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Anti-uropathogenic activity, drug likeness, physicochemical and molecular docking assessment of (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy) acetohydrazide

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ABSTRACT

Objective: To deal with the anti-uropathogenic and in silico screening of (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy)acetohydrazide analogues in order to search the potential anti-uropathogenic agents.

Methods: Three (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy)acetohydrazide analogues were synthesized. Structure elucidation was done using various spectroscopic techniques including infrared radiation, 1hydrogen-nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, *etc*. Physicochemical score, bioactivity score and molecular docking studies were carried out using Lipinski's rule of five, Molinspiration (web based software), Autodock 4.2 tools. *In vitro* anti-uropathogenic activity was carried out against four pathogens named as *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis*, *Proteus mirabilis* and *Escherichia coli* by disc diffusion method and macro-dilution test following their morphological and biochemical characterization.

Results: The formation of (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy)aceto-hydrazide is confirmed from the spectroscopic results. All the compounds were found in compliance with Lipinski's rule of five and exhibited bioactivity score from −0.50 to 0.00. Docking results revealed that compound-1 is forming one hydrogen bond with TYR 576 and two hydrogen bond with GLU 569, while compound-2 is forming one hydrogen bond with ARG 599, and compound-3 forming 0 hydrogen bond. The anti-uropathogenic evaluation exhibited that compound one exhibited better activity against *S. aureus*, while it was found to possess moderate to good activity against both Gram-positive bacteria and Gram-negative bacteria excluding *S. aureus*.

Conclusions: Our study revealed that compound one exhibited better activity than the standard in case of *S. aureus* and moderate to good activity against rest of the pathogens. Molecular docking, physicochemical and bioactivity studies strongly supported the experimental results. From the well obtained results it was concluded that compound-1 can lead as potential anti-uropathogenic agents.

1. Introduction

Multidrug-resistant strains of uropathogenic microorganisms have evolved as unmanageable infections of urinary tract and emerged as the second most common disease after respiratory tract infection [1]. Multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (*S. aureus*), penicillin resistant *Streptococcus pneumoniae* (*S. pneumoniae*), and vancomycin-resistant *Enterococci*, compounded problems in the therapeutics [2,3]. Quinolines and its derivatives have been known to possess diverse pharmacological activities such as antibacterial, antifungal, antimycobacterial, antidepressant, antimalarial, anticonvulsant, antiviral, anticancer, hypotensive and anti-inflammatory activities [4–11]. Quinine, which is extracted from Cinchona bark, has provided the basis for the development of synthetic quinoline-containing drugs, and many of them are presently available such as chloroquine, amodiaquine and mefloquine [12]. Hydrazones owing to their physiological activity and co-ordination capability yielded a number of pharmacophores

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with antiplatelet, antiulcer, antitumor, antiprotozoal, antibacterial and antifungal activities [13–29]. Some of the hydrazone derivatives have been found to represent the potential antimicrobial activity such as 2,5-diformyl-1H-pyrrole bis(methan-1-yl-1-ylidene)dimalonohydrazone [30], Benzylidene/2-aminobenzylidene hydrazides [30], 1,3-benzothiazole-2-yl-hydrazone [31], metal complexes with 2-acetylpyridine phenoxyacetyl hydrazone [32], pyrrole dihydrazones and their metal complexes [33–36], cholic acid hydrazone analogues [37], macrocyclic bis hydrazone [37], 3-oxido-1H-imidazole-4-carbohydrazides [38]. On the other hand, the evaluation of physicochemical parameters of a chemical entity plays a vital role in generation and escalation of bioactivity of chemical entity which is obtained by Molinspiration, web based software [39]. Docking studies were also carried out to find out the interaction of the synthesized compounds with the receptor in comparison to the standard drug Ciprofloxacin. Keeping in mind the importance of quinoline nucleus and hydrazones functionality, we designed our research in such a way that the combination of this functionality together will be important to enhance the biological activity.

2. Materials and methods

Solvents and organic reagents were purchased from Sigma Aldrich, Merck (Germany) and were used without further purification. Melting points (m.p.) were performed using a Mel-Temp instrument, and the results were uncorrected. Precoated aluminium sheets (silica gel 60 F254, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized under UV light. Elemental analyses were performed on Heraeus Vario EL III analyzer. Infrared radiation (IR) spectra were recorded on Perkin–Elmer model 1600 FT-IR RX1 spectrophotometer as KBr discs. 1Hydrogen-nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on Bruker AVANCE 300 spectrometer using $CDCl_3$ and dimethyl sulfoxide (DMSO) as solvents with tetramethylsilane as internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Chemical shift values are given in ppm. Electrospray ionisation mass spectrometry (ESI-MS) was recorded on a Micromass Quattro II triple quadrupole mass spectrometer.

2.1. General procedure for the synthesis of ethyl-2-(quinolin-8-yloxy)acetate (A)

A mixture of 8-hydroxyquinoline (10 mmol), ethyl chloroacetate (10 mmol) and potassium carbonate (15 mmol) in dry acetone (100 mL) was refluxed for 24 h. The reaction mixture was filtered hot and the solvent was distilled off from the filtrate. The crude ester thus obtained was purified by recrystallization from ethanol.

2.2. General procedure for the synthesis of 2-(quinolin-8-yloxy)acetohydrazide (B)

A mixture of A (10 mmol) and hydrazine hydrate (99%, 10 mmol) in ethanol (50 mL) was refluxed for 8 h. The solution on cooling gave a solid mass of hydrazide, which was collected by filtration, and recrystallized from ethanol.

2.3. General procedure for the synthesis of Schiff bases (1–3)

A mixture of compound B (10 mmol), appropriate aldehyde (10 mmol) and few drops of glacial acetic acid in ethanol (50 mL) was refluxed for 12 h. The product was precipitated, collected by filtration and re-crystallized from ethanol.

(E-)-N′-(4-methoxybenzylidene)-2-(quinolin-8-yloxy)aceto-hydrazide (1).

Yellow solid; yield: 80%; m.p. 162–164 °C Anal. Calc. for $C_{19}H_{17}N_3O_3$: C 68.05, H 5.11, N 12.53%, found C 68.12, H 5.14, N 12.54%; IR ν (cm^{-1}): 3134 (NH), 3011 (Ar, C–H), 1720 (C═O), 1612 (C═N); ^{1}H-NMR (DMSO) δ (ppm): 9.14 (s, 1H, NH), 8.12 (s, 1H, CH═N), 7.87 (d, 1H, J = 8.1 Hz, Ar–H), 7.78 (d, 1H, J = 8.1 Hz, Ar–H), 7.68 (m, 1H, Ar–H), 7.64 (m, 2H, Ar–H), 7.56 (d, 1H, J = 9.0 Hz, Ar–H), 7.31 (d, 2H, J = 8.0 Hz, Ar–H), 7.24 (d, 2H, J = 8.0 Hz, Ar–H), 5.68 (s, 2H, –CH_2–), 3.85 (s, 3H, –OCH_3); ^{13}C-NMR (DMSO) δ/ppm: 167.6 (C═O), 165.56 (C═N), 160.72, 158.64, 151.63 (Ct), 150.45, 148.53, 139.27, 131.62, 129.90, 129.52, 129.13, 128.41, 127.22, 119.43, 115.33, 112.52, 55.62 (–OCH_3), 52.21 (–CH_2–); ESI-MS m/z: [M^++H] 336.13.

(E-)-N′-(3,4-dimethoxybenzylidene)-2-(quinolin-8-yloxy) acetohydrazide (2).

Yellow solid; yield: 84%; m.p. 166–168 °C Anal. Calc. for $C_{20}H_{19}N_3O_4$: C 65.74, H 5.24, N 11.50%, found C 65.75, H 5.26, N 11.54%; IR ν (cm^{-1}): 3136 (NH), 3017 (Ar, C–H), 1724 (C═O), 1610 (C═N); ^{1}H-NMR (DMSO) δ (ppm): 9.10 (s, 1H, NH), 8.11 (s, 1H, CH═N), 7.89 (d, 1H, J = 8.2 Hz, Ar–H), 7.74 (d, 1H, J = 8.6 Hz, Ar–H), 7.65 (m, 1H, Ar–H), 7.60 (m, 2H, Ar–H), 7.56 (d, 1H, J = 8.5 Hz, Ar–H), 7.35 (d, 2H, J = 8.0 Hz, Ar–H), 7.30 (d, 2H, J = 7.0 Hz, Ar–H), 5.60 (s, 2H, –CH_2–), 3.81 (s, 3H, –OCH_3), 3.79 (s, 3H, –OCH_3); ^{13}C-NMR (DMSO) δ/ppm: 167.63 (C═O), 165.55 (C═N), 160.76, 158.62, 151.62, 150.47, 148.50, 139.23, 131.61, 129.90, 129.51, 129.13, 128.40, 127.29, 119.43, 115.37, 112.56, 55.60 (–OCH_3), 52.2 (–CH_2–); ESI-MS m/z: [M^++H] 366.14.

(E-)-N′-(3,4,5-methoxybenzylidene)-2-(quinolin-8-yloxy) acetohydrazide (3).

Yellow solid; yield: 83%; m.p. 170–172 °C Anal. Calc. for $C_{21}H_{21}N_3O_5$: C 63.79, H 5.35, N 10.63%, found C 63.76, H 5.35, N 10.65%; IR ν (cm^{-1}): 3130 (NH), 3017 (Ar, C–H), 1725 (C═O), 1614 (C═N); ^{1}H-NMR (DMSO) δ (ppm): 9.10 (s, 1H, NH), 8.14 (s, 1H, CH═N), 7.82 (d, 1H, J = 8.6 Hz, Ar–H), 7.76 (d, 1H, J = 8.8 Hz, Ar–H), 7.65 (m, 1H, Ar–H), 7.62 (m, 2H, Ar–H), 7.54 (d, 1H, J = 9.5 Hz, Ar–H), 7.34 (d, 2H, J = 8.4 Hz, Ar–H), 7.27 (d, 2H, J = 7.0 Hz, Ar–H), 5.63 (s, 2H, –CH_2–), 3.85 (s, 3H, –OCH_3), 3.80 (s, 3H, –OCH_3), 3.78 (s, 3H, –OCH_3); ^{13}C-NMR (DMSO) δ/ppm: 167.67 (C═O), 165.52 (C═N), 160.71, 158.63, 151.60, 150.40, 148.55, 139.24, 131.63, 129.97, 129.56, 129.11, 128.49, 127.27, 119.45, 115.33, 112.52, 55.61 (–OCH_3), 52.23 (–CH_2–); ESI-MS m/z: [M^++H] 336.13.

2.4. Physicochemical properties [40]

Physico-chemical properties of compounds 1–3 and ciprofloxacin were checked with the help of software Molinspiration physicochemical properties calculator available online (www.molinspiration.com). The properties of partition coefficient (log P), molar refractivity, molecular weight, number of heavy atoms, number of hydrogen donor, number of hydrogen acceptor and number of violation were calculated.

2.5. Bioactivity score [41]

The compounds and standard were also checked for the bioactivity score by calculating the activity score for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand. All the parameters were checked with the help of software Molinspiration drug-likeness score online (www.molinspiration.com). Drug likeness score of each compounds was calculated and compared with the specific activity of each compound, and the results were compared with standard drug.

2.6. Biological screening

2.6.1. Isolation and characterization of uropathogens

2.6.1.1. Collections of urine specimen

Midstream urine samples were aseptically collected from patients suspected to have urinary tract infections attending Al-Dawadmi General Hospital, Saudi Arabia. Midstream urine samples (n = 10) were collected in a sterile container. The specimens were transported to the clinical microbiology laboratory of College of Medicine, Al-Dawadmi with the support of ice packs and appropriately stored in refrigerator at 4 °C until analyses were carried out.

2.6.1.2. Total aerobic plate count

The total aerobic plate count of the urine specimens were carried out using serial dilution-pour plate technique. Serial dilutions were carried out by pipetting 1 mL of the urine sample into 9 mL of normal saline solution and then it was serially diluted to 10^{-6}. Following inoculation the culture plates were incubated for overnight at 37 °C and number of CFU was recorded using a colony counter.

2.6.1.3. Selective isolation of pure culture

The urine specimens (n = 4) showing significant bacteriuria based on total aerobic plate counts were inoculated aseptically in different selective media such as Mac Conkey agar, eosin methylene blue agar, blood agar and mannitol salt agar. All the inoculated plates were incubated at 37 °C for overnight. Pure cultures were recovered using repeated periodic sub-culturing method.

2.6.1.4. Maintenance and preservation of pure culture

Pure cultures obtained were maintained in refrigerator for further microbiological analyses on agar slant at 4 °C and were revived periodically. They were preserved in glycerol stocks at −20 °C freezer.

2.6.1.5. Morphological and biochemical characterization of pure cultures

Characterization of all the isolates was carried out on the basis of their morphological features and biochemical characteristics. The biochemical tests were carried out using IMViC test, catalase test, urease test, coagulase test and gas production tests to identify the isolates.

2.6.2. Anti-uropathogenic activity

Organism culture and *in vitro* screening for anti-uropathogenic activity was done by the disk diffusion method with minor modifications. The isolated and biochemically characterized *Escherichia coli* (*E. coli*), *Proteus mirabilis* (*P. mirabilis*), *S. aureus* and *Staphylococcus epidermidis* (*S. epidermidis*) were subcultured in nutrient agar medium and incubated for 18 h at 37 °C. Following the incubation the bacterial cells were suspended, according to the McFarland protocol in saline solution to produce a suspension of about 10^5 CFU/mL. About 10 mL of this suspension was mixed with 10 mL of sterile antibiotic agar at 40 °C and poured on to an agar plate in a laminar flow cabinet. Five paper disks (6.0 mm diameter) were fixed onto nutrient agar plate. One milligram of each test compound was dissolved in 100 mL DMSO to prepare stock solution. From the stock solution different dilutions of each test compound were prepared and poured over disk plate. Ciprofloxacin was used as a standard drug (positive control). DMSO poured disk was used as negative control. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. The zone of inhibition was calculated by antibiotic zone scale. The results were compared with the negative and positive controls and the zone of inhibitions was measured at the minimum inhibitory concentration (MIC). The MIC was evaluated by the macro-dilution test using standard inoculums of 10^5 CFU/mL. Serial dilutions of the test compounds, previously dissolved in DMSO were prepared to final concentrations of 400, 200, 100, 50, 12.5, 6.25 and 3.125 μg/mL. To each tube was added 100 mL of a 24-h-old inoculum. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 18 h, was determined visually after incubation for 18 h, at 37 °C.

2.7. In silico molecular docking studies

The ligands were drawn in ChemDraw Ultra 8.0 (ChemOffice package) and were converted to energy minimized 3D structures for in silico protein–ligand docking using AutoDock Tools [42,43]. All the heteroatoms were removed from the 2VF5.PDB, to make complex receptor free of any ligand before docking. AutoDock requires pre-calculated grid maps, one for each atom type, present in the ligand being docked as it stores the potential energy arising from the interaction with macromolecule. This grid must surround the region of interest (active site) in the macromolecule. In the present study, the binding site was selected based on the amino acid residues, which are involved in binding with glucosamine-6-phosphate of GlcN-6-P synthase as obtained from PDB with ID 2VF5 which would be considered as the best accurate active region as it is solved by experimental crystallographic data [42,43]. Therefore, the grid was centered at the region including all the 12 amino acid residues (Ala602, Val399, Ala400, Gly301, Thr302, Ser303, Cys300, Gln348, Ser349, Thr352, Ser347 and Lys603) that surround active site as in Figures 1 and 2. The grid box size was set at 70, 64, and 56 Å for x, y and z respectively, and the grid center was set to 30.59, 15.822 and 3.497 for x, y and z respectively, which covered all the 12 amino acid residues in the considered active pocket. Docking software AutoDock 4.2 Program supplied with AutoGrid 4.0 and AutoDock 4.0 was used to produce grid maps. The spacing between grid points was 0.375 Å. The Lamarckian genetic algorithm was chosen to search for the best conformers. During the docking process, a maximum of 10 conformers was considered for each compound. All the AutoDock docking runs were performed in Intel Core i3-2330M CPU @ 2.20 GHz of Sony system, with 2 GB DDR2 RAM. AutoDock 4.0 was compiled and run under Microsoft Windows-7 operating system.

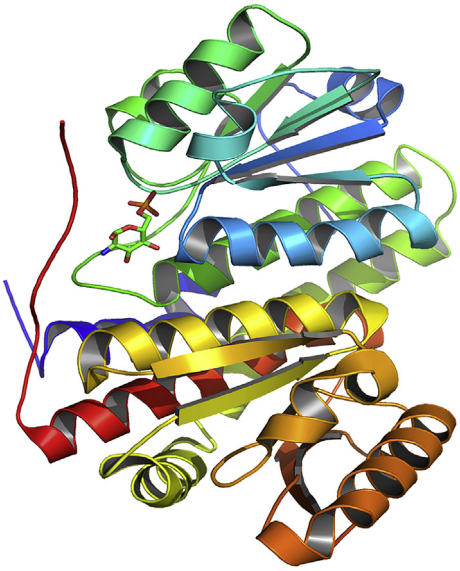

Figure 1. Crystal structure of X chain of GlcN-6-P synthase complex with glucosamine-6-phosphate.

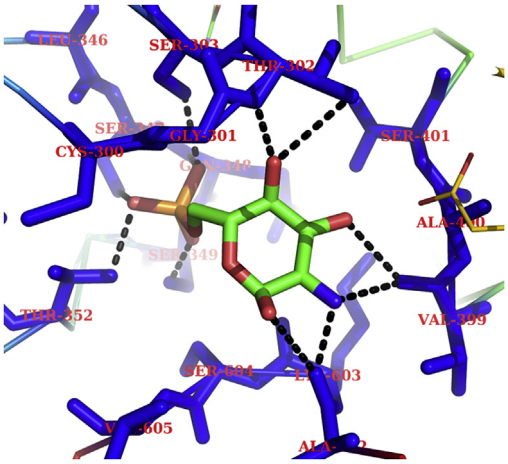

Figure 2. All 12 amino acid residues of active pocket (which were involved in binding with glucosamine-6-phosphate of GlcN-6-P synthase as obtained from PDB with ID 2VF5).

3. Results

3.1. Chemistry

The synthetic route for (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy) acetohydrazide (1–3) is shown in Figure 3, which completes in three steps. In the first ethyl-2-(quinolin-8-yloxy)acetate (A) was formed using ethyl chloroacetate and potassium carbonate. TLC confirmed the conversion and ^{1}H NMR helped in establishing the structure. In addition the signals at 1.11–1.26 ppm, a triplet for CH_3 and 4.10–4.26 ppm, a quartet for CH_2 proton confirmed the condensation reaction. In second step on refluxing in ethanol with hydrazine hydrate gave rise to condensation with hydrazine hydrate to get the acetohydrazide. 2-(quinolin-8-yloxy)acetohydrazide (B). A singlet at around 9.52 ppm confirmed this conversion. In the third step formation of (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy) acetohydrazide (1–3) achieved by simple reaction of B with

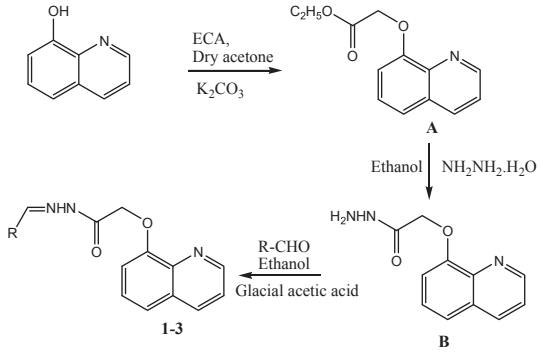

Figure 3. Diagrammatic representation of the scheme followed for the synthesis of compounds 1–3.

appropriate aldehyde. The structure of (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy) acetohydrazide (1–3) are presented in Figure 4. The reaction was monitored by TLC and structures were elucidated using various spectroscopic techniques. The purity of compounds was established from sharp melting points and elemental analysis data. The detailed spectroscopic data were represented in experimental part.

3.2. Physicochemical properties

Lipinski's rule of five states that, in general, an orally active drug has not more than 5 hydrogen bond donors (OH and NH groups), not more than 10 hydrogen bond acceptors (notably N and O), molecular weight under 500 g/mol, partition coefficient log P less than 5, number of violation less than 4 [41]. All the compounds were found in compliance with Lipinski's rule of five and the results are reported in Table 1.

3.3. Bioactivity score

The bioactivity score was calculated for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. For average organic molecule the probability of bioactivity score is more than 0.00 then it is active, −0.50 to 0.0 then moderately active and if less than −0.50 then inactive. Here in our study all the synthesized compounds 1–3 were subjected for bioactivity score presented in Table 2.

3.3.1. Isolation and characterization of uropathogens

The most commonly used criterion for defining significant bacteriuria is the presence of $\geq 10^5$ CFU/mL of urine specimen. Total aerobic plate counts of four out of 10 mid-stream urine

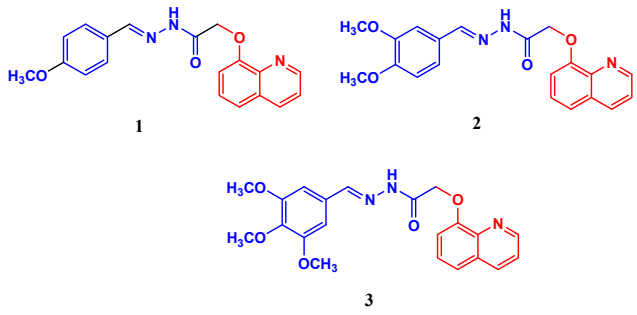

Figure 4. Structure of the compounds 1–3.

Table 1
Representing the physicochemical properties of all the synthesized compounds (1–3) and ciprofloxacin.

Compounds	Physicochemical property score								
	miLogP	TPSA	Natoms	MW	nON	nOHNH	Nviolations	Nrotb	Volume
1	2.682	72.822	25.0	335.363	6	1	0	6	301.266
2	2.672	82.056	27.0	365.389	7	1	0	7	326.812
3	2.657	91.290	29.0	395.415	8	1	0	8	352.357
Ciprofloxacin	−0.701	74.569	24.0	331.347	6	2	0	3	285.460

miLogP: Partition coefficient; TPSA: Topological polar surface area; Natoms: Number of heavy atoms; MW: Molecular weight; nON: Number of hydrogen bond acceptor; nOHNH: Number of hydrogen bond donors; Nviolations: Number of violations; Nrotb: Number of ratable bonds.

Table 2
Representing the bioactivity score of all the synthesized compounds (1–3) and ciprofloxacin.

Compounds	Bioactivity score					
	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	−0.34	−0.69	−0.39	−0.66	−0.50	−0.32
2	−0.33	−0.65	−0.37	−0.64	−0.50	−0.30
3	−0.32	−0.62	−0.34	−0.65	−0.48	−0.28
Ciprofloxacin	0.12	−0.04	−0.07	−0.19	−0.21	0.28

samples processed were found to be $\geq 10^5$ CFU/mL of urine specimen and the other six specimens were showing insignificant bacteriuria.

Urine (CFU/mL) = number of CFU × dilution factor/volume (0.1 mL) of the diluted sample taken.

Four pure bacterial isolates recovered were morphologically and biochemically characterized and designated as *E. coli*, *S. aureus*, *S. epidermidis* and *P. mirabilis* (Table 3 and Figure 5).

3.3.2. Anti-uropathogenic activity

All the compounds (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy) acetohydrazide (1–3) were screened for their *in vitro* anti-uropathogenic activity against the isolated Gram-positive and Gram-negative bacteria. Four different cultures, two each of Gram-negative (*E. coli* and *P. mirabilis*) and Gram-positive (*S. aureus* and *S. epidermidis*) were treated with synthesized compounds using disk diffusion method [44]. The results were compared with positive control, the standard drug ciprofloxacin and negative control, the DMSO poured disk. MIC was evaluated by macrodilution test using standard inoculums of 10^{-5} CFU/mL. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 37 °C. The inhibition zones (mm) of each compound and the minimum concentration at which the inhibition zones appeared are presented in Tables 4 and 5, respectively.

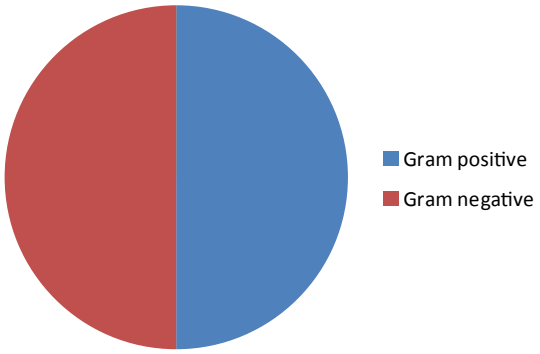

Figure 5. The Gram reaction results against the uropathogens.

3.4. Molecular docking studies

Considering the well obtained *in vitro* results, it was thought worthy to perform molecular docking studies, hence screening the compounds, inculcating both in silico and *in vitro* results.

Table 3
Representing the results of morphological and biochemical characterization of uropathogens.

Uropathogens	Test											
	Coagulase	Catalase	Indole	MR	VP	Citrate	Urease	Gas production	Swarming on blood agar medium	Shape	Size	Arrangement	Gram reaction
S. aureus	+	+	−	+	+	−	−	+	−	Round (Coccus)	Small	Cluster	+
S. epidermidis	−	+	−	+	+	−	−	+	−	Round (Coccus)	Small	Cluster	+
E. coli	−	+	+	+	−	−	−	+	−	Rod Shaped (Bacillus)	Small	Single	−
P. mirabilis	−	+	−	+	−	+	+	−	+	Rod Shaped (Bacillus)	Small	Single or Paired	−

MR: Methyl red test; VP: Voges–Proskauer test.

Table 4

Anti-uropathogenic activity of the synthesized compounds (1–3) (mm).

Compounds	Gram-positive		Gram-negative	
	S. aureus	*S. epidermidis*	*P. mirabilis*	*E. coli*
1	21.55 ± 0.56	21.14 ± 0.44	20.43 ± 0.61	21.82 ± 0.50
2	19.16 ± 0.84	18.35 ± 0.62	20.72 ± 0.99	22.34 ± 0.33
3	18.33 ± 0.53	17.57 ± 0.24	18.36 ± 0.22	20.44 ± 0.11
Ciprofloxacin	21.46 ± 0.31	22.64 ± 0.54	22.24 ± 0.30	23.82 ± 0.47
DMSO	–	–	–	–

Ciprofloxacin was used as positive control and DMSO as negative control measured by the Holo zone test.

Table 5

Representing MIC of the compounds (1–3).

Compounds	Gram positive		Gram negative	
	S. aureus	*S. epidermidis*	*P. mirabilis*	*E. coli*
1	6.25	3.125	6.25	12.5
2	12.50	12.500	12.50	25.0
3	12.50	6.250	12.50	12.5
Ciprofloxacin	6.25	3.125	6.25	12.5

Ciprofloxacin was used as standard drug.

Considering GlcN-6-P synthase as the target receptor, comparative and automated docking studies with newly synthesized candidate lead compounds was performed to determine the best in silico conformation. The Lamarckian genetic algorithm, inculcated in the docking program AutoDock 4.2, was employed to satisfy the purpose. Figure 1 shows the native crystal structure of GlcN-6-P synthase (X chain) in complex with glucosamine-6-phosphate obtained from Protein Data Bank (http://www.pdb.org/pdb/home/home.do) with the PDB ID 2VF5 which was resolved at 2.90 A° using X-ray diffraction [42,43] (Figure 3). All the three synthesized molecules were docked. Figure 6 represents the docked images of all the compounds including the considered standard drug ciprofloxacin.

4. Discussion

The structure elucidation of (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy) acetohydrazide (1–3) was done using various spectroscopic techniques such as Fourier transform infrared spectroscopy, ^{1}H-NMR, ^{13}C-NMR spectral data. The reaction was monitored by TLC and structures were elucidated using various spectroscopic techniques. The purity of compounds was established from sharp melting points and elemental analysis data. Assignment of selected characteristic IR bands provides significant indications for the formation of the (1–3). The band around 1610–1614, 1720–1725 cm^{-1} represents the stretching frequency of C═N and C═O groups, respectively. Similarly the band around 3130-3136 cm^{-1} was assigned to N–H respectively. The structures of compounds were further established on the basis of ^{1}H and ^{13}C-NMR. In ^{1}H-NMR, besides the aromatic region, four prominent signals were observed. The presence of singlet around (9.10–9.14) ppm was assigned to NH proton. Another singlet around (8.11–8.14) ppm due to HC═N proton also confirms the formation of the compounds (1–3). The carbonyl carbon signal was found downshifted to (167.63–167.67) ppm and the nitrile carbon signals due to H–C═N found around 165.52–165.56 ppm which provide strong recommendation for the formation of these compounds. In addition to NMR, the compounds were subjected to ESI-MS analysis for detecting the molecular mass of the compounds. Results obtained for structure elucidation of all the synthesized compounds provide strong recommendation for the complete conversion of these compounds and also supported by the reported literature [9,10,25]. Physicochemical calculations exhibited that all the compounds were found to compliance with Lipinski's rule of five similar to the results obtained by Verma [40]. The bioactivity score was calculated for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. Bioactivity score results revealed that the compounds have bioactivity score between −0.50 and 0.00 which states that these compounds are moderately active according to the bioactivity score evaluation parameters as discussed in the study of Verma [40]. Molecular docking studies state that compound one and the

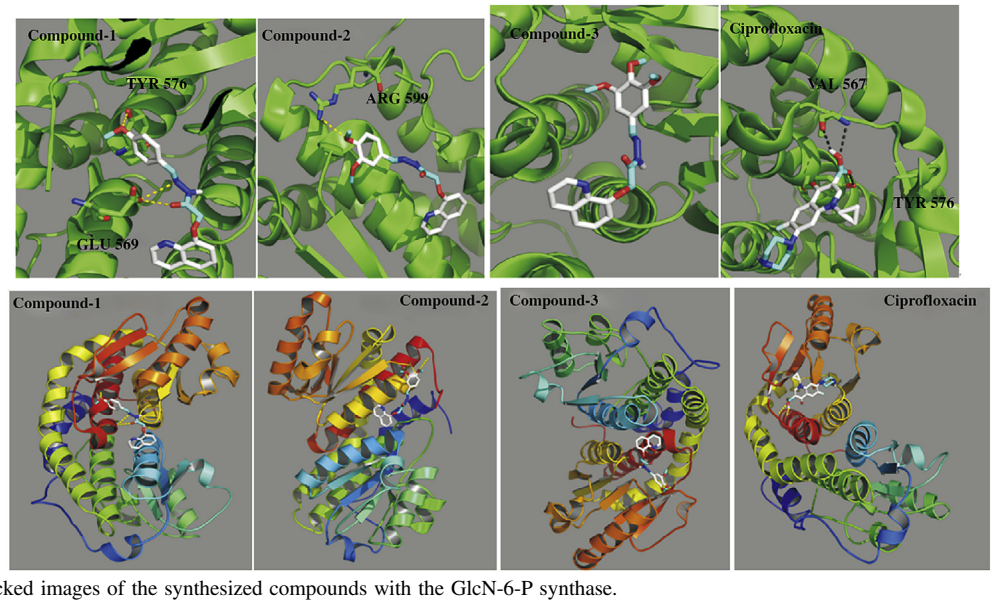

Figure 6. The docked images of the synthesized compounds with the GlcN-6-P synthase.

ciprofloxacin are forming the hydrogen bond with TYR 576, and the results obtained from docking study are found in accordance with the results obtained experimentally and strongly supported by the reported literature [45,46]. The zone of inhibition was measured at the MIC. Anti-uropathogenic activity of the synthesized compounds was evaluated on uropathogens following isolation and characterization. About 50% of the total isolates were found to be of Gram-negative culture while 50% was showing Gram-positive characteristics similar to the reported results [47]. When zone of inhibition was compared with the standard drug, it was observed that compound two and three were found to show 80% resemblance with the standard drug only in case of *E. coli* and exhibiting moderate to good activity against the rest of the pathogens. On the other hand, compound one exhibited better activity against *S. aureus*, while it was found to possess moderate to good activity against both Gram-positive bacteria and Gram-negative bacteria excluding *S. aureus*. The results were found in accordance with the study carried out by Shakir *et al.* [48]. The importance of such work lies in the possibility that the new compound might be more effective against bacteria for which a thorough investigation regarding the structural activity relationship, toxicity and the biological effects would be helpful in designing more potent antibacterial agents for therapeutic use.

The (E-)-N′-(substituted-benzylidene)-2-(quinolin-8-yloxy) acetohydrazide (1–3) was synthesized starting from the simple chemical molecules and their structures were elucidated by various spectroscopic techniques. All the compounds were screened for anti-uropathogenic activity against the isolated and characterized Gram-positive and Gram-negative bacteria. Results revealed that compound one exhibited better activity than the standard in case of *S. aureus* and moderate to good activity against rest of the pathogens. While compound two and three were found moderate to good activity against all Gram-positive and Gram-negative bacteria. All the compounds follow the Lipinski's rule of five and bioactivity score is also in accordance with the experimental results except only in case of compound one. To support the results obtained experimentally docking studies was done and docking results are found in accordance with the experimental results.

Conflict of interest statement

We declare that we have no conflict of interest.

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