Synthesis and antimicrobial activity of substituted 1,4-*bis*-spiro [benzocycloheptene-6(5*h*),3′(3*h*-pyrazol)-5-one]-benzene under microwave irradiation and molecular docking study

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Background and objective
Spiropyrazole derivatives are one of the most bioactive spiro compounds that play a vital role in drug discovery, such as antibacterial, anti-inflammatory, antifungal, antiviral, analgesic, and antidepressant activities. Moreover, microwave as an energy source enhances the reaction rates and improves the regioselectivity. The aim of this study was to synthesize the spiropyrazole derivative compounds. Molecular docking was performed.
Materials and methods
1,3-Dipolar cycloaddition of 6,6′-(1,4-phenylene-*bis*(methanylylidene))-*bis*(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one) to a variety of nitrilimines (generated *in situ* by triethylamine dehydrohalogenation of the corresponding hydrazonoyl halides) under microwave irradiation proceeded regioselectively affording spiro[benzo[7]-annulene-6,3′-pyrazol]-4′-yl)phenyl)-spiro[benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-ones. The structure of the newly synthesized compounds was confirmed on the basis of spectral data and elemental analyses. The antimicrobial activity of the *bis* (spiropyrazoles) derivatives was tested for antimicrobial activity. Molecular docking was performed and analyzed with the molecular modeling environment program.
Results and conclusion
Compound **7f** has high potency against all fungi (except *Candida albicans*) and bacterium species (except *Pseudomonas aeruginosa*) compared with the reference drug fungicide amphotericin B and the standard bactericides ampicillin and gentamicin. Docking of the most active antibacterial compounds **7f** and **7g** against the dihydropteroate synthase enzyme gave comparable scores for hydrogen bond interaction (–22.9123, –17.5995 kcal/mol(and binding mode to the reference antibiotic sulfamethoxazole (–13.00 kcal/mol).

Keywords:
1-benzosuberone, hydrazonoyl halides, microwave irradiation, regioselectivity, spiropyrazoles

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Introduction

Spiropyrazole derivatives belong to one of the most bioactive spiro compounds as some derivatives show antibacterial [1,2], antifungal [3], anti-inflammatory [4,5], analgesic [6], and herbicide activities [7]. Moreover, microwave as an energy source enhances the reaction rates and improves the regioselectivity [8–11]. One of the most important methods to synthesize spiropyrazoles is 1,3-dipolar cycloaddition of the nitrilimines (generated *in situ* from the corresponding hydrazonoyl halides) to the exocyclic double bond [12–15]. Moreover, *bi*-pyrazole derivatives show a wide range of antitumor [16], anti-inflammatory [17], cytotoxic [18,19], antiallergic [20], cardiovascular [21], and diuretic [22] activities. From the above observation, the present work deals with the reaction of nitrilimines (**5**) (generated *in situ* from the corresponding hydrazonoyl halides) with 6,6′-(1,4-phenylene-*bis* (methanylylidene))-*bis*(6,7,8,9-tetrahydro-5*H*-benzo[7] annulen-5-one) (**3**) under microwave irradiation to synthesize spiro[benzo[7]annulene-6,3′-pyrazol]-4′-yl-phenyl-spiro[benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-ones.

Materials and methods

Chemistry

Melting points were measured with an IA 9000-series digital melting-point apparatus (Bibby Sci. Lim. Stone, Staffordshire, UK). IR spectra were recorded in potassium bromide discs on FTIR 8101 PC infrared spectrophotometers (Shimadzu, Tokyo, Japan). NMR spectra were recorded on a Mercury VX-300 NMR

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spectrometer (Varian, Inc., Karlsruhe, Germany) operating at 300 MHz (^{1}H-NMR) and run in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCeMS-QP1000 EX mass spectrometer (Tokyo, Japan) at 70 eV. Elemental analyses were measured using an ElementarVario LIII CHNS analyzer (elementar Analysensysteme GmbH, Hanau, Germany). Micro wave was carried out in an ElmaRD-7700 apparatus (Singen, German). Antimicrobial activity was evaluated by the (Elementar Analysen system Gmb H, Hanau , Germany) Regional Center for Mycology and Biotechnology, Al-Azhar University (Cairo, Egypt). Hydrazonoyl halides (**4**) were prepared as previously reported in the literature [23,24].

Synthesis of 6,6′-(1,4-phenylene-*bis*(methanylylidene)) *bis*(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one) (3)

A mixture of 1-benzosuberone (**1**) (1.6 g, 10 mmol) and terephthalaldehyde (**2**) (5 mmol) in absolute ethanol (25 ml) in the presence of KOH (1 g) was refluxed under pressurized microwave irradiation for 5 min. The separated solid was collected and crystallized from dimethylformamide to give compound **3** as white crystals; 85% yield; melting point (m.p.) 260–262°C (DMF/ethanol); IR υ_{max} 1693(CO)/cm; ^{1}H NMR (DMSO-d_6) 1.08–3.10 (m, 12H, 6CH_2), 7.09–7.70 (m, 12H, Ar-H), 7.85 (s, 2H, olefinic CH); MS *m/z* (%) 419 (M^+, +1, 26), 418 (M^+, 88), 417 (62), 298 (100), 260 (86), 131 (48), 128 (38), 103 (52), 91 (49), 90 (39), 77 (43). Anal. calcd. for $C_{30}H_{26}O_2$ (418.54): C, 86.09; H, 6.26. Found: C, 86.23; H, 6.12.

Reaction of 1,4-*bis*[2-(3,4-dihydro-(2*H*)-1-oxo-naphthalenyl)methylene]benzene 3 with hydrazonoyl halides (4)

A mixture of **3** (2.5 mmol) and the appropriate hydrazonoyl halide **4a–g** (5 mmol) in dry benzene (30 ml) containing triethylamine (7.5 mmol) was irradiated in a pressurized microwave (17.2 bar, 140°C) at a power of 300 W for 15–30 min as evidenced by TLC. The reaction mixture was filtered when hot to remove the triethylamine hydrochloride, and then concentrated to 10 ml and cooled overnight. The separated solid was collected and crystallized from the suitable solvent to afford the corresponding products **7a–g**.

4′-(4-(5-Oxo-2′,5′-diphenyl-2′,4′,5,7,8,9-hexahydrospiro [benzo[7]annulene-6,3′-pyrazol]-4′-yl)phenyl)-2′,5′-diphenyl-2′,4′,8,9-tetrahydrospiro[benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-one (7a)

Yellow solid; yield 74%; m.p. 150–152°C (ethanol); IR υ_{max} 1678(CO)/cm; ^{1}H NMR (DMSO-d_6) 1.17–2.86 (m, 12H, 6CH_2), 5.33 (s, 2H, 2-pyrazole-H), 6.94–7.70 (m, 32H, Ar-H); MS *m/z* (%) 807 (M^+, +1, 17), 806 (M^+, 17), 613 (35), 493 (59), 167 (45), 131 (38), 91 (100), 84 (52), 77 (86). Anal. calcd. for $C_{56}H_{46}N_4O_2$ (806.36): C, 83.35; H, 5.75; N, 6.94. Found: C, 83.23; H, 5.56; N, 6.73.

2′-(4-Nitrophenyl)-4′-(4-(2′-(4-nitrophenyl)-5-oxo-5′-phenyl-2′,4′,5,7,8,9-hexahydrospiro[benzo[7]annulene-6,3′-pyrazol]-4′-yl)phenyl)-5′-phenyl-2′,4′,8,9-tetrahydrospiro[benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-one (7b)

Yellow solid; yield 55%; m.p. 214–216°C (dioxane); IR υ_{max} 1698(CO)/cm; ^{1}H NMR (DMSO-d_6) 1.18–2.88 (m, 12H, 6CH_2), 4.75 (s, 2H, 2-pyrazole-H), 6.78–7.69 (m, 30H, Ar-H). Anal. calcd. for $C_{56}H_{44}N_6O_6$ (896.33): C, 74.98; H, 4.94; N, 9.37. Found: C, 74.70; H, 4.76; N, 9.21.

4′-(4-(5-Oxo-2′-phenyl-5′-((E)-styryl)-2′,4′,5,7,8,9-hexahydrospiro[benzo[7]annulene-6,3′-pyrazol]-4′-yl) phenyl)-2′-phenyl-5′-((E)-styryl)-2′,4′,8,9-tetrahydrospiro [benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-one (7c)

Yellow solid; yield 64%; m.p. 222–224°C (dioxane/ethanol); IR υ_{max} 1711(CO)/cm; ^{1}H NMR (DMSO-d_6) 0.97–2.83 (m, 12H, 6CH_2), 5.18 (s, 2H, 2-pyrazole-H), 6.60–7.64 (m, 36H, Ar-H, 4CH); MS *m/z* (%) 859 (M^+, +1, 14), 858 (M^+, 12), 418 (52), 299 (100), 260 (50), 247 (36), 141 (36), 131 (48), 128 (50), 115 (57), 103 (71) 90 (41), 89 (29), 77 (31). Anal. calcd. for $C_{60}H_{50}N_4O_2$ (858.39): C, 83.89; H, 5.87; N, 6.52. Found: C, 83.65; H, 5.71; N, 6.40.

2′-(4-Nitrophenyl)-4′-(4-(2′-(4-nitrophenyl)-5-oxo-5′-(thiophen-2-yl)-2′,4′,5,7,8,9-hexahydrospiro[benzo[7] annulene-6,3′-pyrazol]-4′-yl)phenyl)-5′-(thiophen-2-yl)-2′,4′,8,9-tetrahydrospiro[benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-one (7d)

Yellow solid; yield 54%; m.p. 200–202°C (ethanol), IR υ_{max} 1688(CO)/cm;^{1}H NMR (DMSO-d_6) 1.08–2.98 (m, 12H, 6CH_2), 5.34 (s, 2H, 2-pyrazole-H), 6.97–7.66 (m, 26H, Ar-H); MS *m/z* (%) 908 (M^+, 2), 568 (15), 298 (39), 169 (33), 148 (85), 122 (73), 121 (39), 119 (30), 105 (88), 103 (73), 91 (61), 77 (73), 55 (100). Anal. calcd. for $C_{52}H_{40}N_6O_6S_2$ (908.25): C, 68.71; H, 4.44; N, 9.25. Found: C, 68.54; H, 4.65; N, 9.18.

5′-(4-Chlorophenyl)-4′-(4-(5′-(4-chlorophenyl)-5-oxo-2′-phenyl-2′,4′,5,7,8,9-hexahydrospiro[benzo[7]annulene-6,3′-pyrazol]-4′-yl)phenyl)-2′-phenyl-2′,4′,8,9-tetrahydrospiro[benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-one (7e)

Dark yellow solid; yield 69%; m.p.: 124–126°C (ethanol); IR υ_{max} 1681(CO)/cm; ^{1}H NMR (DMSO-d_6) 1.05–2.90 (m, 12H, 6CH_2), 5.34 (s, 2H, 2-pyrazole-H), 6.94–7.56 (m, 30H, Ar-H); MS *m/z* (%) 878 (M^+, +4, 29), 877 (M^+, +3, 54), 876

(M$^+$, +2,42), 756 (42), 437 (33), 312 (58), 144 (42), 121 (42), 111 (33), 105 (50), 91 (67), 77 (100). Anal. calcd. for $C_{56}H_{44}Cl_2N_4O_2$ (875.89): C, 76.79; H, 5.06; N, 6.40. Found: C, 76.58; H, 5.06; N, 6.40%.

5′-(Ethoxycarbonyl)-4′-(4-(5′-(ethoxycarbonyl)-5-oxo-2′-phenyl-2′,4′,5,7,8,9-hexahydrospiro[benzo[7]annulene-6,3′-pyrazol]-4′-yl)phenyl)-2′-phenyl-2′,4′,8,9-tetrahydrospiro[benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-one (7f)

Dark orange solid; yield 64%; m.p. 210–212°C (ethanol); IR υ_{max} 1693, 1666(2CO)/cm; ^{1}H NMR (DMSO-d_6) 1.0 (t, 6H, 2CH_3), 1.08–3.09 (m, 12H, 6CH_2), 4.12 (q, 4H, 2CH_2), 4.85 (s, 2H, 2-pyrazole-H), 7.12–7.70 (m, 22H, Ar-H); MS *m*/*z* (%) 798 (M$^+$, 25), 797 (15), 299 (40), 260 (35), 132 (55), 104 (70), 91 (60), 90 (45), 77 (90), 55 (100). Anal. calcd. for $C_{50}H_{46}N_4O_6$ (798.34): C, 75.17; H, 5.80; N, 7.01. Found: C, 75.03; H, 5.65; N, 7.23.

5′-(Ethoxycarbonyl)-4′-(4-(5′-(ethoxycarbonyl)-5-oxo-2′-(4-bromophenyl)-2′,4′,5,7,8,9-hexahydrospiro[benzo[7]annulene-6,3′-pyrazol]-4′-yl)phenyl)-2′-(4-bromophenyl)-2′,4′,8,9-tetrahydrospiro[benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-one (7g)

Brown solid, yield 68%; m.p. 82–84°C (dioxane/ethanol); IR υ_{max} 1732, 1699(2CO)/cm; ^{1}H NMR (DMSO-d_6) 1.01 (t, 6H, 2CH_3), 1.18–3.09 (m, 12H, 6CH_2), 4.15 (q, 4H, 2CH_2), 4.85 (s, 2H, 2-pyrazole-H), 7.09–7.72 (m, 20H, Ar-H); MS *m*/*z* (%) 953 (M$^+$, –1, 5), 952 (7), 454 (10), 439 (10), 316 (14), 284 (14), 239 (14), 186 (13), 156 (16), 129 (25), 98 (29), 97 (31), 91 (22), 85 (33), 77 (20), 57 (100). Anal. calcd. for $C_{50}H_{44}Br_2N_4O_6$ (954.16): C, 62.77; H, 4.64; N, 5.86. Found: C, 62.54; H, 4.56; N, 5.71.

Antimicrobial activity

Agar diffusion well method to determine the antimicrobial activity

The microorganism inoculums were uniformly spread using a sterile cotton swab on a sterile Petri dish malt extract agar (for fungi) and nutrient agar (for bacteria). One hundred milliliter of each sample was added to each well (6-mm diameter holes cut in the agar gel, 20-mm agar from one another). The systems were incubated for 24–48 h at 37°C (for bacteria) and at 28°C (for fungi). After incubation, the growth of microorganisms was observed. Inhibition of bacterial and fungal growth was measured in millimeter. Tests were performed in triplicate [25,26].

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the samples was estimated for each tested organism in triplicate using concentrations of the samples (1000–0.007 μg/ml). Nutrient broth was added followed by a loopful of the test organism previously diluted to 0.5, and McFarland turbidity standard was introduced to the tubes. A tube containing only broth media was seeded with the test organism to serve as control. Tubes containing tested organism cultures were then incubated at 37°C for 24 h, whereas the other fungal cultures were incubated at 25–30°C for 48 h. Microbial growth was indicated by the presence of turbidity of the well. The lowest concentration showing no growth was taken as the MIC [27].

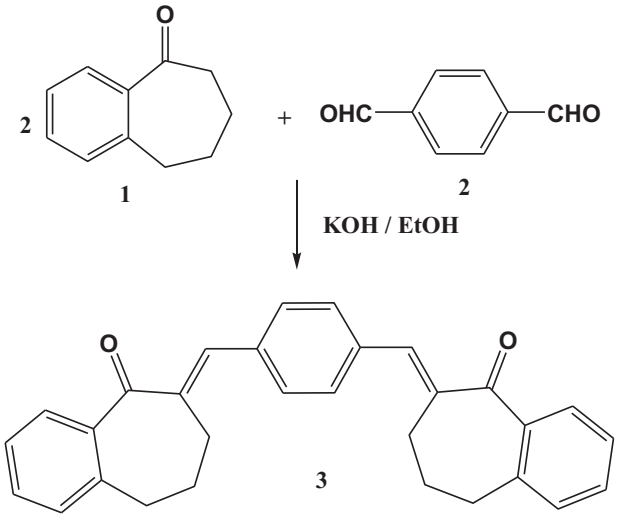
Results and discussion

Chemistry

6,6′-(1,4-Phenylene-*bis*(methanylylidene))-*bis* (6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one) (**3**) was prepared from condensation of two mole equivalents of benzosuberone (**1**) with terephthalaldehyde (**2**) in ethanolic potassium hydroxide solution under microwave irradiation for 5 min (Scheme 1). The ^{1}H NMR spectrum of compound **3** showed one singlet signal at 7.85 ppm due to olefinic protons. Moreover, the mass spectrum of compound **3** showed the expected molecular ion peak at 418 (88%). The IR spectrum of the isolated product **3** revealed the carbonyl absorption bands at υ 1693/cm.

We were interested here to investigate the reaction of nitrilimines with the *bis*(aryledine) derivative **3** under microwave irradiation. Reaction of **3** with nitrilimines (**5**) [generated *in situ* through triethylamine dehydrohalogenation of the corresponding hydrazonoyl halides (**4**)] in 1 : 2 molar ratio in dry benzene under

Scheme 1



Synthesis of 6,6′-(1,4-phenylene-*bis*(methanylylidene)),*bis*(6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one) (**3**).

microwave irradiation for 15–30 min gave the dicyclo adduct **7** rather than **6** or **8**. The IR spectra of the isolated products **7** revealed the carbonyl absorption bands in the region υ 1711–1666/cm. The appearance of one singlet signal in ^{1}H NMR spectra at δ=5.34–4.75 assignable to the pyrazole H-4 ruled out the formation of the isomeric dicyclo adduct **6** or **8** (Scheme 2) [28,29].

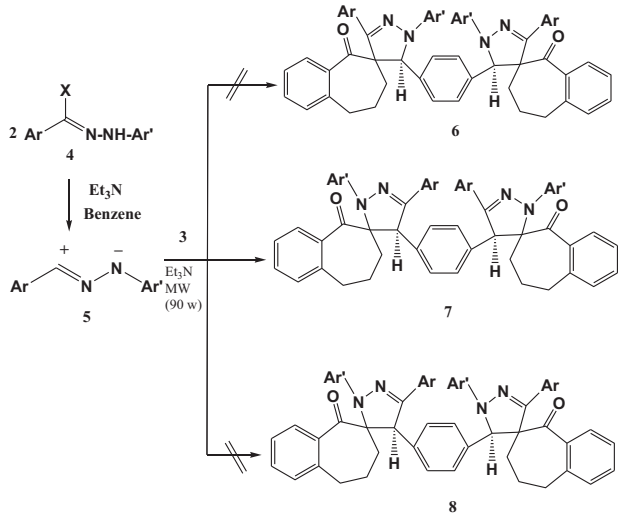
Antimicrobial activity

Screening of the newly synthesized products **7a–g** was carried out using four fungal strains – namely, *Aspergillus fumigatus*, *Penicillium italicum*, *Candida albicans*, and *Geotrichum candidum* – and four bacteria species, including Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, and Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*. The results in Table 1 indicate that compound **7f** has high potency against all fungi (except *C. albicans*) and bacterium species (except *P. aeruginosa*) compared with the reference fungicide amphotericin B and the standard bactericides ampicillin and gentamicin. In addition, compounds **7g**, **7d**, **7c**, and **7e** showed significant activities against all tested microorganisms (except *C. albicans* and *P. aeruginosa*), whereas the compounds **7a** and **7b** have moderate activity against the same microorganisms (Table 1). Moreover, it was found that compound **7f** exhibited the MIC against all tested microorganisms, especially fungus *A. fumigatus* and *P. italicum* (MIC=0.9 and 3.9 μg/ml, respectively) (Table 2). This is may be due to the presence of the ethoxycarbonyl group (COOEt) and the Ph group. Moreover, we note that compound **7g** showed less activity compared with **7f**. This may be related to the presence of bromine in p-position of the phenyl group.

Molecular docking study

Drug resistance is one of the most common problems that decreases the clinical utility of virtually all marketed

Scheme 2



Ar /Ar': a, Ph / Ph; b, Ph / 4-$NO_2C_6H_4$; c, PhCH=CH / Ph; d, 2-thienyl / 4-$NO_2C_6H_4$; e, 4-ClC_6H_4 / Ph; f, COOEt / Ph; g, COOEt / 4-BrC_6H_4

Synthesis of 6,6′-(spiro[benzo[7]annulene-6,3′-pyrazol]-4′-yl)phenyl)-spiro[benzo[7]annulene-6,3′-pyrazol]-5(7*H*)-ones (**7a–g**).

Table 2 Minimum inhibitory concentration (μg/ml) of the most active compounds

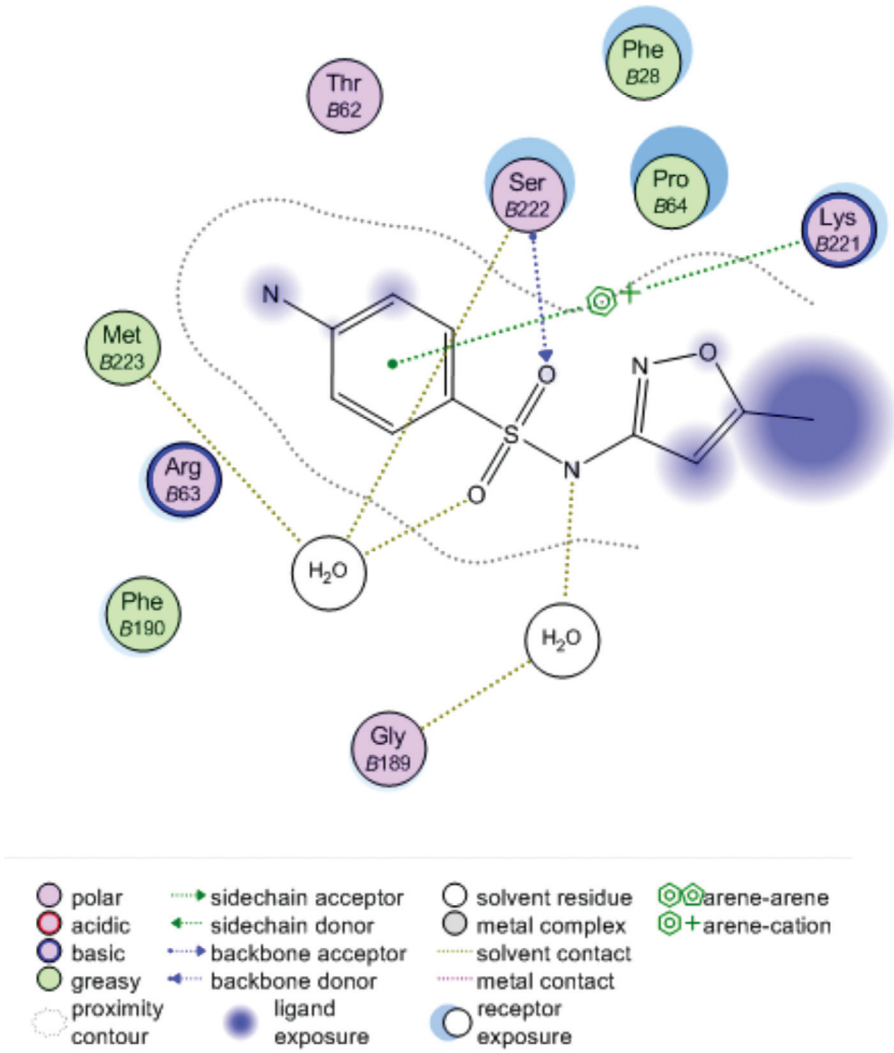
Tested microorganisms	7c	7d	7f	7g	Standard compound.
Fungi					Amphotericin B
Aspergillus fumigatus	16.4	8.9	0.9	3.9	1.95
Penicillium italicum	28.4	15.6	3.9	7.8	3.9
Candida albicans	NA	NA	NA	NA	7.81
Geotrichum candidum	9.4	1.95	0.5	0.9	0.06
Gram-positive bacteria					Ampicillin
Staphylococcus aureus	3.9	0.9	0.2	0.5	0.12
Bacillus subtilis	1.9	0.5	0.1	0.2	0.007
Gram-negative Bacteria					Gentamicin
Pseudomonas aeruginosa	NA	NA	NA	NA	31.25
Escherichia coli	125	31.3	15.6	125	1.95

Table 1 Preliminary antimicrobial activity of the tested compounds 7a–g

Microorganisms	7a	7b	7c	7d	7e	7f	7g	Standard compound (30 μg/ml)
Fungi								Amphotericin B
Aspergillus fumigatus	14.6	14.5	19.1	19.9	18.4	22.0	20.8	23.7
Penicillium italicum	15.2	17.1	16.4	18.0	16.8	20.8	17.8	21.9
Candida albicans	NA	NA	NA	NA	NA	NA	NA	19.8
Geotrichum candidum	15.4	13.4	19.9	21.8	18.2	22.5	22.7	28.7
Gram-positive bacteria								Ampicillin
Staphylococcus aureus	13.4	12.6	20.1	22.3	19.6	23.9	22.7	27.4
Bacillus subtilis	12.6	13.0	24.1	24.5	20.4	26.4	24.2	32.4
Gram-negative bacteria								Gentamicin
Pseudomonas aeruginosa	NA	NA	NA	NA	NA	NA	NA	17.3
Escherichia coli	10.5	11.2	13.6	16.9	12.5	15.1	14.0	22.3

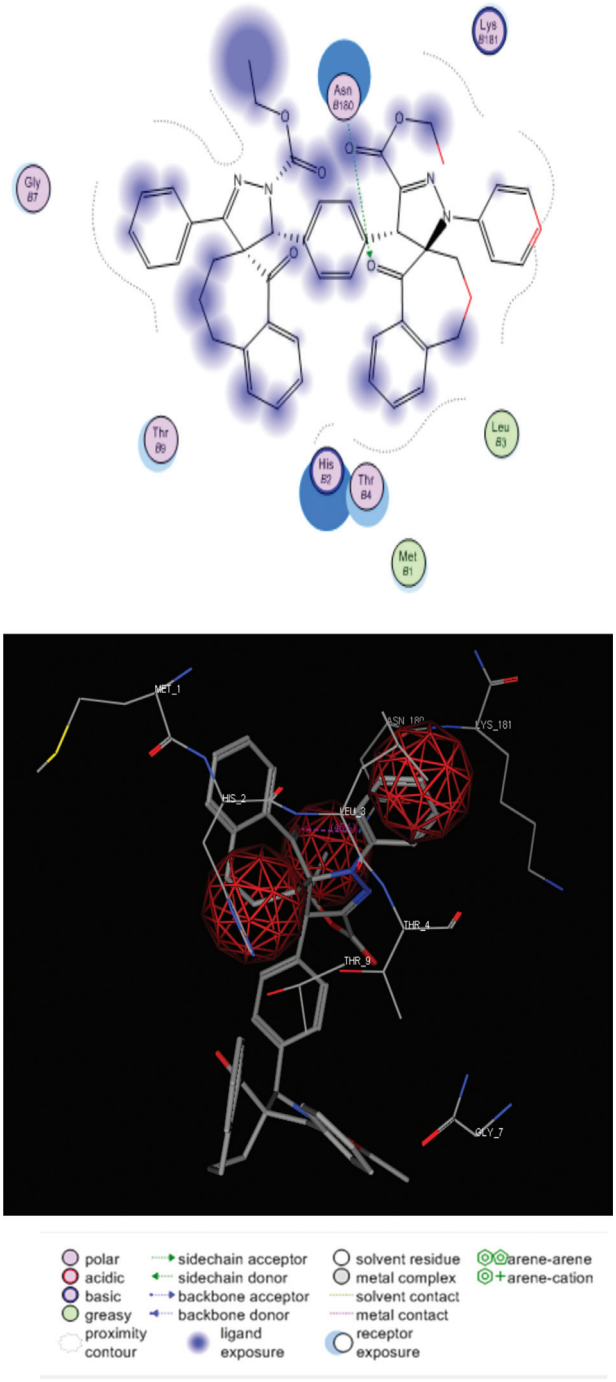
antibacterial agents [30]. Unfortunately, sulfonamide class was one victim of this phenomenon [31,32]. Sulfonamide class (sulfa drugs) works by targeting the dihydropteroate synthase (DHPS) essential in the folate pathway in bacteria, which catalyzes the condensation of 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate (DHPP) with p-aminobenzoic acid in the production of the folate intermediate, 7,8-dihydropteroate [33]. Sulfa drugs can target both Gram-positive and Gram-negative infection. However, resistance mutations have severely compromised the usefulness of these drugs [31]. Thus, searching for new drugs became important. The molecular docking study is performed and analyzed with the molecular modeling environment (MOE) program. The actively synthesized compounds **7f** and **7g** are enquired for the binding affinity of the DHPS enzyme receptor [protein data bank (PDB) code: 3TZF] [34] for the purpose of lead optimization; to study the interaction between compounds **7f** and 7**g** and the DHPS receptor, docking calculations were carried out using standard default variables for the MOE program. The binding affinity was evaluated from the binding free energies (*S*-score, kcal/mol), hydrogen bonds, and root mean square deviation values. Compounds **7f** and **7g** were docked into the comparable groove of the binding site of the native cocrystallize ligand. Scoring in MOE software was carried out using the London dG scoring function and enhanced using two different refinement methods; the force-field and grid-min poses were updated to ensure that refined poses satisfy the specified conformations. Rotatable bonds were allowed; the best 10 poses were retained and analyzed for the binding pose's best score. Energy was minimized through force-field MMFF94 optimization with a gradient of 0.0001 for determining low-energy conformations with the most favorable geometry

Figure 1



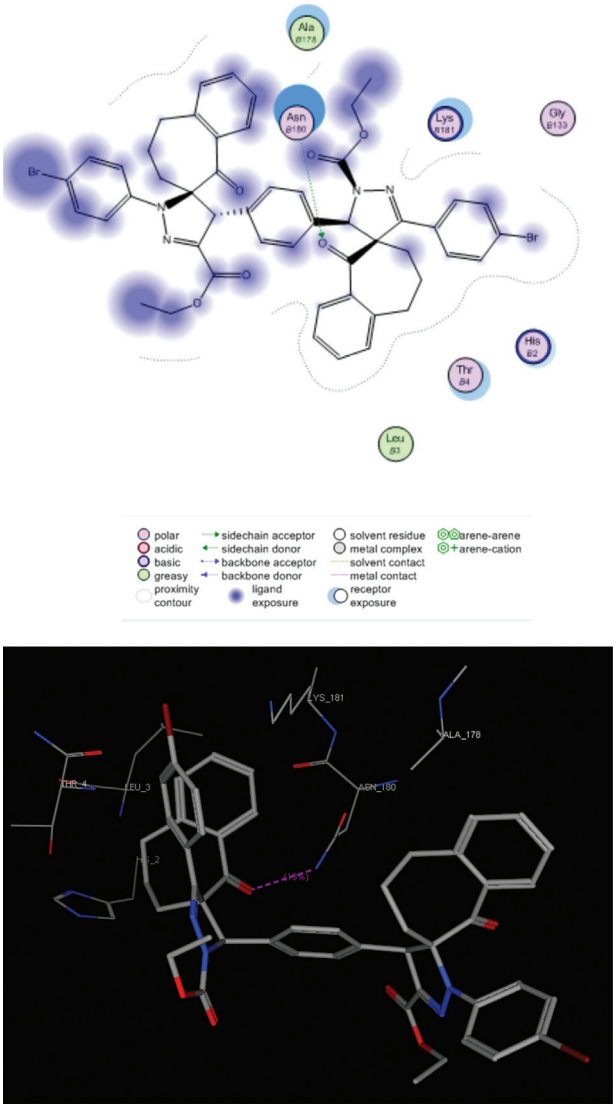
The ligand interaction and the binding mode of the native ligand sulfamethaxazole (O8D) showed one H-bond donor with HOH 333 with a distance of 2.76 (black color); it bonded with one H-bond acceptor with SER 222 at a distance of 2.9 (blue color) and one H-bond acceptor with HOH 289 (black color) depicted as hatched line. It gave a score of −13.0424.

Figure 2



The ligand interaction and the binding mode of the compound **7f**. It binds with one H-bond acceptor with ASN 180 at a distance of 2.57. It gives a score of –22.9123 kcal/mol greater than that of the cocrystallized ligand.

Figure 3



The ligand interaction and the binding mode of the compound **7g**. It binds with one H-bond acceptor with ASN 180 at a distance of 2.46. It gave a score of –17.5995 kcal/mol greater than that of the cocrystallized ligand.

(lowest energy). The crystal structures of DHPS enzyme receptor in complex with sulfamethoxazole ligand were obtained from the PDB (http://www.rcsb.org/pdb/explore.do?structureId=3TZF; PDB code: 3TZF). Partial charges and hydrogen atoms were added to the protein to assign ionization states and position of hydrogen atoms in the macromolecular structure using protonation 3D application in MOE (Figs 1–3).

Conclusion

6,6′-(1,4-Phenylene-*bis*(methanylylidene))-*bis*(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one) (**3**) was prepared. Reaction of **3** with nitrilimines (**5**) [generated *in situ* through triethylamine dehydrohalogenation of the appropriate hydrazonoyl halides (**4**)] in 1 : 2 molar ratio in dry benzene under microwave irradiation for 15–30 min gave dicyclo adduct **7** rather than **6** or **8**. The structure of all compounds was established based on spectral data and elemental analysis. Compounds **7g**, **7d**, **7c**, and **7e** showed significant activities against all tested microorganisms (except *C. albicans* and *P. aeruginosa*), whereas other compounds **7a** and **7b** have moderate activity against the same microorganisms. It was found that compound **7f**

exhibits the MIC against all tested microorganisms, especially fungus *A. fumigatus* and *P. italicum* (MIC=0.9 and 3.9 μg/ml, respectively). This may be due to the presence of the ethyl carboxylate group (COOEt) and the Ph group. We also noted that compound **7g** shows less activity compared with **7f**, which may be related to the presence of bromine in p-position of the phenyl group. Docking of the most active antibacterial compounds **7f** and **7g** against the dihydroperorate synthase enzyme gave comparable scores for hydrogen bond interaction (−22.9123, −17.5995 kcal/mol(and binding mode to the reference antibiotic sulfamethoxazole (−13.0424 kcal/mol).

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Conflicts of interest

There are no conflicts of interest.

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