Synthesis, antioxidant, and antimicrobial activities of new 2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitriles, (1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanones, and (1,3,4-oxadiazol-2-yl)-1,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonitriles: QSAR and molecular docking analysis

Fatma A. Bassyouni[a,b], Hanaa A. Tawfik[a], Ahmed R. Hamed[b,c], Maha M. Soltan[b,c], Mahmoud ElHefnawi[d], Ahmed A. ElRashedy[d], Maysa E. Moharam[e] and Mohamed Abdel Rehim[f]

[a]Department of Chemistry of Natural and Microbial Products, [b]Pharmaceutical Research Group, Center of Excellence for Advanced Sciences, [c]Department of Chemistry of Medicinal Plants, [d]Biomedical Informatics and Chemo Informatics Group, Department of Informatics and Systems, Centre of Excellence for Advanced Sciences, [e]Department of Microbial Chemistry, National Research Centre, Dokki, Cairo, Egypt and [f]Department of Analytical Chemistry, Stockholm University, Stockholm, Sweden

Correspondence to Fatma A. Bassyouni, PhD, Department Chemistry of Natural and Microbial Products and Pharmaceutical Research Group, Center of Excellence for Advanced Sciences, National Research Centre, 12311 Cairo, Egypt
Tel: +02 1118596967; fax: +0202 33370931; e-mail: fatma.nrc@hotmail.com

Received 19 February 2012
Accepted 6 June 2012

Egyptian Pharmaceutical Journal
2012,11:80–92

Objectives
A new series of 2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**6a**,**b**), (1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl) methanone (**9**–**11**), and (1,3,4-oxadiazol-2-yl)-1,5-dihydro-[1,2,4]triazolo[1,5-*a*] pyridine-8-carbonitrile (**14**–**16**) derivatives were synthesized and evaluated for their antioxidant and antimicrobial activities; in addition, their quantitative structure–activity relationships and molecular docking were investigated.

Methods
The target compounds **6a**,**b** were synthesized by the following method: reaction of 5,6-dimethyl-1H-benzoimidazole-2-carbohydrazide (**2**) with 4-(dimethyl amino)benzaldehyde or anthracene-9-carbaldehyde yielded Schiff's bases **3a**,**b**, which were reacted with ethyl cyanoacetate to yield 1H-pyrazole-4-carbonitriles **4a**,**b**; *N*-methylation of **4a**,**b** afforded **5a**,**b**, which reacted with 4-aminoantipyrine to give **6a**,**b**. In addition, 5-benzoyl-1H-benzo[d]imidazole-2-carbohydrazide (**8**) or 8-cyano-6-isocyano-5-oxo-7-phenyl-1,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-2-carbohydrazide (**13**) reacted with different carboxylic acids such as crotonic acid, 3,4-diaminobenzoic acid, and 6-hydroxy-4-methoxybenzofuran-5-carboxylic acid to form compounds **9**–**11** and **14**–**16**, respectively. The synthesized compounds were evaluated for their antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, and the diffusion plate method for antimicrobial activity.

Results and conclusion
Among other tested compounds, compounds **15**, **11**, and **10** possessed the highest antioxidant activity, whereas compounds **4a**, **5b**, **6b**, **10**, and **11** displayed high activity against *Staphylococcus aureus*, *Salmonella typhimurium*, and *Candida albicans*. The quantitative structure–activity relationships of the studied compounds **4a**, **4b**, **5b**, **6b**, **10**, **11**, **14**, **15**, and **16** indicated a high correlation ($r^2 = 0.82$) between the predicted and actual activities as obtained from molecular descriptors and the inhibitory activity of this set of tested molecules measured as antioxidant activity. Moreover, the three-dimensional (3D) pharmacophore was generated, and docking of the most active antibacterial compound **4a** against the dihydropteroate synthase enzyme gave comparable scores for hydrogen bond interaction (– 13.5 kcal/mol) and binding mode to the reference antibiotic sulfamethoxazole (– 13.00 kcal/mol).

Keywords:
antibacterial, antioxidant, benzimidazoles, molecular docking, quantitative structure–activity relationships, triazoles

Egypt Pharm J 11:80–92

1687-4315

Introduction

Benzimidazoles have a wide range of biological activities such as anticancerous and fungicidal activities; they also serve as antioxidants and central nervous system depressants [1–6]. A large number of benzotriazole derivatives are currently used in clinical applications [7–9]. In addition, they are of great value as intermediates

1687-4315 © 2012 Division of Pharmaceutical and Drug Industries Research, National Research Centre DOI: 10.7123/01.EPJ.0000422113.69898.e0

and final products in organic synthesis [10]. Fused heterocyclic compounds containing 1,2,4-triazoles have biological potency such as central nervous system depressant [11], antifungal [12], antiviral, and antibacterial activities [13]. Therefore, triazole derivatives have consistently attracted scientific and practical interest because of their widely varying chemical properties, synthetic versatility, and pharmacological activities [14,15].

Herein, we aim to prepare a new series of 2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile, (1,3,4-oxadiazol-2-yl-1H-benzo[d]imidazol-5-yl)(phenyl) methanone, and (1,3,4-oxadiazol-2-yl)-1,5-dihydro-[1,2,4]triazolo [1,5-*a*] pyridine-8-carbonitrile derivatives and evaluate them for their antioxidant and antibacterial activities. In addition, the quantitative structure–activity relationships (QSAR) of this series will be investigated.

Experimental

Chemistry

All melting points were determined using the Electro thermal capillary (Stuart, SMP10, UK) melting point apparatus and were uncorrected. ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were measured in DMSO-d_6 using a JEOL-500 spectrometer (Japan) with Me_4Si as an internal standard. Mass spectra were obtained using a Finigan gas chromatography–mass spectrometry (USA) at 70 eV. The IR spectra (4000–400 cm^{-1}) were recorded using KBr pellets in a Jasco FT/IR 300 E Fourier transform infrared spectrophotometer (USA) and in the 500–100 cm^{-1} region using polyethylene-sandwiched nujol mulls on a Perkin Elmer FT-IR 1650 spectrophotometer (Norwalk, USA). Elemental analyses were carried out at the Micro analytical Laboratory of the National Research Centre, Cairo, Egypt. Silica gel thin-layer chromatography cards were purchased from Merck (Darmstadt, Germany) (silica gel precoated aluminum cards with fluorescent indicator at 254 nm). Visualization was performed by illumination with a UV light source. Compounds **1**, **7**, and **12** were prepared according to the reported literature [16].

*5,6-Dimethyl-ethyl-2-benzimidazole carboxylate (**1**)*

Yield 9.1 g (84%), melting point (MP) 180–182°C. ^{1}H NMR δ:1.30 (3H, t, CH_3), 2.15 (6H, s, $2CH_3$), 4.10–4.30 ($_2$H, q, CH_2), 10.70 (1H, s, NH benzimidazole), 7.22–7.46 (2H, m, Ar-H). IR (KBr; cm^{-1}): 3350 (NH), 1730 (C = O), 1640 (C = N). MS: $m/z = 218$ [M^+]. Anal. calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.09; H, 6.40; N, 12.80.

*Synthesis of 5,6-dimethyl-1,3-benzimidazole-2-carboxyhydrazide (**2**)*

5,6-Dimethyl-ethyl-2-benzimidazole carboxylate (**1**; 0.025 mol, 5.40 g) was dissolved in absolute ethanol (30 ml). The hydrazine hydrate (0.050 mol, 1 ml) was added dropwise under stirring and refluxed for 6 h. The reaction mixture was cooled to room temperature and poured into ice-cold water. The solid formed was filtered, washed with water, dried, and then recrystallized from absolute ethanol to yield 4.15 g (80%), MP 230–232°C. ^{1}H NMR δ: 2.15 (6H, s, $2CH_3$), 5.56 (2H, br, NH_2, D_2O– exchangeable), 9.56 (1H, s, NH), 10.70 (1H, s, NH benzimidazole), 7.22–7.46 (2H, m, Ar-H). IR (KBr; cm^{-1}): 3340–3350 (NH), 3290 (NH_2), 1725 (C = O), 1640 (C = N). MS: $m/z = 208$ [M^+]. Anal. calcd for $C_{10}H_{12}N_4O$: C, 58.81; H, 5.92; N, 27.43. Found: C, 58.86; H, 5.87; N; 27.48.

*General procedure for the synthesis of N′-(4-(dimethylamino) benzylidene)-5,6-dimethyl-1H-benzo[d]imidazole-2-carbohydrazide (**3a**) and N′-(anthracen-9-ylmethylene)-5, 6-dimethyl-1H-benzo[d]imidazole-2-carbohydrazide (**3b**).*

To a solution of 5,6-dimethyl-1,3-benzimidazole-2-carboxyhydrazide (**2**; 1 mmol, 0.208 g) in absolute ethanol (30 ml), the appropriate aromatic aldehydes, namely 4-dimethyl amino benzaldehyde (1 mmol, 0.133 g) and anthracene-9-carbaldehyde (1 mmol, 0.206 g), were added with a few drops of piperidine and refluxed under stirring for 7 h. After cooling, the product was poured into crushed ice; the formed solid was filtered, washed with water, and recrystallized from absolute ethanol to give **3a** and **3b**.

***N′*-(4-(Dimethylamino)benzylidene)-5,6-dimethyl-1H-benzo [d]imidazole-2-carbohydrazide (*3a*)**

Yield 0.22 g (86%), MP 166–168°C. ^{1}H NMR δ: 2.15 (6H, s, $2CH_3$), 3.17 (6H, s, N–CH_3), 6.88–7.10 (4H, m, Ar-H), 7.22–7.46 (2H, m, Ar-H), 8.10 (1H, s, N = CH), 9.60 (1H, s, NH), 10.70 (1H, s, NH benzimidazole). C^{13} NMR: 19.80 (CH_3), 40.50 (N–CH_3), 110.30, 115.50, 123.50, 125.30, 130.50, 135.90 (Ar-CH), 145.80 (C = N). IR (KBr; cm^{-1}): 3350–3360 (NH), 1720 (C = O), 1640 (C = N). Anal. calcd for $C_{19}H_{21}N_5O$: C, 68.04; H, 6.31; N, 20.88. Found: C, 68.09; H, 6.38; N, 20.84.

*N′-(Anthracen-9-ylmethylene)-5,6-dimethyl-1H-benzo[d] imidazole-2-carbohydrazide (**3b**)*

Yield 0.32 g (84%), MP 190–192°C. ^{1}H NMR δ: 2.15 (6H, s, $2CH_3$), 7.20–7.46 (2H, m, Ar-H), 7.50–7.95 (9H, m, Ar-H), 8.10 (1H, s, N = CH), 9.60 (1H, s, NH), 10.70 (1H, s, NH benzimidazole), C^{13} NMR: 19.80 (CH_3), 115.50, 110.30, 123.50, 125.30, 128.20, 130.50, 131.80, 135.90 (Ar-CH), 145.80 (N = CH), 142.30 (C = N), 155.50 (C = O). IR (KBr; cm^{-1}): 3350–3365 (NH), 1720 (C = O), 1640 (C = N). Anal. calcd for $C_{25}H_{20}N_4O$: C, 76.51; H, 5.14; N, 14.28. Found: C, 76.56; H, 5.18; N, 14.33.

*General procedure for the synthesis of 2-(5,6-dimethyl-1H-benzo[d]imidazole-2-carbonyl)-5-(4-(dimethylamino) phenyl)-3-oxo-2,3-dihydro-1H-pyrazole-4-carbonitrile (**4a**) and 5-(anthracen-9-yl)-2-(5,6-dimethyl-1H-benzo[d]imidazole-2-carbonyl)-3-oxo-2,3-dihydro-1H-pyrazole-4-carbonitrile (**4b**)*

Compound **3a** (1 mmol, 0.335 g) or **3b** (1 mmol, 0.392 g) was dissolved in dry benzene (25 ml) and ethyl cyanoacetate (1 mmol, 0.15 ml), and a few drops of TEA were added. The reaction mixture was heated under reflux with stirring for 5 h. It was cooled to room temperature and poured into ice-cold water. The precipitate was filtered off and purified by crystallization from chloroform to form compounds **4a** or **4b**.

*2-(5,6-Dimethyl-1H-benzo[d]imidazole-2-carbonyl)-5-(4-(dimethylamino)phenyl)-3-oxo-2,3-dihydro-1H-pyrazole-4-carbonitrile (**4a**)*

Yield 0.3 g (84%), MP 242–244°C. ^{1}H NMR δ:2.15 (6H, s, 2CH_3), 6.85–7.10 (4H, m, Ar-H), 7.20–7.46 (2H, m, Ar-H), 10.70 (1H, s, NH benzimidazole), 11.20 (1H, s, NH pyrazole). C^{13} NMR: 19.80 (CH_3), 40.50 N(CH_3)$_2$, 115.90 (CN), 110.50, 123.50, 125.30, 130.50, 131.80, 135.90 (Ar-CH), 142.30 (C = N), 150.50 (C = O), 165.20 (C = O). IR (KBr; cm^{-1}): 3370–3380 (NH), 1710–1720 (C = O), 1640 (C = N), 2150 (CN). MS: *m/z* = 401[M$^+$ + 1]. Anal. calcd for $C_{22}H_{20}N_6O_2$: C, 65.99; H, 5.03; N, 20.99. Found: C, 65.95; H, 5.07; N, 20.96. MS: *m/z* = 401[M$^+$ + 1].

*5-(Anthracen-9-yl)-2-(5,6-dimethyl-1H-benzo[d]imidazole-2-carbonyl)-3-oxo-2,3-dihydro-1H-pyrazole-4-carbonitrile (**4b**)*

Yield 0.35 g (80%), MP 210–212°C. ^{1}H NMR δ: 2.15 (6H, s, 2CH_3), 3.17–3.30 [6H, s, N–(CH_3)$_2$], 7.20–7.46 (2H, m, Ar-H), 7.50–7.95 (9H, m, Ar-H), 10.70 (1H, s, NH), 11.20 (1H, s, NH pyrazole). C^{13} NMR: 19.80 (CH_3), 32.40 (CH_3), 115.90 (CN), 110.50, 123.50, 125.30, 130.50, 131.80, 135.90 (Ar-CH), 142.30 (C = N), 150.50 (C = O), 165.20 (C = O). IR (KBr; cm^{-1}): 3354–3360 (NH), ^{1}710–1720 (C = O), 1640 (C = N), 2165 (CN). MS: *m/z* = 458 [M$^+$ + 1]. Anal. calcd for $C_{28}H_{19}N_5O_2$: C, 73.51; H, 4.19; N, 15.31. Found: C, 73.56; H, 4.24; N, 15.36.

*General procedure for the synthesis of 5-(4-(dimethylamino)phenyl)-3-oxo-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (5a) and 5-(anthracen-9-yl)-3-oxo-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**5b**)*

Method A: A solution of methyl iodide (2 ml) in *n*-hexane (2 ml; 1/1, v/v) was added to the reaction mixture of compound **4a** (1 mmol, 0.40 g) or **4b** (1 mmol, 0.45 g) and sodium hydride/DMF (0.1 g sodium hydride/1.0 ml DMF) and stirred at room temperature. The reaction was stopped by the careful addition of a few drops of water followed by 20 ml of water under stirring at room temperature for 8 h. The product was extracted with 30 ml of *n*-hexane, dried with anhydrous sodium sulfate, filtered, and the solvent was evaporated under vacuum to afford **5a** or **5b** in yields up to 78–80%, respectively.

Method B: A mixture of compound **4a** (1 mmol, 0.40 g) or **4b** (1 mmol, 0.45 g), anhydrous K_2CO_3 (0.01 mol, 1.0 g), and dimethyl carbonate (DMC; 0.03 mol, 2.5 ml) in DMF (10 ml) was refluxed for 3 h. The reaction mixture was cooled to room temperature, following which ice water was added; the precipitated solid was filtered, dried, and crystallized from ethanol to afford **5a** or **5b** in yields up to 85–88%, respectively.

*5-(4-(Dimethylamino)phenyl)-3-oxo-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**5a**)*

Yield 0.36 g (88%) by method B, MP 234–236°C. ^{1}H NMR δ: 2.15 (6H, s, 2CH_3), 4.00 (3H, s, CH_3), 6.88–7.10 (4H, m, Ar-H), 7.20–7.46 (2H, m, Ar-H), 10.70 (1H, s, NH), 11.20 (1H, s, NH pyrazole). IR (KBr; cm^{-1}): 3320 (NH), 1710–1720 (C = O), 1640 (C = N). MS: *m/z* = 413 [M$^+$ – 1]. Anal. calcd for $C_{23}H_{22}N_6O_2$: C, 66.65; H, 5.35; N, 20.28. Found: C, 66.70; H, 5.40; N; 20.25. MS: *m/z* = 414 [M$^+$].

*5-(Anthracen-9-yl)-3-oxo-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**5b**)*

Yield 0.40 g (85%) by method B, MP 205–207°C. ^{1}H NMR δ: 2.15 (6H, s, 2CH_3), 4.00 (3H, s, CH_3), 7.20–7.46 (2H, m, Ar-H), 7.50–7.95 (9H, m, Ar-H), 10.70 (1H, s, NH), 11.20 (1H, s, NH pyrazole). IR (KBr; cm^{-1}): 3345 (NH), 1710–1720 (C = O), 1640 (C = N). MS: *m/z* = 471 [M$^+$]. Anal. calcd for $C_{29}H_{21}N_5O_2$: C, 73.87; H, 4.49; N, 14.85. Found: C, 73.84; H, 4.55; N, 14.90.

*General procedure for the synthesis of 3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylimino)-5-(4-(dimethylamino)phenyl)-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**6a**) and 5-(anthracen-9-yl)-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylimino)-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**6b**)*

Compound **5a** (1 mmol, 0.41 g) or **5b** (1 mmol, 0.47 g) was dissolved in absolute ethanol (25 ml), followed by the addition of 4-aminoantipyrine (1 mmol, 0.20 g) in the presence of acetic acid (2 ml). The reaction mixture was heated under reflux for 8 h. After cooling, the solvent was evaporated under vacuum and the precipitated product was crystallized from ethanol to afford **6a** or **6b**.

*3-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylimino)-5-(4-(dimethylamino)phenyl)-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**6a**)*

Yield 0.5 g (85%), MP 168–170°C. ^{1}H NMR δ: 2.10 (6H, s, 2CH_3), 4.00 (3H, s, CH_3), 6.85–7.10 (4H, m, Ar-H), 7.20–7.46 (2H, m, Ar-H), 10.70 (1H, s, NH). IR (KBr; cm^{-1}): 3358 (NH), 1640–1645 (C = N), 1690, 1685 (C = O), 2100 (CN). MS: *m/z* = 598 [M$^+$ – 1]. Anal. calcd for $C_{34}H_{33}N_9O_2$: C, 68.10; H, 5.55; N, 21.02. Found: C, 68.02; H, 5.50; N, 20.96.

*5-(Anthracen-9-yl)-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylimino)-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**6b**)*

Yield 0.54 g (83%) MP 177–179°C. ^{1}H NMR δ: 2.10 (6H, s, 2CH_3), 4.00 (3H, s, CH_3), 7.20–7.46 (2H, m, Ar-H), 7.50–7.95 (9H, m, Ar-H), 10.90 (1H, s, NH). IR (KBr; cm^{-1}): 3369 (NH), 1640–1665 (C = N), 1690, 1680 (C = O), 2120 (CN). Anal. calcd for. $C_{40}H_{32}N_8O_2$: C, 73.15; H, 4.91; N, 17.06. Found: C, 73.11; H, 4.87; N, 16.96.

*Synthesis of compounds **7** and **12***

Compounds **7** and **12** were synthesized using the same procedure as that described for the synthesis of

compound **1** and were obtained in 77 and 74% yields, respectively.

Ethyl 5-benzoyl-1H-benzo[d]imidazole-2-carboxylate (**7**): Yield (77%), MP 158–160 °C. ^{1}H NMR δ: 2.25 (3H, s, CH_3), 3.80 (2H, q, CH_2), 7.4 0–7.60 (2H, m, Ar-H), 7.80–7.90 (4H, m, Ar-H), 11.80 (1H, s, NH). IR (KBr; cm^{-1}): 3320 (NH), 1700 (C = O), 1660 (C = N). Anal. calcd for $C_{16}H_{14}N_2O_3$: C, 68.00, H, 6.90, N, 9.90. Found: C, 68.15; H, 6.82; N, 9.81.

*Synthesis of compounds **8** and **13***

Compounds **8** and **13** were synthesized by adopting the general procedure used for the preparation of compound **2** and were obtained in 75 and 78% yields, respectively.

5-Benzoyl-1H-benzo[d]imidazole-2-carbohydrazide (**8**): Yield (75%), MP 182–184°C. ^{1}H NMR δ: 5.60 (2H, s, NH_2), 7.4 0–7.60 (2H, m, Ar-H), 7.80–7.90 (4H, m, Ar-H), 11.80 (1H, s, NH). IR (KBr; cm^{-1}): 3320 (NH), 3250 (NH_2), 1700 (C = O), 1660 (C = N). Anal. calcd for $C_{15}H_{12}N_4O$: C, 68.18; H, 5.30; N, 10.60. Found: C, 68.1; H, 5.23; N, 10.61.

*General procedure for the synthesis of compounds **9–11***

To a solution of compound **8** (3 mmol, 0.792 g) in dry DMF (5 ml), $POCl_3$ was added dropwise (6 mmol, 1 ml), followed by 3 mmol of each acid, namely crotonic acid (3 mmol, 0.30 g), 3,4-diaminobenzoic acid (3 mmol, 0.75 g), and 6-hydroxy-4-methoxybenzofuran-5-carboxylic acid (3 mmol, 0.63 g). The reaction mixture was stirred at room temperature for 15 min, thereafter at 80–90°C for 5 h. After cooling, the reaction mixture was poured into crushed ice and neutralized by $NaHCO_3$ (20%). The precipitated product **9**, **10**, or **11** was filtered, washed with ice water, dried, and crystallized from ethanol.

Phenyl(2-(5-(prop-1-enyl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)methanone (**9**): Yield 0.25 g (75%), MP 223–225°C. ^{1}H NMR δ: 2.20 (3H, d, CH_3), 5.80 (1H, d, CH), 5.95 (1H, m, CH), 7.4 0–7.60 (2H, m, Ar-H), 7.80–7.90 (4H, m, Ar-H), 11.80 (1H, s, NH). IR (KBr) cm^{-1}: 3320 (NH), 1700 (C = O), 1640–1645 (C = N). MS: $m/z = 330$ [M$^+$]. Anal. calcd for $C_{19}H_{14}N_4O_2$: C, 69.08; H, 4.27; N, 16.96. Found: C, 69.12; H, 4.20; N, 16.90.

(2-(5-(3,4-Diaminophenyl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(Phenyl) methanone (**10**): Yield 0.308 g (78%), MP 188–190 °C. ^{1}H NMR δ: 5.60 (2H, m, NH_2), 6.80–7.10 (3H, m, Ar-H), 7.40–7.60 (2H, m, Ar-H), 7.80–7.90 (4H, m, Ar-H), 11.80 (1H, s, NH). IR (KBr; cm^{-1}): 3320 (NH), 1700 (C = O), 1640–1650 (C = N). MS: $m/z = 396$ [M$^+$]. Anal. calcd. for $C_{22}H_{16}N_6O_2$: C, 66.66; H, 4.07; N, 21.20. Found: C, 66.71; H, 21.25; N, 8.12.

(2-(5-(6-Hydroxy-4-methoxybenzofuran-5-yl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**11**): Yield 0.346 g (77%), MP 198–200°C. ^{1}H NMR δ: 2.35 (3H, s, CH_3), 3.60 (3H, s, OCH_3), 6.40–6.60 (2H, m, CH furan), 6.70 (1H, m, CH furan), 6.90 (1H, m, CH aromatic), 7.40–7.60 (4H, m, Ar-H), 7.80–7.90 (2H, m, Ar-H), 11.80 (1H, s, NH). IR (KBr; cm^{-1}): 3320 (NH), 1700 (C = O), 1640–1655 (C = N). Anal. calcd for $C_{25}H_{16}N_4O_5$: C, 66.30; H, 3.54; N, 12.39. Found: C, 66.39; H, 3.59; N, 12.45.

Ethyl 8-cyano-6-isocyano-5-oxo-7-phenyl-1,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-2-carboxylate (**12**): Yield 0.215 g (74%), MP 236–238°C. ^{1}H NMR δ: 2.20 (3H, s, CH_3), 3.80 (2H, q, CH_2), 7.30–7.75 (4H, m, Ar-H), 10.90 (1H, s, NH). IR (KBr; cm^{-1}): 3350 (NH), 1720 (C = O), 2120–2127 (CN). Anal. calcd f or $C_{16}H_{11}N_5O_3$: C, 63.90; H, 3.78; N, 24.05. Found: C, 63.82; H, 3.70; N, 24.10.

8-Cyano-6-isocyano-5-oxo-7-phenyl-1,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-2-carbohydrazide (**13**): Yield 0.25 g(78%), MP193–195°C. ^{1}H NMR δ: 5.80 (1H, m, CH), 5.90 (1H, d, CH), 6.50 (2H, s, NH_2), 7.30–7.75 (4H, m, Ar-H), 9.80 (1H, s, NH), 10.90 (1H, s, NH). IR (KBr; cm^{-1}): 3320 (NH), 3210 (NH_2), 1710 (C = O), 2150–2155 (CN). Anal. calcd for $C_{14}H_9N_7O2$: C, 52.33; H, 2.80; N, 34.89. Found: C, 52.28; H, 2.72; N, 34.82.

*General procedure for the synthesis of compounds **14–16***

To a solution of compound **13** (1 mmol, 0.321 g) in dry DMF, $POCl_3$ (5 ml) was added dropwise (3 mmol, 0.50 ml), followed by the addition of 1 mmol of each of the following carboxylic acids: crotonic acid, 3,4-diaminobenzoic acid, or 6-hydroxy-4-methoxybenzofuran-5-carboxylic acid. The reaction mixture was stirred at room temperature for 15 min and then at 80–90°C for 6 h. After cooling, the reaction mixture was poured into crushed ice and neutralized by $NaHCO_3$ (20%); the precipitated products **14**, **15**, or **16** were filtered, washed with ice water, dried and, crystallized from methanol.

6-Isocyano-5-oxo-7-phenyl-2-(5-(prop-1-enyl)-1,3,4-oxadiazol-2-yl)-1,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-8-carbonitrile (**14**): Yield 0.30 g (82%), MP 239–241°C. ^{1}H NMR δ: 2.28 (3H, d, CH_3), 5.80 (1H, m, CH), 5.90 (1H, d, CH), 7.30–7.75 (4H, m, Ar-H), 10.90 (1H, s, NH). IR (KBr; cm^{-1}): 3320 (NH), 1710 (C = O), 2150–2156 (CN). MS: $m/z = 369$ [M$^+$]. Anal. calcd for $C_{19}H_{11}N_7O_2$: C, 61.79; H, 3.00; N, 26.55. Found: C, 61.84; H, 3.06; N, 26.59.

2-(5-(3,4-Diaminophenyl)-1,3,4-oxadiazol-2-yl)-6-isocyano-5-oxo-7-phenyl-1,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-8-carbonitril (**15**): Yield 0.34 g (80%), MP 220–222°C. ^{1}H NMR δ: 5.60 (2H, m, NH_2), 6.90–7.10 (3H, m, Ar-H), 7.30–7.75 (5H, m, Ar-H), 10.90 (1H, s, NH). IR (KBr; cm^{-1}): 3320 (NH), 1710 (C = O), 2140–2150 (CN). Anal. calcd for $C_{22}H_{13}N_9O_2$: C, 60.69; H, 3.01; N, 28.95. Found: C, 60.75; H, 3.06; N, 29.05.

1,5-Dihydro-2-(5-(6-hydroxy-4-methoxybenzofuran-5-yl)-1,3,4-oxadiazol-2-yl)-6-isocyano-5-oxo-7-phenyl-[1,2,4]triazolo[1,5-a]pyridine-8-carbonitrile (**16**): Yield 0.40 g (81%), MP 230–232°C. ^{1}H NMR δ: 2.35 (3H, s, CH_3), 3.60 (3H, s, OCH_3), 6.40–6.60 (2H, m, CH furan), 6.70 (1H, m, CH furan), 6.90 (1H, m, CH aromatic), 7.30–7.75 (5H, m, Ar-H), 10.90 (1H, s, NH). IR (KBr; cm^{-1}): 3320 (NH), 1710 (C = O), 2150–2156 (CN). Anal. calcd for-$C_{25}H_{13}N_7O_5$: C, 61.09; H, 2.64; N, 19.96. Found: C, 61.15; H, 2.70; N, 20.09.

Results and discussion

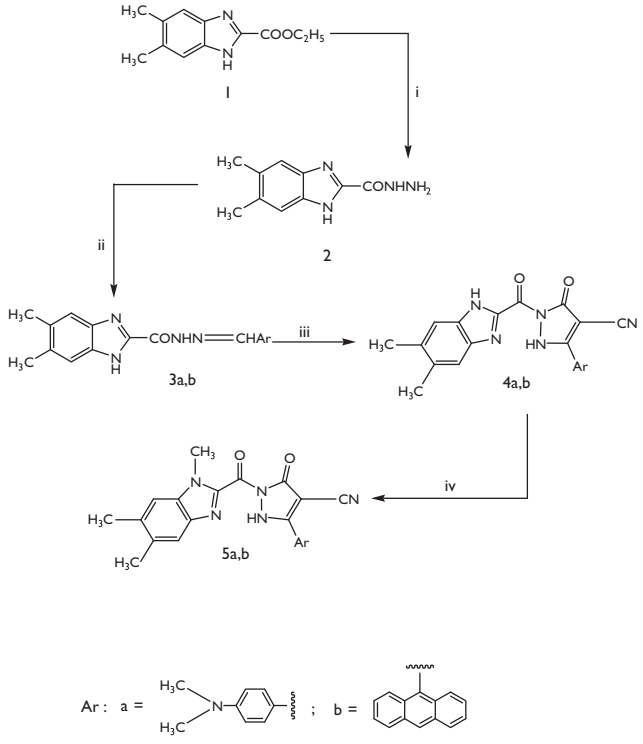
Compound 5,6-dimethyl-1H-benzo[d]imidazole-2-carbohydrazide (**2**) was prepared by the condensation reaction of 5,6-dimethyl-ethyl-2-benzimidazole carboxylate (**1**) with hydrazine hydrate in the presence of absolute ethanol. Schiff's bases *N*′-(4-(dimethylamino)benzylidene)-5,6-dimethyl-1H-benzo[d]imidazole-2-carbohydrazide (**3a**) and *N*′-(anthracen-9-ylmethylene)-5,6-dimethyl-1H-benzo [d]imidazole-2-carbohydrazide (**3b**) were obtained by the reaction of **2** with 4-(dimethylamino) benzaldehyde and anthracene-9-carbaldehyde in ethanol, respectively, in the presence of a few drops of piperidine. The target compounds 2-(5,6-dimethyl-1H-benzo[d]imidazole-2-carbonyl)-5-(4-(dimethylamino)phenyl)-3-oxo-2,3-dihydro-1 H-pyrazole-4-carbonitrile (**4a**) and 5-(anthracen-9-yl)-2-(5,6-dimethyl-1H-benzo[d]imidazole-2-carbonyl)-3-oxo-2,3-dihydro-1H-pyrazole-4-carbonitrile (**4b**) were synthesized by the reaction of **3a** and **3b** with ethyl cyanoacetate in ethanol in the presence of triethylamine, respectively. Methylation of **4a** and **4b** was achieved by their reaction with methyl iodide or DMC that yielded 5-(4-(dimethylamino)phenyl)-3-oxo-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**5a**) and 5-(anthracen-9-yl)-3-oxo-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**5b**), respectively (Scheme 1). Their structures were established on the basis of elemental analysis and spectral data.

Furthermore, compounds **6a** or **6b** were synthesized by the following reaction: **5a** and **5b** reacted with 4-aminoantypyrine in ethanol and in the presence of catalytic amounts of acetic acid to give 3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylimino)-5-(4-(dimethylamino)phenyl)-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**6a**) and -5-(anthracen-9-yl)-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylimino)-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**6b**), respectively (Scheme 2).

In Scheme 3, ethyl 5-benzoyl-1H-benzo[d]imidazole-2-carboxylate (**7**) and 5-benzoyl-1H-benzo[d]imidazole-2-carbohydrazide (**8**) were prepared according to the method used for the synthesis of compounds **1** and **2**. Compounds **9–11** were formed by intermolecular cyclization of the hydrazide derivative **8** through a condensation reaction with crotonic acid, 3,4-diaminobenzoic acid, and 6-hydroxy-4-methoxybenzofuran-5-carboxylic acid in the presence of $POCl_3$ and DMF that yielded the corresponding substituted oxadiazol derivatives identified as phenyl(2-(5-(prop-1-enyl)-1,3,4-oxadiazol-2-yl)-1H-benzo [d]imidazol-5-yl)methanone (**9**), 2-(5-(3,4-diaminophenyl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**10**), and (2-(5-(6-hydroxy-4-methoxybenzofuran-5-yl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**11**), respectively. The structures of compounds **9–11** were confirmed on the basis of their elemental analysis and spectral data (cf. Experimental data).

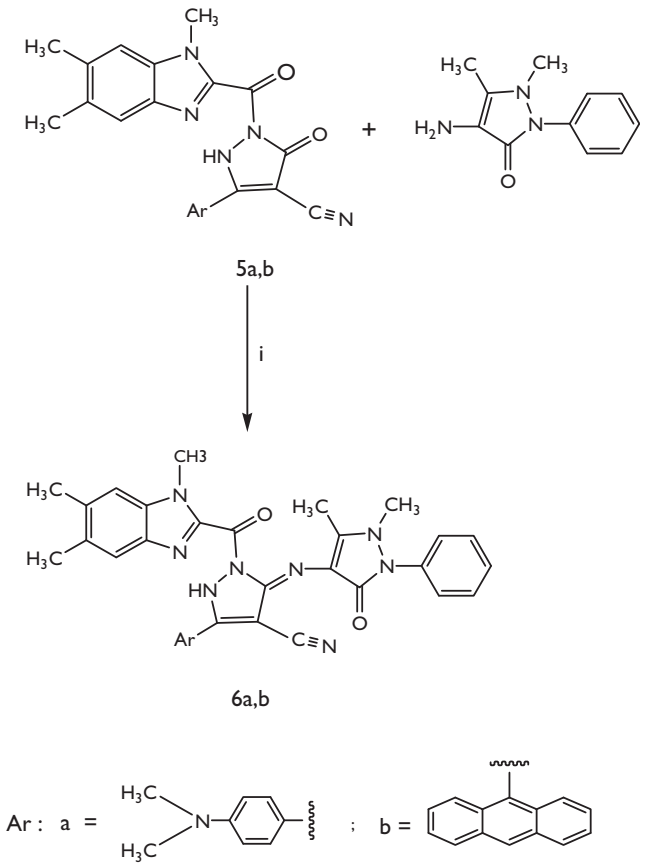
Moreover, the condensation reaction of compound **12** with hydrazine hydrate yielded 8-cyano-6-isocyano-5-oxo-7-phenyl-1,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-2-car-

Scheme 1



Synthesis of compounds **1**–**5a**,**b**. *Condition and reagents*: (i) $NH_2NH_2 \cdot H_2O$/ethanol/reflux, (ii) ArCHO/ethanol/piperidine/reflux, (iii) ethyl cyanoacetate/ethanol/TEA/reflux, (iv) method A: MeI/NaH/DMF/RT, method B: DMC/K_2CO_3/DMF/reflux.

Scheme 2



Synthesis of compounds **6a**,**b**. *Condition and reagents*: (i) **e**thanol/acetic acid/reflux.

bohydrazide (**13**), which was reacted with crotonic acid, 3,4-diaminobenzoic acid, or 6-hydroxy-4-methoxybenzofuran-5-carboxylic acid in the presence of $POCl_3$ in DMF to produce the corresponding derivatives 6-isocyano-5-oxo-7-phenyl-2-(5-(prop-1-enyl)-1,3,4-oxadiazol-2-yl)-1,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonitrile (**14**), 2-(5-(3,4-diaminophenyl)-1,3,4-oxadiazol-2-yl)-6-isocyano-5-oxo-7-phenyl-1,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonitrile (**15**), or 1,5-dihydro-2-(5-(6-hydroxy-4-methoxybenzofuran-5-yl)-1,3,4-oxadiazol-2-yl)-6-isocyano-5-oxo-7-phenyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonitrile (**16**), respectively (Scheme 3). The structure of compounds **14–16** was assigned on the basis of their elemental analyses and spectral data.

Biological screening

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) scavenging assay

Compounds **4a**, **4b**, **5a**, **5b**, **6b**, **10**, **11**, **14**, **15**, and **16** were prepared in DMSO as 10 × stocks from each of the tested concentrations (0, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} mol/l) and briefly sonicated when necessary in an ultrasonic water bath. The compounds were submitted for testing to determine the effective concentration of the compound producing 50% scavenging of the DPPH (EC_{50}). Two reference radical scavengers, quercetin, and gallic acid were tested in the assay as positive controls. The method used in the present study was based on previously published methods in literature. The compound stock solutions (15 µl/well) were pipetted in duplicates into 96-well plates. The assay was started with the addition of DPPH reagent (0.004% w/v) in methanol (135 µl/well). Appropriate negative controls were simultaneously run using methanol as a correction for the optical density of colored compounds at 540 nm. The plate was immediately shaken for 30 s and incubated in the dark for 30 min at room temperature. The remaining DPPH was measured in a microplate reader (BMG Fluostar Optima, Ortenberg, Germany) at 540 nm. The percentage of antioxidant activity (%AA) was calculated using the following equation:

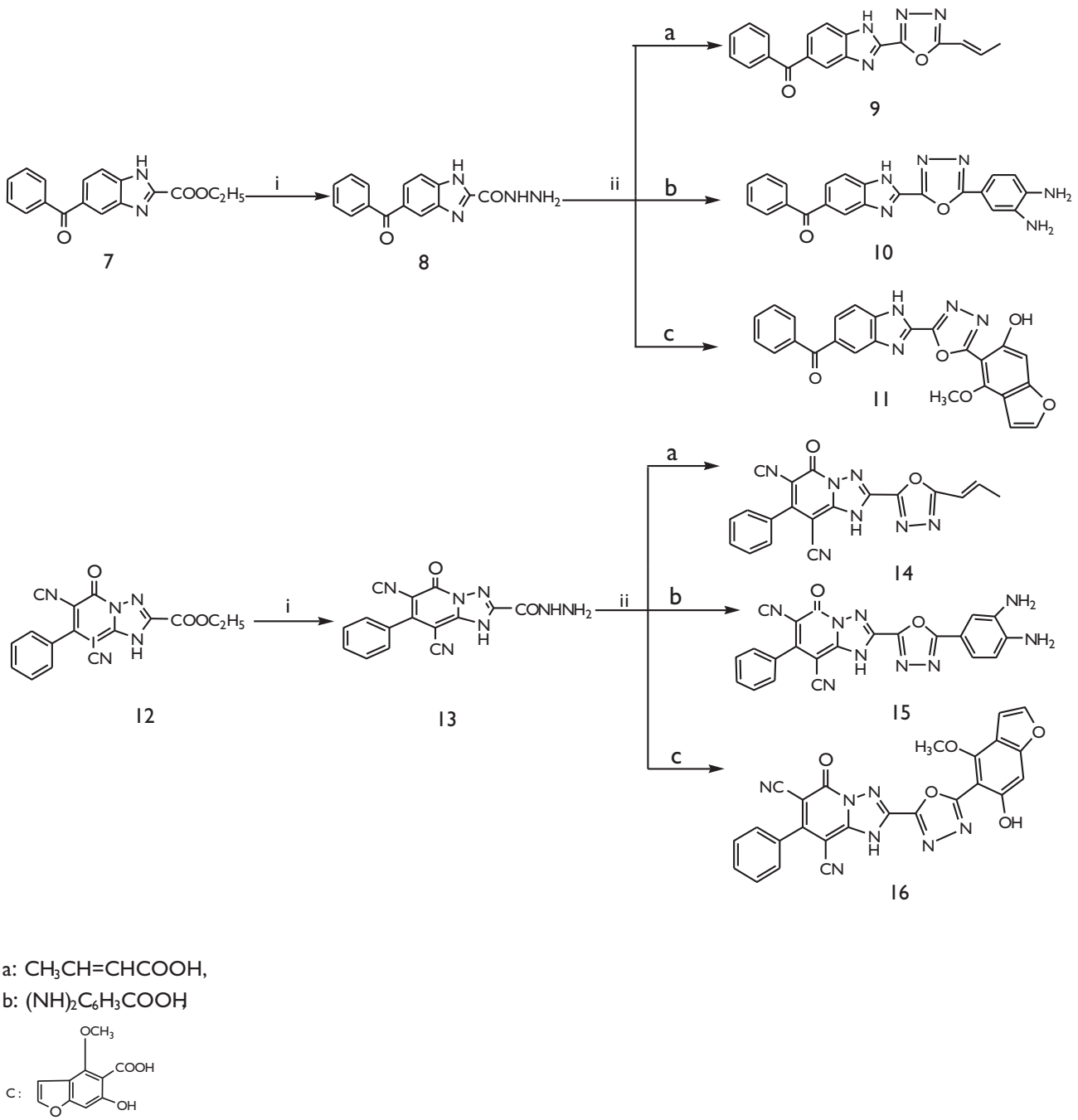
$$\%\ \text{Antioxidant activity}\,(\%\,\text{AA}) = 100 \times \frac{[\text{OD}_{540\,\text{nm}}\,(\text{blank}) - \text{OD}_{540\,\text{nm}}\,(\text{sample})]}{\text{OD}_{540\text{nm}}\,(\text{blank})}.$$

Statistical analysis

Regression analysis was used to determine the EC_{50} values for each compound using the concentration–%AA relationship. All data were represented as the mean value of the duplicate absorbance measurement.

Table 1 illustrates the antioxidant effects of the tested compounds **4a**, **4b**, **5a**, **5b**, **6b**, **10**, **11**, **14**, **15**, and **16**,

Scheme 3



a: $CH_3CH{=}CHCOOH$,

b: $(NH)_2C_6H_3COOH$

c:

Synthesis of compounds **7–11** and **12–16**. *Condition and reagents*: (i) $NH_2NH_2.H_2O$/ethanol/reflux, (ii) $POCl_3$/DMF/ (a) crotonic acid, (b) 3,4-diaminobenzoic acid, (c) 6-hydroxy-4-methoxybenzofuran-5-carboxylic acid.

represented as EC_{50} values. The compounds showed antioxidant activities against the DPPH radical in the following order of higher activity (lower EC_{50}): **15** > **11** > **10** > **16** > **14** > **4b** > **5a** > **6b** > **5b** > **4a**. As shown in Table 1, compounds 2-(5-(3,4-diaminophenyl)-1,3,4-oxadiazol-2-yl)-6-isocyano-5-oxo-7-phenyl-1,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonitrile (**15**), (2-(5-(6-hydroxy-4-methoxybenzofuran-5-yl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**11**), and 2-(5-(3,4-diaminophenyl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**10**) showed the highest antioxidant activity compared with other tested compounds and their activities were comparable with the activities of the reference antioxidants quercetin and gallic acid.

Antimicrobial activity

The antibacterial and antifungal activities of the tested compounds **4a**, **4b**, **5b**, **6a**, **6b**, **10**, **11**, **14**, **15**, and **16** were estimated using the diffusion plate method. A sterilized filter paper disc saturated with a measured quantity (25 µl) of the sample (1 mg/ml) was placed on a plate (9 cm in diameter) containing a solid bacterial

medium (nutrient agar) or a fungal medium (potato dextrose agar) that had been seeded with the spore suspension of the test organism. After incubation of the bacterial culture at 37°C for 24 h (in the case of fungi, 25°C for 72 h), the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism (% inhibition = sample inhibition zone (cm)/plate diameter × 100). All measurements were taken with methanol as a solvent, which has zero inhibitory activity [22–26].

The antimicrobial activity of the tested compounds **4a**, **4b**, **5b**, **6a**, **6b**, **10**, **11**, **14**, **15**, and **16** was examined with gram-positive bacteria *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*, gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*, and the fungus *Candida albicans*. The obtained results were compared with that of the reference antibiotic amoxicillin that was purchased from Pfizer (Cairo, Egypt).

Table 2 shows that compounds 2-(5,6-dimethyl-1H-benzo[d]imidazole-2-carbonyl)-5-(4-(dimethylamino)phenyl)-3-oxo-2,3-dihydro-1H-pyrazole-4-carbonitrile (**4a**) and (2-(5-(6-hydroxy-4-methoxybenzofuran-5-yl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**11**) were the most active compounds against *S. typhimurium*, whereas compounds 5-(anthracen-9-yl)-3-oxo-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**5b**) and 5-(anthracen-9-yl)-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylimino)-2-(1,5,6-trimethyl-1H-benzo[d]imidazole-2-carbonyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (**6b**) exhibited high antimicrobial activity against *S. aureus*, reaching an 18 mm clear zone; other compounds showed variable antibacterial activity with different extents. With respect to *C. albicans*, compounds 2-(5-(3,4-diaminophenyl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**10**) and (2-(5-(6-hydroxy-4-methoxybenzofuran-5-yl)-1,3,4-oxadiazol-2-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**11**) exhibited significantly high activity against the species.

Table 1 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (cell-free system) of compounds 4a, 4b, 5a, 5b, 6b, 10, 11, 14, 15, and 16 represented as EC_{50} values

Compounds	DPPH EC_{50} (μmol/l)
4a	1135.0
4b	186.1
5a	227.9
5b	412.7
6b	361.8
10	32.9
11	27.8
14	133.8
15	23.3
16	90.4
Quercetin	8.9
Gallic acid	7.1

DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; EC_{50}, effective concentration of the compound producing 50% scavenging of the DPPH.

Antioxidant quantitative structure–activity relationships study

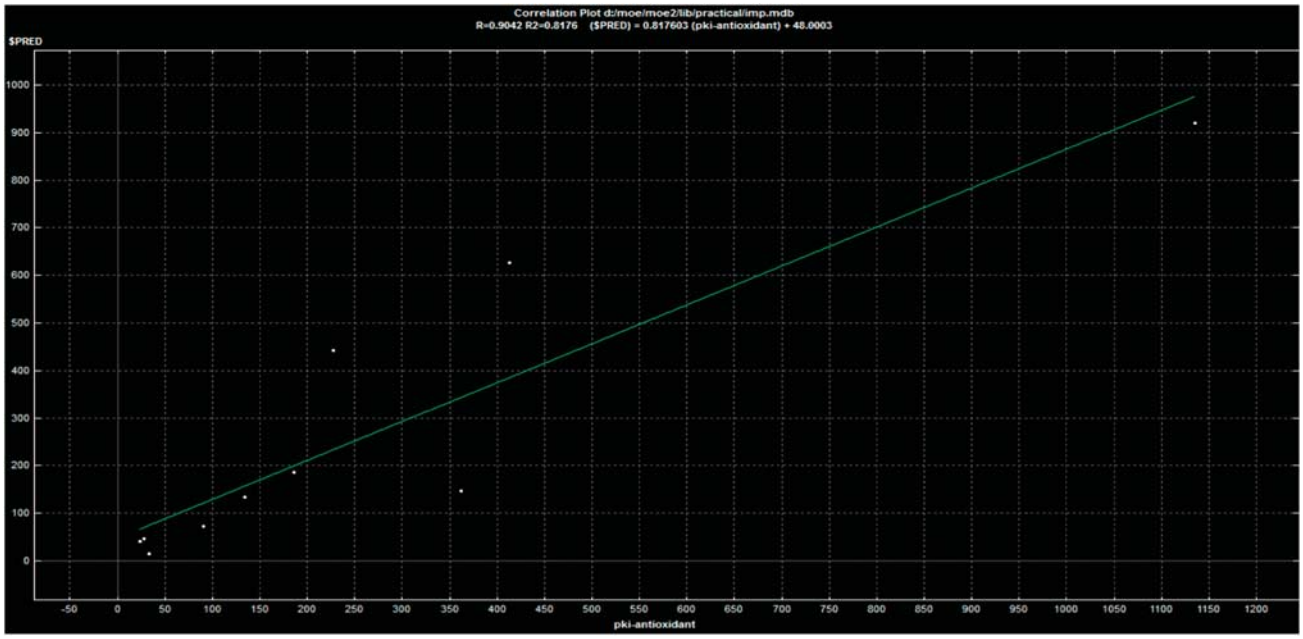
The flexagen algorithm develops predictive pharmacophore models with both activity and structural data of the training set molecules [27]. To guarantee the construction of robust models, it is crucial to generate a representative training set with sufficient coverage of both biological and chemical spaces occupied by the original data set. Therefore, diversity sampling of the original data set by considering both activity and structural information is the premise for rational selection of training sets. Furthermore, simultaneous inclusion of several similar compounds in the training sets should be avoided, as it may only provide redundant information and bias the resulting model toward those similar structures. Other important guidelines are in the literature [28]. Pharmacophoric hypotheses are important tools in drug design and discovery as they provide excellent insights into ligand macromolecule recognition. However, their predictive value as three-dimensional (3D)-QSAR models is limited by steric clashes and bioactivity enhancing or reducing auxiliary groups. This point, combined with the fact that pharmacophore modeling of antioxidant activity furnished numerous binding hypotheses of comparable success criteria, prompted us to use classical QSAR analysis to search for the best combination of pharmacophore(s) and 2D descriptors capable of explaining bioactivity variation. Furthermore, 3D QSAR modeling was implemented in the current case as grounds of competition to select the best pharmacophore(s) that could explain bioactivity variation across the whole training list.

All computational work was carried out using the molecular operating environment (MOE) program (Chemical Computing Group Inc., Quebec, Canada; 2008). All compounds were drawn using Chem (Cambridge Soft Corporation, Cambridge, Massachusetts, USA). Draw 11,

Table 2 The antibacterial and antifungal activities of the tested compounds 4a, 4b, 5b, 6a, 6b, 10, 11, 14, 15, and 16

		Inhibition zone diameter (mm/mg sample)										
Microorganism	Gram-stain reaction	**4a**	**4b**	**5b**	**6a**	**6b**	**10**	**11**	**14**	**15**	**16**	Amoxicillin
Bacillus cereus	Positive	10	7	9	7	10	8	8	8	–	10	22
Bacillus subtilis	Positive	–	–	10	7	9	8	7	9	–	8	25
Escherichia coli	Negative	7	12	8	8	–	–	–	–	10	–	22
Pseudomonas aeruginosa	Negative	10	9	11	11	13	9	11	11	–	10	30
Staphylococcus aureus	Positive	10	10	18	10	18	9	9	10	–	10	16
Candida albicans	Fungi	–	–	–	–	–	17	14	–	12	10	25
Salmonella typhimurium	Negative	40	–	–	–	11	10	16	10	–	11	20

Figure 1



Correlation plot showing a linear relationship between the actual and predicted activities of compounds **4a**, **4b**, **5a**, **5b**, **6b**, **10**, **11**, **14**, **15**, and **16**.

and energy was minimized by force-field MMFF94 × optimization with a gradient of 0.0001 for determining the low-energy conformations with the most favorable (lowest energy) geometry.

The purpose of a QSAR descriptor is to calculate properties of molecules that serve as numerical descriptions or to characterize molecules that are correlated with activity. The descriptors computed using MOE could be classified into three classes: 2D descriptors based on the atom and connection information of the molecule, internal 3D descriptors (i3D) based on the 3D information on each molecule and variant to rotation and translation of the conformation, and external descriptors (x3D) based on the 3D information, fitting the antioxidant activity of the dependent variable pK_i to that of the independent variables, namely the molecular descriptors.

Partial least squares

Partial least squares analysis was used to derive linear equations from the resulting matrices. Leave one out cross-validation was used to select the number of principal components and to calculate the cross-validated statistics. Regression analysis modules of statistical analysis tool were used to build the 3D QSAR models. Regression analysis was carried out using the pK_i activity (antioxidant activity) as the dependent variable and the calculated descriptor as the predicted variable. As shown in the Blow correlation plot, a linear relationship exists between the actual and predicted activities; the correlation coefficient is 0.817603 (Fig. 1).

Principal component analysis

The 2D molecular properties of **4a**, **4b**, **5a**, **5b**, **6b**, **10**, **11**, **14**, **15**, and **16** were estimated using the 'calculate molecular properties' protocol implemented in the QSAR module. The PCA method was then applied using the 'calculate principal components' protocol in the 'library analysis' module to extract three principal components. The program initially generated more than 400 descriptors for each compound and it is predicted that some of the descriptors are highly correlated. Therefore, the PCA method was applied to reduce the dimensionality of the descriptor space and alleviate the correlations [29]. Basically, the PCA method is a mathematical procedure that converts multiple sets of possibly correlated variables into a few orthogonal 'principal components' that are usually linear combinations of the correlated variables, each corresponding to an axis in multiple-dimensional space, as represented by the following equation:

$$\mathrm{PC}_i = \sum_{j=1}^{v} C_{ij} x_j, \qquad (1)$$

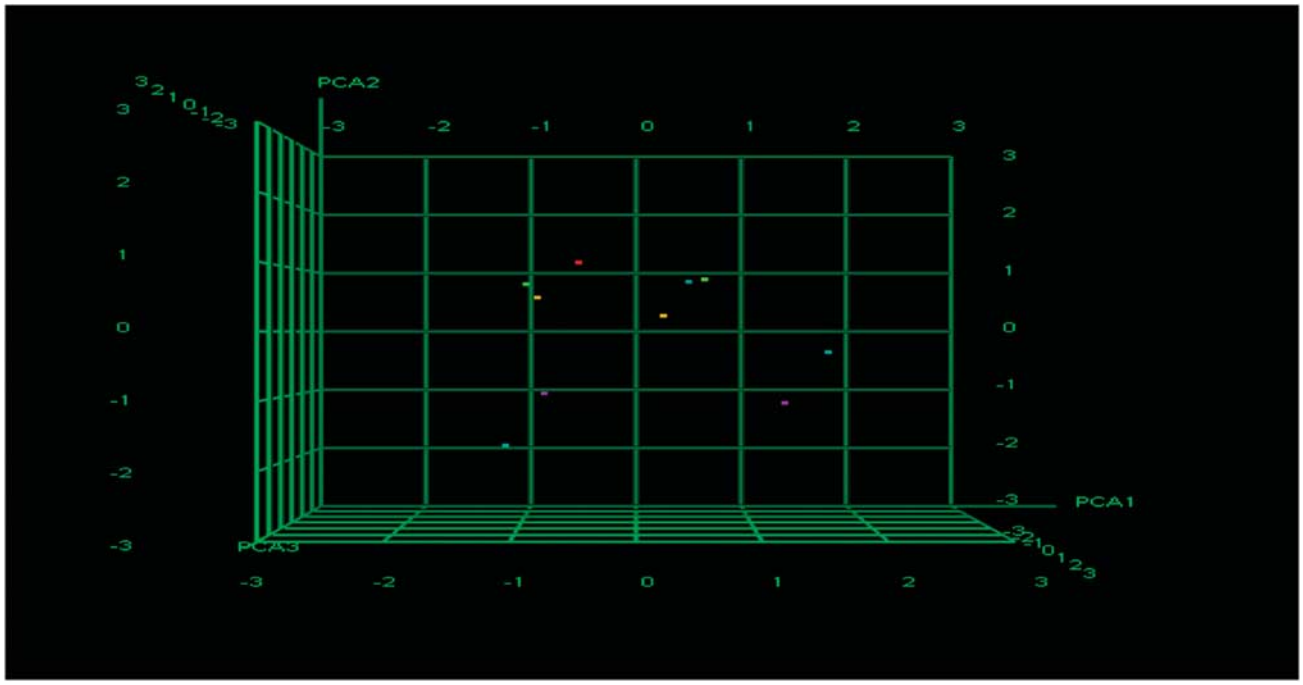
where PC_i is the principle component, C_{ij} is the coefficient of the variable x_j, and is the number of variables.

For clear graphical representation of the molecular diversity, we extracted three principal components that account for the variation in the descriptor space and plotted the molecules as discrete spots in a 3D coordinated system (Fig. 2).

Pharmacophore generation

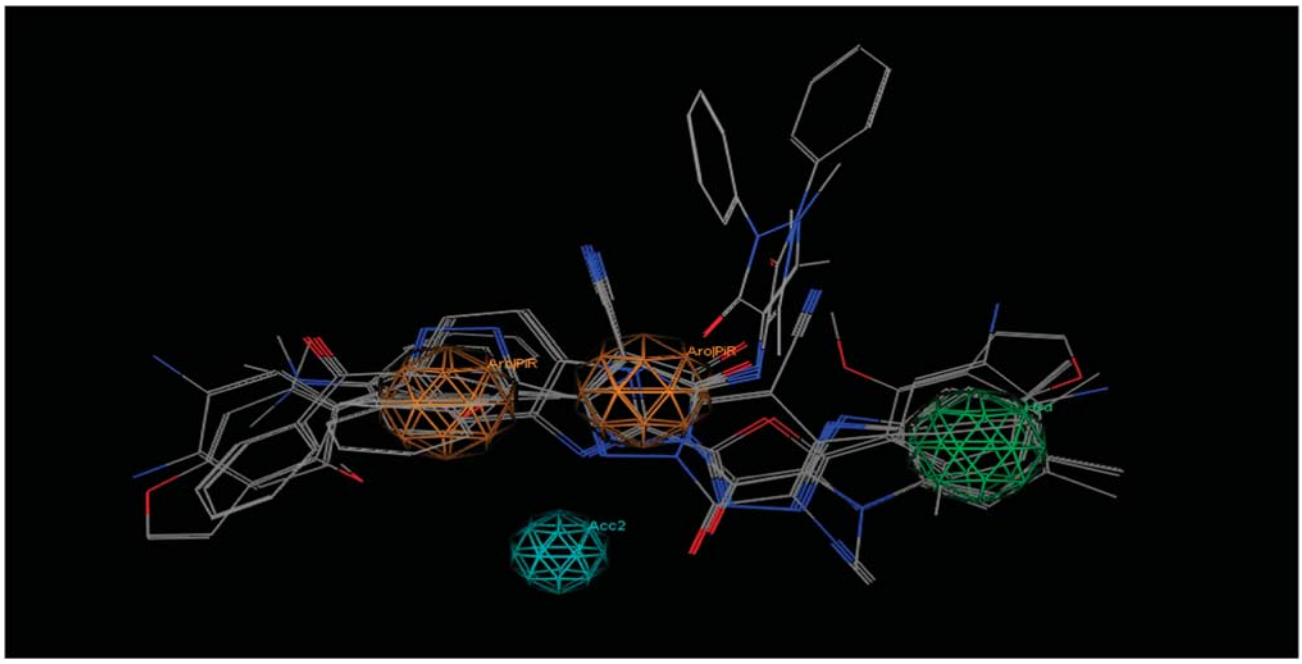
The pharmacophore generation protocol used a flex align algorithm [30]. The features of the hydrogen bond acceptor, hydrogen bond donor, hydrophilic or hydrophobic aromatic center, and the aromatic ring were predefined using a stochastic search conformation, and the parameter of 'maximum excluded volumes' (MEV)

Figure 2



The three-dimensional scatter plot is a visual representation of the molecules as described by the three selected principal components (PCA1, PCA2, and PCA3). Each point corresponds to a molecule and is colored according to the molecule's pK_i value.

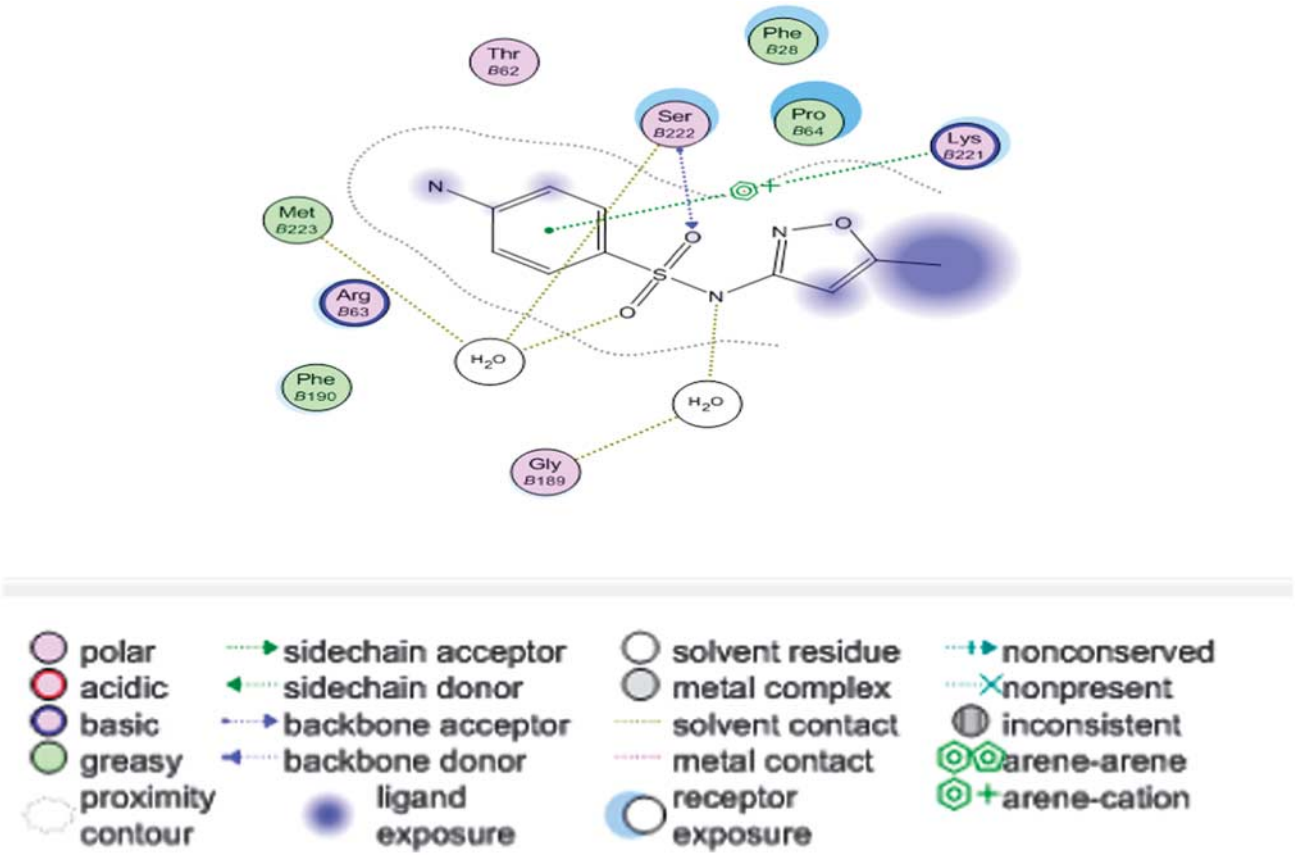
Figure 3



The three-dimensional quantitative structure–activity relationships pharmacophore generated from compounds **4a**, **4b**, **5a**, **5b**, **6b**, **10**, **11**, **14**, **15**, and **16** showed a hydrophobic center (green), two aromatic planar ring centers (orange), and a hydrogen bond acceptor (blue).

had a value of 5. The fundamental approach for pharmacophore elucidation described herein is to exhaustively search for all pharmacophore queries that induce good overlay of most of the active molecules (Fig. 3). Thus, the plausibility of the pharmacophore is measured by overlay of actives, and the relationship with activity is measured by classification accuracy and HipHop/HypoGen methods described in attempt to

Figure 4



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.

relate common feature geometries with activity or complexity [31].

Sensitivity and specificity

The sensitivity and specificity of the models should be established during validation to assess the propensity of the QSAR models for correct qualitative prediction of the dependent variable [32]. Sensitivity can be calculated as follows:

$$\text{Sensitivity}\ (\%) = 100 \times \frac{\text{TP} - \text{FN}}{\text{TP}}. \qquad (2)$$

Specificity can be calculated as follows:

$$\text{Specificity}\ (\%) = 100 \times \frac{\text{TP} - \text{FP}}{\text{TP}}. \qquad (3)$$

TP – FN represents the number of corrected predictions, TP represents the number of true positives, and TN represents the number of true negatives.

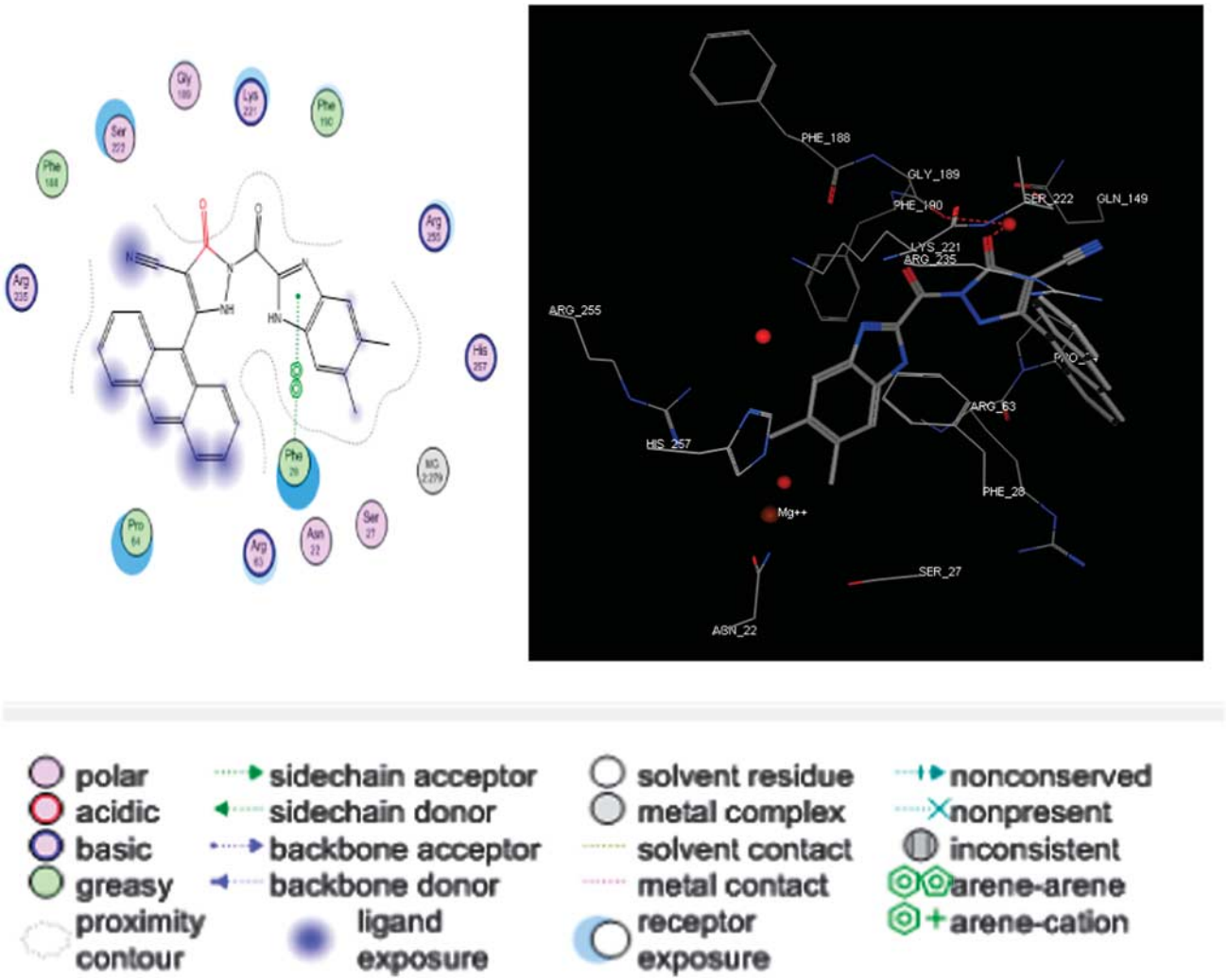
To confirm the probability of identifying true selective molecules, sensitivity and specificity tests were performed on the observed versus predicted selectivity values for the dataset. The observed values for sensitivity and specificity were 90 and 93%, respectively. These results were in accordance with those expected from the earlier examination of probability of predicting the potency profile for the antioxidant activity of the tested compounds **4a**, **4b**, **5a**, **5b**, **6b**, **10**, **11**, **14**, **15**, and **16**.

Antibacterial molecular docking study

Molecular docking was performed and analyzed with the MOE program. 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. Compound **4a** docked into the same groove of the binding site of the native cocrystallize ligand. Scoring in MOE software was performed 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 (lowest energy) geometry.

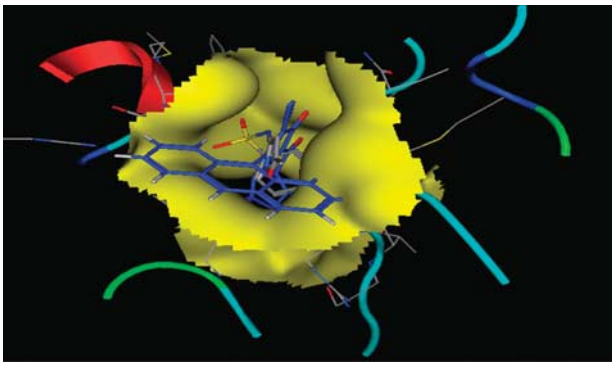
The antibiotic sulfamethaxazole was used as a reference for the docking study. It inhibits the dihydropteroate synthase enzyme, a key enzyme in the folate pathway.

Figure 5



The ligand interaction and the binding mode of the compound **4a**. It binds with one H-bond the acceptor with HOH 333 at a distance of 0.96. It gave a score of −13.5451 kcal/mol greater than that of the cocrystallized ligand.

Figure 6



Superposition of compound **4a** (blue color). The cocrystallized ligand sulfamethaxazole (O8D; gray color) showed binding with HOH 333 as a cocrystallized ligand.

Compound	Score	H-bond involved
Ligand	−13.0424	HOH 333, SER 222, HOH 289
4a	−13.5451	HOH 333

Compound **4a** was investigated for its binding affinity with the dihydropteroate synthase receptor (pdb 3TZF) [33] for the purpose of lead optimization and to study the interaction between compound **4a** and the dihydropteroate synthase receptor (Figs 4–6).

Conclusion

QSAR between the molecular structure and the inhibitory antioxidant activity of the synthesized compounds **4a**, **4b**, **5a**, **5b**, **6b**, **10**, **11**, **14**, **15**, and **16** were studied. Compounds **15**, **11**, and **10** displayed the highest antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. The antimicrobial activity showed that compound **4a** was the most active against *S. typhimurium* and its activity exceeded the activity of the reference antibiotic amoxicillin. Compounds **5b** and **6b** exhibited high antimicrobial activity against *S. aureus*, whereas compounds **10** and **11** showed significantly high activities against *C. albicans*. The molecular modeling study showed that compound **4a** gave score of −13.5451 kcal/mol, which was greater than the score of the cocrystallized ligand sulfamethoxazole (−13.0 kcal/mol).

Acknowledgements

The authors thank the National Research Center, Cairo, Egypt, and the Science and Technology Development fund (STDF Grant No.: 1169) for financial support.

Conflicts of interest

There are no conflicts of interest.

References

1 Refaat HM. Synthesis and anticancer activity of some novel 2-substituted benzimidazole derivatives. Eur J Med Chem 2010; 45:2949–2956.

2 Spasov AA, Yozhitsa IN, Bugaeva LI, Anisimova VA. Benzimidazole derivatives: spectrum of pharmacological activity and toxicological properties (a review). Pharm Chem J 1999; 33:232–243.

3 Perston PN. *In the chemistry of heterocyclic compounds, benzimidazoles and congeneric tricyclic compounds. Vol. 40, part 2*. New York: John Wiley & Sons Interscience; 1981.

4 Laryea D, Gullbo J, Isaksson A, Larsson R, Nygren P. Characterization of the cytotoxic properties of the benzimidazole fungicides, benomyl and carbendazim, in human tumour cell lines and primary cultures of patient tumour cells. Anticancer Drugs 2010; 21:33–42.

5 Kopańska K, Najda A, Zebrowska J, Chomicz L, Piekarczyk J, Myjak P, Bretner M. Synthesis and activity of 1H-benzimidazole and 1H-benzotriazole derivatives as inhibitors of *Acanthamoeba castellanii*. Bioorg Med Chem 2004; 12:2617–2624.

6 Ayhan-Kilcigil G, Kus C, Çoban T, Can-Eke B, Iscan M. Synthesis and antioxidant properties of novel benzimidazole derivatives. J Enzyme Inhib Med Chem 2004; 19:129–135.

7 Carta A, Sanna P, Palomba M, Vargiu L, La Colla M, Loddo R. Synthesis and antiproliferative activity of 3-aryl-2-(1H-benzotriazol-1-yl)acrylonitriles. Part III. Eur J Med Chem 2002; 37:891–900.

8 Boido A, Vazzana I, Mattioli F, Sparatore F. Antiinflammatory and antinociceptive activities of some benzotriazolylalkanoic acids. Farmaco 2003; 58:33–44.

9 Biagi G, Calderone V, Giorgi I, Livi O, Scartoni V, Baragatti B, Martinotti E. Some structural changes on triazolyl-benzotriazoles and triazolyl-benzimidazolones as potential potassium channel activators. III. Farmaco 2001; 56:841–849.

10 Katritzky AR, Lan X, Yang JZ, Denisko OV. Properties and synthetic utility of *n*-substituted benzotriazoles. Chem Rev 1998; 98:409–548.

11 Parmar SS, Gupta AK, Singh HH, Gupta TK. Benzimidazolyl-1,2,4(H)-triazoles as central nervous system depressants. J Med Chem 1972; 15:999–1000.

12 Hwang LC, Tu CH, Wang JH, Lee GH. Synthesis and molecular structure of 6-amino-3-benzylmercapto-1,2,4-triazolo[3,4-*f*][1,2,4]triazin-8(7H)-one. Molecules 2006; 11:169–176.

13 Hiremath SP, Ullagaddi A, Shivaramayya K, Purohit MG. Amino acid derivatives, VI: synthesis, antiviral, and antimicrobial evaluation of amino acid esters bearing a 1,2,3-triazolo[4,5-*d*]pyrimidinedione side chain. Indian J Heterocycl Chem 1999; 3:145–148.

14 Shivarama Holla B, Veerendra B, Shivananda MK, Poojary B. Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. Eur J Med Chem 2003; 38:759–767.

15 Kritsanida M, Mouroutsou A, Marakos P, Pouli N, Papakonstantinou-Garoufalias S, Pannecouque C, *et al*. Synthesis and antiviral activity evaluation of some new 6-substituted 3-(1-adamantyl)-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles. Farmaco 2002; 57:253–257.

16 John BW. The chemistry of the benzimidazoles. Chem Rev 1951; 84: 397–541.

17 Klein ATJ, Holschbach M. Labelling of the solvent DMSO as side reaction of methylations with n.c.a. [11C]CH 3I. Appl Radiat Isot 2001; 55:309–313.

18 Ouk S, Thiébaud S, Borredon E, Chabaud B. *N*-methylation of nitrogen-containing heterocycles with dimethyl carbonate. Syn Commun 2005; 35:3021–3026.

19 Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia tarapotensis*. J Nat Prod 2001; 64:892–895.

20 Nara K, Miyoshi T, Honma T, Koga H. Antioxidative activity of bound-form phenolics in potato peel. Biosci Biotechn Biochem 2006; 70:1489–1491.

21 Hamed A. In ''Investigation of multiple cytoprotective actions of some individual phytochemicals and plant extracts''. Chapter 7: Development of cell-free and intracellular screening systems for testing antioxidant properties of plant extracts. PhD Thesis (Biomedical Sciences), the University of Nottingham, United Kingdom; 2009.

22 Pelczar MJ, Chan EC, Kruz NR. *Microbiology*. 5th ed. New Delhi: Tata McGraw-Hill Publishing Company Ltd.; 2006.

23 Grayer RJ, Harborne JB. A survey of antifungal compounds from higher plants, 1982–1993. Phytochemistry 1994; 37:19–42.

24 Irobi ON, Moo-Young M, Anderson WA, Daramola SO. Antimicrobial activity of bark extracts of *Bridelia ferruginea* (Euphorbiaceae). J Ethnopharmacol 1994; 43:185–190.

25 Jawetz E, Melnick JL, Adelberg EA. *Review of Medical Microbiology*. Los Atos, California: Lang Medical Publication; 1974.

26 Muanza DN, Kim BW, Euler KL, Williams L. Antibacterial and antifungal activities of nine medicinal plants from Zaire. Int J Pharmacogn 1994; 32:337–345.

27 Fang C, Xiao Z, Guo Z. Generation and validation of the first predictive pharmacophore model for cyclin-dependent kinase 9 inhibitors. J Mol Graphics Modell 2011; 29:800–808.

28 Labute P, Williams C, Feher M, Sourial E, Schmidt JM. Flexible alignment of small molecules. J Med Chem 2001; 44:1483–1490.

29 Kurogi Y, Güner OF. Pharmacophore modeling and three-dimensional database searching for drug design using catalyst. Curr Med Chem 2001; 8:1035–1055.

30 Leach AR. *Molecular Modeling: principles and applications*. 2nd ed. New York: Prentice Hall Pearson Ed. Ltd.; 2001.

31 Guner OF. *Pharmacophore perception, development and use in drug design*. La Jolla, CA: International University Line; 2000.

32 Walker JD, Carlsen L, Jaworska J. Improving opportunities for regulatory acceptance of QSARs: the importance of model domain, uncertainty, validity and predictability. QSAR and Combinatorial Science 2003; 22:346–350.

33 Yun M-K, Wu Y, Li Z, Zhao Y, Waddell MB, Ferreira AM, *et al*. Catalysis and sulfa drug resistance in dihydropteroate synthase. Science 2012; 335:1110–1114.