H, cyclopropyl-H), 3.43–3.31 (m, 4H, piperazine-4H), 2.92–2.81 (m, 4H, piperazine-4H), 1.40–1.30 (m, 2H, cyclopropyl-H), 1.20–1.11 (m, 2H, cyclopropyl-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 177.02, 167.01, 163.60, 153.68, 147.44, 145.69, 139.10, 133.18, 132.73, 131.35, 127.26, 123.06, 119.84, 112.31, 107.98, 105.08, 52.40, 51.88, 49.67, 35.44, 8.29; MS m/z calcd for $C_{26}H_{23}ClFN_5O_4$ [M^+]: 523.14, found: 523.63; Anal. calcd for $C_{26}H_{23}ClFN_5O_4$: C, 59.60; H, 4.42; N, 13.37; found: C, 59.43; H, 4.437; N, 13.48.

4.2. Molecular docking study

Docking simulation study was performed using Molecular Operating Environment (MOE®) version 2008.10 (Chemical Computing Group Inc., Montreal, Canada). [45] All minimizations were performed with MOE until a RMSD gradient 0.01 Kcal mol^{-1} $A^{\circ-1}$ with MMFF94X force-field. For this purpose, tested compounds docked into the binding pocket of active site of urease obtained from protein data bank (PDB: 1E9Y). [42] Preparation of compounds for docking was achieved via building their 3D structure by MOE and database formation. Target compounds were subjected to a conformational search, and all conformers were subjected to energy minimization. 3D Protonation of structures, surfaces and maps were taken before docking. Docking was applied for the most active compounds **5b** and **5e**.

Flexible ligand–rigid receptor docking of the most stable conformers was done with MOE-DOCK using triangle matcher as the placement method, London dG as the scoring function, and refinement of the results was achieved using force field energy. Docking results will appear in a **DBV** window (dock.mdb). The **S** field, that the docking poses are ranked by the MM/GBVI binding free energy calculation is identical to the **E_refine** score. Use the **Database browser** for comparing docking poses to the ligand in the co-crystallized structure. Thirty of the most stable docking models for each ligand were retained with the best scored conformation.

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