Synthesis and Antifungal Activity of 1-[(2-Benzyloxy)Phenyl]-2-(Azol-1-yl)Ethanone Derivatives: Exploring the Scaffold Flexibility

Saeed Emami[1],*, Motahare Kazemi-Najafabadi[1], Soughra Pashangzadeh[1], Alireza Foroumadi[2], Mohammad Ali Faramarzi[3], Nasrin Samadi[4], Mehraban Falahati[5], Roohollah Fateh[5] and Mahtab Ashrafi-Khozani[5]

[1]*Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran*
[2]*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran*
[3]*Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
[4]*Department of Drug and Food Control, Faculty of Pharmacy and Pharmaceutical Quality Assurance Research Center, Tehran University of Medical Sciences, Tehran, Iran*
[5]*Department of Parasitology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*Corresponding author: Dr. Saeed Emami, sd_emami@yahoo.com

Based on the *N*-(phenethyl)azole backbone of azole antifungals, we designed 1-[(2-benzyloxy)phenyl]-2-(azol-1-yl)ethanone derivatives 2 and 3, containing benzyloxyphenyl scaffold of croconazole. Also these compounds can be considered as flexible analogs, resulted from C2–C3 disconnection of 3′-chloro-3-imidazolylflavanone 1, recently described as antifungal agent. Thus, in this report, we describe the synthesis of 1-[(2-benzyloxy)phenyl]-2-(azol-1-yl)ethanone derivatives 2 and 3 and their biological evaluation against different pathogenic fungi. By comparing the antifungal activity profile of flexible compounds 2 and 3 with that of rigid analog 1, it can be inferred that lower susceptibilities (higher minimum inhibitory concentrations) were observed with flexible compounds. However, among the synthesized compounds, 1-[2-(2,4-dichlorobenzyloxy)phenyl]-2-(1*H*-imidazol-1-yl)ethanone hydrochloride (2g) showed comparable or more potent antifungal activity in comparison with fluconazole as a standard drug.

Key words: 1,2,4-triazole, 1*H*-imidazole, antifungal activity, azole antifungals, conformational study

In recent decades, the incidence of opportunistic fungal infections has greatly increased as a result of an increasing number of immunocompromised hosts, such as patients suffering from tuberculosis, infected with human immunodeficiency virus, and undergoing organ or bone marrow transplantations and cancer chemotherapy (1). Clinically, in spite of the introduction of several echinocandins (such as caspofungin and micafungin) and azoles (such as itraconazole and voriconazole) as new antifungal agents, the therapeutic management of life-threatening fungal infections still remains a challenge (2). The most important problem in the treatment for life-threatening fungal infections is the spread of drug-resistance mainly in complicated patients who are chronically subjected to antimycotic therapy. Thus, currently available antifungal drugs do not meet the increasing requirements of managing infection in the complicated patients. Moreover, the antifungal spectrum, pharmacokinetics, and toxicity profiles of the available antifungals are far from satisfactory. Therefore, development of new antifungal agents has been constantly required in the pharmaceutical research and industry (3).

Among the several classes of antifungal agents, azoles are the most widely used antifungal agents with specified mechanism of action and targeting. This situation has led to an ongoing search for new azoles, and several novel azoles, for example posaconazole, ravuconazole, and albaconazole are currently in different stages of clinical trials (3,4).

To develop new azole derivatives as potential antifungal agents, previously we described new *trans*-3-imidazolylflavanones, which consisted of a *N*-(phenethyl)azole scaffold (Figure 1) as the common pharmacophore of azole antifungals (5). Among the synthesized compounds, 3′-chloro-analog of 3-imidazolylflavanone (compound **1**, Figure 1) exhibited better profile of antifungal activity against the strains of fungi tested (5). On the other hand, croconazole (Figure 1) is a well-established drug for the treatment for many mycotic infections (6,7). A critical survey of the structure of azole class of antifungals reveals that most of them contain *N*-(phenethyl)azole moiety linked with benzylether side chain, while croconazole is characterized by having a *N*-(phenylvinyl)azole skeleton instead of *N*-(phenethyl)azole. Another distinct structural feature of this molecule is the attachment of the benzylether moiety to phenyl ring rather than to the ethyl linker.
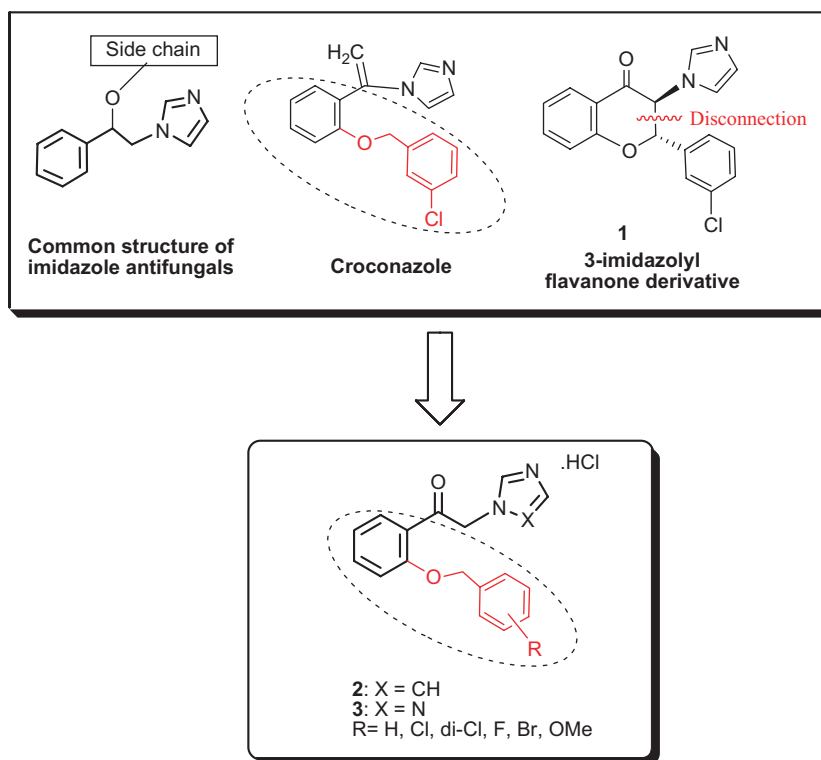
Figure 1: Design of new azole antifungals, 1-[(2-benzyloxy)phenyl]-2-(azol-1-yl)ethanone derivatives **2** and **3**.

Based on the *N*-(phenethyl)azole backbone of azole antifungals, we designed 1-[(2-benzyloxy)phenyl]-2-(azol-1-yl)ethanone derivatives **2** and **3** (Figure 1), containing benzyloxyphenyl scaffold of croconazole. Also, these compounds can be considered as flexible analogs of 3-imidazolylflavanone **1** resulted from C2–C3 disconnection. Thus, in this report, we describe the synthesis of 1-[(2-benzyloxy)phenyl]-2-(azol-1-yl)ethanone derivatives **2** and **3** and their biological evaluation against different pathogenic fungi.

Experimental Section

Chemistry
Chemical reagents and all solvents used in this study were purchased from Merck AG Chemical and used without further purification. The 1-(2-hydroxyphenyl)-2-(1*H*-imidazol-1-yl)ethanone **6** and 1-(2-hydroxyphenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanone **8** were prepared from 2'-hydroxyacetophenone **4** according to the literature methods (8). Melting points were determined in open glass capillaries using Bibby Stuart Scientific SMP3 apparatus (Stuart Scientific, Stone, UK) and are uncorrected. NMR spectra were recorded using a Bruker 500 spectrometer (Bruker, Rheinstetten, Germany), and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. Elemental analyses were carried out on a HERAEUS CHN-O rapid elemental analyzer (GmbH, Hanav, Germany) for C, H, and N, and the results are within ±0.4% of the theoretical values. The progress of reactions was monitored by thin-layer chromatography (TLC) analysis on silica gel 60 F254 plates (Merck). Visualization was made with UV light (254 nm) or iodine. Yields are based on purified material and were not optimized.

General procedure for the synthesis of imidazole derivatives (2a–h)
To a mixture of compound **6** (1.5 mmol, 303 mg) and K_2CO_3 (1.5 mmol, 207 mg) in acetone (15 mL), appropriate benzyl halide (1.5 mmol) was added and refluxed for 10–44 h. After the completion of the reaction, the solvent was evaporated under reduced pressure and the residue was washed with water. The crude product was dissolved in the least amount of absolute ethanol (2 mL) and treated with 37% aqueous HCl (0.2 mL). The solution was cooled at a freezer prior to gradual addition of diethyl ether to complete the precipitation of the hydrochloride salts **2a–h**, which were collected by filtration and washed with diethyl ether.

1-[2-(Benzyloxy)phenyl]-2-(1*H*-imidazol-1-yl)ethanone hydrochloride (2a)
Yield 9%; mp 106–108 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.40 (s, 2H, OCH$_2$), 5.79 (s, 2H, NCH$_2$), 7.12 (t, 1H, *J* = 7.5 Hz, H-5), 7.33–7.47 (m, 4H, H-3, and phenyl H), 7.59–7.68 (m, 5H, H-4, phenyl H, imidazole H-4, imidazole H-5), 7.87 (dd, 1H, *J* = 8.0 and 1.5 Hz, H-6), 9.0 (br s, 1H, imidazole H-2).

1-[2-(2-Fluorobenzyloxy)phenyl]-2-(1*H*-imidazol-1-yl)ethanone hydrochloride (2b)
Yield 22%; mp 146–147 °C; IR (KBr, 1/cm): 3394, 3027, 1677, 1598, 1575, 1480, 1454, 1346, 1280, 1223, 1203, 1165, 1000, 841, 763, 648. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.43 (s, 2H, OCH$_2$), 5.71 (s, 2H, NCH$_2$), 7.15 (dt, 1H, *J* = 7.5 and 1.0 Hz, H-5), 7.23–7.32 (m, 2H, H-3 and H-3'), 7.40–7.48 (m, 2H, H-4' and H-5'), 7.62–7.74 (m, 4H,

imidazole H-4, imidazole H-5, H-6′ and H-4), 7.86 (dd, 1H, $J = 7.75$ and 1.75 Hz, H-6), 9.01 (s, 1H, imidazole H-2).

1-[2-(3-Fluorobenzyloxy)phenyl]-2-(1*H*-imidazol-1-yl)ethanone hydrochloride (2c)

Yield 20%; mp 195–196 °C; IR (KBr, 1/cm): 3413, 3034, 2802, 1677, 1596, 1574, 1479, 1452, 1374, 1346, 1225, 1205, 1112, 1006, 757, 692. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.43 (s, 2H, OCH$_2$), 5.83 (s, 2H, NCH$_2$), 7.14 (dt, 1H, $J = 7.5$ and 0.5 Hz, H-5), 7.17–7.22 (m, 1H, H-4′), 7.32 (d, 1H, $J = 8.5$ Hz, and H-3), 7.43–7.51 (m, 3H, H-2′, H-5′, and H-6′), 7.64–7.72 (m, 3H, imidazole H-4, imidazole H-5, and H-4), 7.88 (dd, 1H, $J = 7.5$ and 1.5 Hz, H-6), 9.03 (br s, 1H, imidazole H-2).

1-[2-(4-Fluorobenzyloxy)phenyl]-2-(1*H*-imidazol-1-yl)ethanone hydrochloride (2d)

Yield 10%; mp 188–189 °C; IR (KBr, 1/cm): 3405, 3031, 1677, 1602, 1574, 1511, 1479, 1454, 1346, 1281, 1227, 1200, 1163, 1000, 840, 757, 647. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.37 (s, 2H, OCH$_2$), 5.76 (s, 2H, NCH$_2$), 7.12 (dt, 1H, $J = 7.75$ and 0.5 Hz, H-5), 7.24 (dd, 2H, $J = 7.0$ and 2.0 Hz, H-2′ and H-6′), 7.35 (d, 1H, $J = 8.0$ Hz, H-3), 7.61–7.69 (m, 5H, imidazole H-4, imidazole H-5, H-4, H-3′, and H-5′), 7.86 (dd, 1H, $J = 7.75$ and 1.75 Hz, H-6), 8.99 (br s, 1H, imidazole H-2).

1-[2-(3-Chlorobenzyloxy)phenyl]-2-(1*H*-imidazol-1-yl)ethanone hydrochloride (2e)

Yield 10%; mp 215–216 °C; IR (KBr, 1/cm): 3417, 3037, 2957, 1675, 1665, 1598, 1573, 1480, 1454, 1346, 1255, 1225, 1205, 1165, 1005, 758. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.42 (s, 2H, OCH$_2$), 5.82 (s, 2H, NCH$_2$), 7.14 (t, 1H, $J = 7.75$ Hz, H-5), 7.31 (d, 1H, $J = 8.0$ Hz, H-3), 7.4 (dt, 1H, $J = 8.0$ and 1.5 Hz, H-4′), 7.46 (t, 1H, $J = 7.5$ Hz, H-5′), 7.59 (d, 1H, $J = 7.5$ Hz, H-6′), 7.63–7.71 (m, 4H, H-4, H-2′, imidazole H-4, and imidazole H-5), 7.88 (dd, 1H, $J = 8.0$ and 2.0 Hz, H-6), 9.00 (br s, 1H, imidazole H-2).

1-[2-(4-Chlorobenzyloxy)phenyl]-2-(1*H*-imidazol-1-yl)ethanone hydrochloride (2f)

Yield 16%; mp 210–211 °C; IR (KBr, 1/cm): 3124, 3023, 1670, 1599, 1478, 1454, 1344, 1223, 1198, 1161, 1089, 994, 758. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.39 (s, 2H, OCH$_2$), 5.79 (s, 2H, NCH$_2$), 7.12 (dt, 1H, $J = 7.62$ and 0.75 Hz, H-5), 7.33 (d, 1H, $J = 8.0$ Hz, H-3), 7.47 (d, 2H, $J = 8.5$ Hz, H-2′, and H-6′), 7.62–7.69 (m, 5H, imidazole H-4, imidazole H-5, H-3′, H-5′, and H-4), 7.87 (dd, 1H, $J = 8.0$ and 2.0 Hz, H-6), 9.04 (br s, 1H, imidazole H-2).

1-[2-(2,4-Dichlorobenzyloxy)phenyl]-2-(1*H*-imidazol-1-yl)ethanone hydrochloride (2g)

Yield 20%; mp 215–216 °C; IR (KBr, 1/cm): 3433, 3031, 2821, 1673, 1598, 1574, 1480, 1454, 1370, 1346, 1306, 1226, 1200, 1164, 1013, 836, 760. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.43 (s, 2H, OCH$_2$), 5.74 (s, 2H, NCH$_2$), 7.18 (dt, 1H, $J = 7.5$ and 0.7 Hz, H-5), 7.38 (dd, 1H, $J = 8.5$ and 0.5 Hz, H-3), 7.52 (dd, 1H, $J = 8.0$ and 2.0 Hz, H-5′), 7.64 (br s, 1H, imidazole H-5), 7.68 (br s, 1H, imidazole H-4), 7.71 (dt, 1H, $J = 7.8$ and 1.8 Hz, H-4), 7.75 (d, 1H, $J = 2.5$ Hz, H-3′), 7.77 (d, 1H,

$J = 8.5$ Hz, H-6′), 7.89 (dd, 1H, $J = 8.0$ and 2.0 Hz, H-6), 9.00 (br s, 1H, imidazole H-2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 58.22, 67.42, 114.01, 119.47, 121.53, 123.40, 123.59, 127.76, 129.20, 130.73, 131.94, 132.61, 133.78, 134.00, 136.01, 136.70, 158.28, 190.99.

1-[2-(3,4-Dichlorobenzyloxy)phenyl]-2-(1*H*-imidazol-1-yl)ethanone hydrochloride (2h)

Yield 23%; mp 235–236 °C; IR (KBr, 1/cm): 3423, 3017, 2653, 1667, 1599, 1574, 1477, 1451, 1367, 1345, 1294, 1219, 1199, 1160, 1029, 1007, 868, 759, 642, 630. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.41 (s, 2H, OCH$_2$), 5.82 (s, 2H, NCH$_2$), 7.15 (t, 1H, $J = 7.5$ Hz, H-5), 7.31 (d, 1H, $J = 8.5$ Hz, H-3), 7.62 (dd, 1H, $J = 8.0$ and 2.0 Hz, H-6′), 7.65–7.73 (m, 4H, H-4, H-5′, imidazole H-4, and imidazole H-5), 7.89 (dd, 1H, $J = 7.75$ and 1.75 Hz, H-6), 7.90 (d, 1H, $J = 1.5$ Hz, H-2), 9.03 (br s, 1H, imidazole H-2).

General procedure for the synthesis of triazole derivatives (3a–h)

To a mixture of compound **8** (1.5 mmol, 305 mg) and K$_2$CO$_3$ (1.5 mmol, 207 mg) in acetone (15 mL), appropriate benzyl halide (1.5 mmol) was added and refluxed for 24–44 h. After the completion of the reaction, the solvent was evaporated under reduced pressure and the residue was washed with water. The crude product was dissolved in the least amount of absolute ethanol (2 mL) and treated with 37% aqueous HCl (0.2 mL). The solution was cooled at a freezer prior to gradual addition of diethyl ether to complete the precipitation of the hydrochloride salts **3b**–**h**, which were collected by filtration and washed with diethyl ether. In the case of compound **3a**, the crude product was dissolved in absolute ethanol (1.5 mL) and treated with 65% aqueous HNO$_3$ (0.2 mL). After the addition of diethyl ether (2 mL), the mixture was refrigerated to complete the precipitation of the nitrate salt **3a**, which was collected by filtration and washed with diethyl ether.

1-[2-(Benzyloxy)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanone nitrate (3a)

Yield 7%; mp 116–119 °C; IR (KBr, 1/cm): 3427, 3121, 3049, 1686, 1597, 1447, 1419, 1403, 1384, 1307, 1229, 1116, 1010, 761, 647. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.35 (s, 2H, OCH$_2$), 5.74 (s, 2H, NCH$_2$), 7.10 (t, 1H, $J = 7.5$ Hz, H-5), 7.27–7.47 (m, 4H, H-3, and phenyl H), 7.59 (dd, 2H, $J = 7.5$ and 0.5 Hz, phenyl H), 7.64 (dt, 1H, $J = 7.0$ and 0.5 Hz, H-4′), 7.78 (dd, 1H, $J = 7.5$ and 0.5 Hz, H-6), 8.13 (s, 1H, triazole H-5), 8.67 (s, 1H, triazole H-3).

1-[2-(4-Bromobenzyloxy)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanone hydrochloride (3b)

Yield 21%; mp 209–210 °C; IR (KBr, 1/cm): 3425, 3006, 1671, 1598, 1480, 1429, 1308, 1289, 1229, 1206, 1167, 1012, 755, 633. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.34 (s, 2H, OCH$_2$), 5.73 (s, 2H, NCH$_2$), 7.10 (dt, 1H, $J = 7.5$ and 1.0 Hz, H-5), 7.31 (dd, 1H, $J = 8.0$ and 0.5 Hz, H-3), 7.47 (d, 2H, $J = 8.5$ Hz, H-2′, and H-6′), 7.62 (d, 2H, $J = 8.5$ Hz, H-3′, and H-5′), 7.63 (m, 1H, H-4), 7.76 (dd, 1H, $J = 8.0$ and 1.5 Hz, H-6), 8.11 (br s, 1H, triazole H-5), 8.66 (br s, 1H, triazole H-3). ^{13}C NMR (125 MHz, DMSO-d_6) δ 59.02, 69.44, 113.96, 121.12, 124.38, 128.62, 130.16, 132.79, 135.33, 158.20, 192.47.

1-[2-(2-Fluorobenzyloxy)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanone hydrochloride (3c)

Yield 40%; mp 137–138 °C; IR (KBr, 1/cm): 3453, 3005, 1670, 1598, 1481, 1455, 1346, 1227, 1205, 1168, 1010, 759, 641. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.41 (s, 2H, OCH$_2$), 5.67 (s, 2H, NCH$_2$), 7.13 (dt, 1H, J = 7.5 and 1.0 Hz, H-5), 7.24–7.32 (m, 2H, H-3, and H-3'), 7.41 (dd, 1H, J = 8.0 and 0.5 Hz, H-6'), 7.43–7.48 (m, 1H, H-5'), 7.65–7.74 (m, 2H, H-4, and H-4'), 7.78 (dd, 1H, J = 7.5 and 1.75 Hz, H-6), 8.12 (s, 1H, triazole H-5), 8.66 (s, 1H, triazole H-3). ^{13}C NMR (125 MHz, DMSO-d_6) δ 58.82, 64.63, 113.92, 115.62, 121.32, 123.04, 124.52, 130.78, 135.53, 145.28, 150.14, 158.19, 192.25.

1-[2-(3-Methoxybenzyloxy)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanone hydrochloride (3d)

Yield 22%; mp 111–112 °C; IR (KBr, 1/cm): 3428, 3003, 2868, 1673, 1599, 1586, 1492, 1447, 1435, 1367, 1294, 1273, 1230, 1208, 1148, 1024, 1004, 851, 780, 768, 626. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.72 (s, 3H, OCH$_3$), 5.31 (s, 2H, OCH$_2$), 5.76 (s, 2H, NCH$_2$), 6.89 (ddd, 1H, J = 8.0, 2.75 and 1.0 Hz, H-4'), 7.10 (dt, 1H, J = 7.5 and 0.5 Hz, H-5), 7.13 (d, 1H, J = 7.5 Hz, H-6'), 7.17 (dd, 1H, J = 2.0 and 1.5 Hz, H-2'), 7.31 (t, 1H, J = 7.5 Hz, H-5'), 7.32 (d, 1H, J = 7.5 Hz, H-3), 7.63 (dt, 1H, J = 8.0 and 2.0 Hz, H-4), 7.77 (dd, 1H, J = 8.0 and 1.75 Hz, H-6), 8.12 (s, 1H, triazole H-5), 8.68 (s, 1H, triazole H-3).

1-[2-(3-Chlorobenzyloxy)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanone hydrochloride (3e)

Yield 35%; mp 153–154 °C; IR (KBr, 1/cm): 3060, 1694, 1600, 1577, 1496, 1458, 1445, 1367, 1340, 1301, 1271, 1218, 979, 783, 757, 642. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.36 (s, 2H, OCH$_2$), 5.76 (s, 2H, NCH$_2$), 7.10 (ddd, 1H, J = 8.5, 7.75 and 0.5 Hz, H-5), 7.29 (dd, 1H, J = 8.5 and 0. 5 Hz, H-3), 7.40 (ddd, 1H, J = 7.5, 2.0, and 1.5 Hz, H-6'), 7.44 (dt, 1H, J = 7.5 and 0.5 Hz, H-5'), 7.56 (dt, 1H, J = 7.5 and 1.5 Hz, H-4'), 7.63 (ddd, 1H, J = 8.5, 0.8 and 1.5 Hz, H-4), 7.67 (dt, 1H, J = 1.5 and 0.5 Hz, H-2'), 7.77 (dd, 1H, J = 7.75 and 1.75 Hz, H-6), 8.11 (s, 1H, triazole H-5), 8.65 (s, 1H, triazole H-3).

1-[2-(4-Chlorobenzyloxy)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanone hydrochloride (3f)

Yield 11%; mp 189–190 °C; IR (KBr, 1/cm): 3436, 1669, 1596, 1491, 1450, 1361, 1296, 1228, 1212, 1117, 1003, 820, 761, 630. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.32 (s, 2H, OCH$_2$), 5.72 (s, 2H, NCH$_2$), 7.10 (dt, 1H, J = 7.5 and 1.0 Hz, H-5), 7.30 (dd, 1H, J = 8.0 and 0.5 Hz, H-3), 7.55 (d, 2H, J = 8.5 Hz, H-2', H-6'), 7.59–7.66 (m, 3H, H-3', H-5', and H-4), 7.76 (dd, 1H, J = 8.0 and 2.0 Hz, H-6), 8.08 (s, 1H, triazole H-5), 8.62 (s, 1H, triazole H-3).

1-[2-(2,4-Dichlorobenzyloxy)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanone hydrochloride (3g)

Yield 38%; mp 204–205 °C; IR (KBr, 1/cm): 3402, 3066, 1690, 1599, 1578, 1497, 1475, 1370, 1302, 1266, 1214, 1038, 865, 759, 749, 641. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.38 (s, 2H, OCH$_2$), 5.67 (s, 2H, NCH$_2$), 7.14 (dt, 1H, J = 7.5 and 0.75 Hz, H-5), 7.34 (dd, 1H, J = 8.5 and 0.5 Hz, H-3), 7.50 (dd, 1H, J = 8.25 and 2.2 Hz, H-5'), 7.66 (ddd, 1H, J = 8.5, 7.5 and 1.5 Hz, H-4), 7.72–7.81 (m, 3H, H-3', H-6', H-6), 8.07 (br s, 1H, triazole H-5), 8.60 (br s, 1H, triazole H-3).

1-[2-(3,4-Dichlorobenzyloxy)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanone hydrochloride (3h)

Yield 29%; mp 150–151 °C; IR (KBr, 1/cm): 3395, 3119, 1690, 1599, 1488, 1473, 1447, 1407, 1368, 1295, 1215, 1132, 1001, 873, 820, 747, 642. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.35 (s, 2H, OCH$_2$), 5.76 (s, 2H, NCH$_2$), 7.11 (dt, 1H, J = 7.5 and 0.5 Hz, H-5), 7.28 (dd, 1H, J = 8.5 and 0.5 Hz, H-3), 7.58–7.66 (m, 2H, H-4, H-6'), 7.67 (d, 1H, J = 8.0 Hz, H-5'), 7.77 (dd, 1H, J = 8.0 and 1.5 Hz, H-6), 7.89 (d, 1H, J = 1.5 Hz, H-2'), 8.13 (br s, 1H, triazole H-5), 8.86 (s, 1H, triazole H-3).

Antifungal activity assay

The minimum inhibitory concentrations (MICs) of synthetic compounds against *Candida albicans* ATCC 10231, *Candida glabrata*, ATCC 90030, *Saccharomyces cerevisiae* NCYC 694, *S. cerevisiae* ATCC 9763, *S. cerevisiae* PTCC 5177, *Aspergillus niger* PTCC 5011, and *Microsporum gypseum* PTCC 5070 were determined by microdilution method using plates with 96 U-shaped wells (9).

A stock solution (512 μg/mL) of compounds was prepared in Sabouraud dextrose broth (SDB) using 10% v/v DMSO. Then, 200 μL of the prepared solution was transferred into the first well in each row and serially diluted by mixing with 100 μL of SDB in subsequent wells. A stock fungal suspension (1×10^3 CFU/mL) was prepared from a 24-h-old culture. Then, aliquot of 100 μL of fungal suspension was added to each well to reach the final inoculum size of 0.5×10^3 CFU/mL. After 24, 48, and 72 h of incubation at 35 °C, the plates were tested for the absence or presence of visible growth in comparison with that of the drug-free control well. The end point for MIC is the lowest concentration of the compound at which the test strain does not demonstrate visible growth.

Minimum fungicidal concentration (MFC) values were determined by subculturing 20 μL of each well that showed complete inhibition (100% or an optically clear well), from the last positive well (growth similar to that for the growth control well) and from the growth control (drug-free medium) onto Sabouraud dextrose agar plates. The plates were incubated at 35 °C until the growth was seen in the growth control subculture. The MFC was the lowest drug concentration that showed either no growth or fewer than three colonies to obtain approximately 99–99.5% killing activity (10).

Results and Discussion

Chemistry

The synthetic pathway to compounds **2** and **3** is outlined in Scheme 1. The starting 2′-hydroxyacetophenone **4** was obtained from phenol according to the method reported in the literature (11). 2′-Hydroxyacetophenone **4** was selectively brominated on the α-position using copper (II) bromide in refluxing CHCl$_3$-AcOEt to give 2-bromo-2′-hydroxyacetophenone **5** (12). Reaction of imidazole with compound **5** in DMF at 40–45 °C afforded 1-(2-hydroxyphenyl)-2-
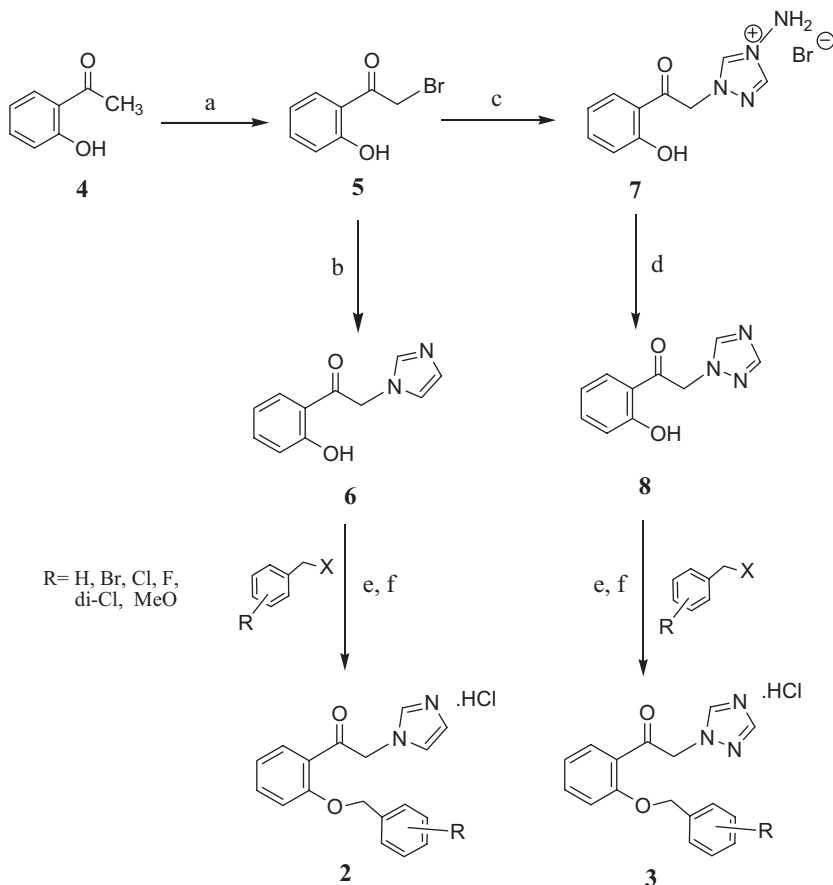
Scheme 1: Synthesis of compounds **2a–h** and **3a–h**. *Reagents and conditions*: (a) CuBr$_2$, CHCl$_3$-EtOAc, reflux; (b) imidazole, DMF, 40–45 °C; (c) 4-amino-1,2,4-triazole, 2-propanol, reflux; (d) NaNO$_2$, HCl; (e) K$_2$CO$_3$, acetone, reflux; (f) HCl, EtOH-Et$_2$O.

(1*H*-imidazol-1-yl)ethanone **6** (8). *O*-Benzylation of compound **6** was conducted with appropriate benzyl halide derivative in the presence of K$_2$CO$_3$ in refluxing acetone. The viscous oily product was dissolved in ethanol and treated with 37% HCl to give target compounds **2a–h** as hydrochloride salts (Table 1).

For regio-selective preparation of 1-substituted-1,2,4-triazole derivatives, 4-amino-1,2,4-triazole is used as a protected synthetic equivalent of 1,2,4-triazole. Thus, reaction of 4-amino-1,2,4-triazole with 2-bromo-2′-hydroxyacetophenone **5** yielded pure aminotriazolium salt **7**, which on subsequent deamination with nitrous acid afforded exclusively the 1-(2-hydroxyphenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanone **8** (13). Compound **8** was converted to *O*-benzyl ether derivatives by refluxing with appropriate benzyl halide in the presence of K$_2$CO$_3$ in acetone. The viscous oily product was dissolved in ethanol and treated with 37% HCl to give target compounds **3** as hydrochloride salts (Table 1).

Antifungal activity

Antifungal activity of imidazole and triazole derivatives (**2a–h** and **3a–h**, respectively) was evaluated against different strains of *Candida* and *S. cerevisiae* as yeast, *A. niger* as a mold, and *M. gypseum* as a dermatophyte. For all fungi, the susceptibility test method

used was a microplate broth dilution method described previously (9). The MIC values were determined at 24 and 48 h for *Candida* and *Saccharomyces* strains, and 48 and 72 h for *A. niger* and *M. gypseum* in comparison with fluconazole as a reference drug (Table 2).

The *in vitro* antifungal susceptibility assay indicated that compounds **2c**, **2g**, and **2h** showed respectable growth inhibition activity against *Candida* and *Saccharomyces* strains. The activities of compounds **2c** and **2h** against yeasts was comparable with that of fluconazole (MICs = 32–256 μg/mL). Compound **2g** was also equipotent to fluconazole in the term of activity against yeasts, with the exception of activity against *S. cerevisiae* PTCC 5177. The activity of compound **2g** against the latter strain was at least 8-fold more than that of fluconazole.

Moreover, compound **2g** showed good activity against *A. niger* and *M. gypseum* with MIC values of 32 and 8 μg/mL after 72 h of incubation, while the standard drug fluconazole showed no activity at the concentrations of less than 128 μg/mL against *A. niger* and *M. gypseum*. Triazole derivative **3h** was able to inhibit the growth of *M. gypseum* at concentrations more than 48 μg/mL. Also, the MIC value of compounds **2h**, **3b**, and **3f** against this dermatophyte was 64 μg/mL.

Table 1: Structures and physical properties of the synthesized compounds **2a–h** and **3a–h**

.HCl
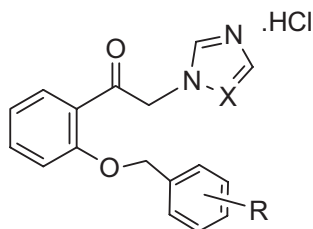
Compound	X	R	Formula	MW	Reaction time (h)	Yield[a] (%)	Mp (°C)
2a	CH	H	$C_{18}H_{16}FN_2O_2 \cdot HCl$	328.621	29	9	106–108
2b	CH	2-F	$C_{18}H_{15}FN_2O_2 \cdot HCl$	346.612	42	22	146–147
2c	CH	3-F	$C_{18}H_{15}FN_2O_2 \cdot HCl$	346.612	42	20	195–196
2d	CH	4-F	$C_{18}H_{15}FN_2O_2 \cdot HCl$	346.612	42	10	188–189
2e	CH	3-Cl	$C_{18}H_{15}ClN_2O_2 \cdot HCl$	362.582	44	10	215–216
2f	CH	4-Cl	$C_{18}H_{15}ClN_2O_2 \cdot HCl$	362.582	29	16	210–211
2g	CH	2,4-Cl₂	$C_{18}H_{14}Cl_2N_2O_2 \cdot HCl$	396.93	25	20	215–216
2h	CH	3,4-Cl₂	$C_{18}H_{14}Cl_2N_2O_2 \cdot HCl$	396.93	10	23	235–236
3a[b]	N	H	$C_{17}H_{15}N_3O_2 \cdot HNO_3$	352.12	24	7	116–119
3b	N	4-Br	$C_{17}H_{14}Br\,N_3O_2 \cdot HCl$	407.53	40	21	209–210
3c	N	2-F	$C_{17}H_{14}FN_3O_2 \cdot HCl$	347.61	42	40	137–138
3d	N	3-OCH₃	$C_{18}H_{17}N_3O_3 \cdot HCl$	359.63	39	22	111–112
3e	N	3-Cl	$C_{17}H_{14}ClN_3O_2 \cdot HCl$	363.58	44	35	153–154
3f	N	4-Cl	$C_{17}H_{14}ClN_3O_2 \cdot HCl$	363.58	29	11	189–190
3g	N	2,4-Cl₂	$C_{17}H_{13}Cl_2N_3O_2 \cdot HCl$	397.54	25	38	204–205
3h	N	3,4-Cl₂	$C_{17}H_{13}Cl_2N_3O_2 \cdot HCl$	397.54	25	29	150–151

[a]The yields are for two steps; preparation and salt formation.
[b]Compound **3a** was prepared as nitrate salt.

Table 2: Antifungal activity (minimum inhibitory concentrations, μg/mL) of compounds **2a–h** and **3a–h** in comparison with that of rigid analog **1** and standard drug fluconazole

| Compound	C. albicans ATCC 10231 24 h	C. albicans ATCC 10231 48 h	C. glabrata ATCC 90030 24 h	C. glabrata ATCC 90030 48 h	Saccharomyces cerevisiae ATCC 9763 24 h	Saccharomyces cerevisiae ATCC 9763 48 h	S. cerevisiae PTCC 5177 24 h	S. cerevisiae PTCC 5177 48 h	S. cerevisiae NCYC 694 24 h	S. cerevisiae NCYC 694 48 h	Aspergillus niger PTCC 5011 48 h	Aspergillus niger PTCC 5011 72 h	Microsporum gypseum PTCC 5070 48 h	Microsporum gypseum PTCC 5070 72 h
2a	256	>256	>128	>128	128	256	>64	>64	256	256	>64	>64	>64	>64
2b	>128	>128	>128	>128	256	256	32	64	256	256	>64	>64	>64	>64
2c	128	256	256	>256	64	256	20	64	64	128	>64	>64	>64	>64
2d	>128	>128	>128	>128	256	256	64	>64	256	256	>64	>64	>64	>64
2e	>128	>128	>128	>128	128	>256	64	>64	256	256	>64	>64	>64	>64
2f	>128	>128	>128	>128	64	128	64	>64	64	128	>64	>64	>64	>64
2g	256	256	64	256	64	64	4	4	64	64	16	32	8	8
2h	256	>256	64	64	64	64	16	48	64	64	>64	>64	64	64
3a	–	–	–	–	–	–	>64	>64	–	–	>64	>64	>64	>64
3b	>128	>128	>128	>128	>256	>256	>64	>64	>128	>128	>64	>64	64	64
3c	>128	>128	>128	>128	256	>256	>64	>64	>128	>128	>64	>64	>64	>64
3d	>128	>128	>128	>128	256	256	>64	>64	>128	>128	>64	>64	>64	>64
3e	>128	>128	>128	>128	256	>256	>64	>64	>128	>128	>64	>64	>64	>64
3f	–	–	–	–	–	–	>64	>64	–	–	>64	>64	64	64
3g	>128	>128	>128	>128	256	>256	>64	>64	>128	>128	>64	>64	>64	>64
3h	>128	>128	>128	>128	256	>256	0.13	0.13	>128	>128	>64	>64	48	48
1	8	32	256	>256	2	8	0.75	0.75	2	4	–	16[a]	–	2[a]
Fluconazole	128	256	64	128	32	64	32	64	32	64	>128	>128	>128	>128

[a]Data from Ref. (5).
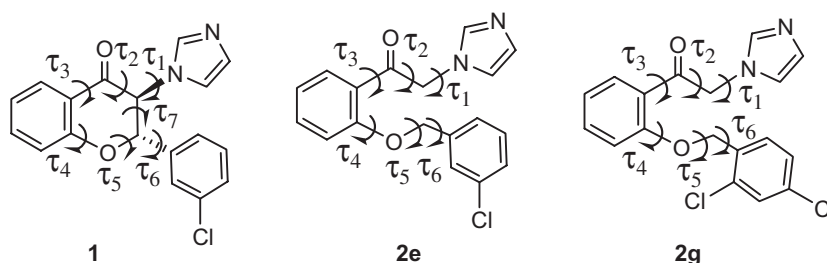
Figure 2: Selected compounds and torsion angles for conformational analysis.

As expected, antifungal susceptibility profile of imidazole compounds was better than that of triazole derivatives. Furthermore, the comparison of MIC values of imidazole series indicated that the dichlorobenzyl analogs exhibited more inhibitory activity against all tested fungi. A survey in the structures of traded imidazole antifungals reveals that dichlorobenzyl scaffold is more common in these agents (e.g., miconazole, oxiconazole, and isoconazole).

In this study, the determination of MICs was primarily used to explore of the biological properties of target compounds. Whether MICs are the best *in vitro* predictors of *in vivo* or clinical response to antifungal agents is uncertain. However, it has been demonstrated that MFCs may be better predictors than MICs of therapeutic failure of some antifungal agents (10). The MFC is the lowest concentration of an antifungal agent that causes at least a 3-log10 reduction in the number of surviving cells (as compared with the initial preincubation concentration). A drug is considered fungicidal when the difference between the MIC and the MFC is lower than three double dilutions (14). In our study, compounds **2g** and **2h** that displayed a better growth inhibition (MICs = 4–48 μg/mL) against *S. cerevisiae* PTCC 5177 were selected for MFC determination. The resulted MFC values of compounds **2g** and **2h** against *S. cerevisiae* PTCC 5177 were more than 128 μg/mL; therefore, these compounds showed a fungistatic character against yeasts.

Conformational study

Previously, we described new *trans*-3-imidazolylflavanones, which consisted of a *N*-(phenethyl)azole scaffold, as conformationally constrained azole antifungals. Among the synthesized compounds, 3′-chloro-analog of 3-imidazolylflavanone (compound **1**) exhibited better profile of antifungal activity against strains of fungi tested. In this work, we introduced imdazole and triazole derivatives of the 1-[(2-benzyloxy)phenyl]-2-(azol-1-yl)ethanone (compounds **2** and **3**), which can be considered as flexible analogs of 3-imidazolylflavanone **1** resulted from C2–C3 disconnection. By comparing the antifungal activity profiles of flexible compounds **2** and **3** with that of rigid analog **1**, it can be inferred that lower susceptibilities (higher MICs) were observed with flexible compounds. Meanwhile, the most direct analog of **1** (compound **2e**) proved to be among the least active compounds (Table 2).

To gain some insight into molecular structures of the least active compound **2e** and the most active one **2g** in comparison with the conformationally restricted imidazolylflavanone **1**, a straightforward conformational study was conducted by molecular mechanics (MM+) methods using the HyperChem ver. 7 software package. The selected compounds **1**, **2e** and **2g** (Figure 2) were built
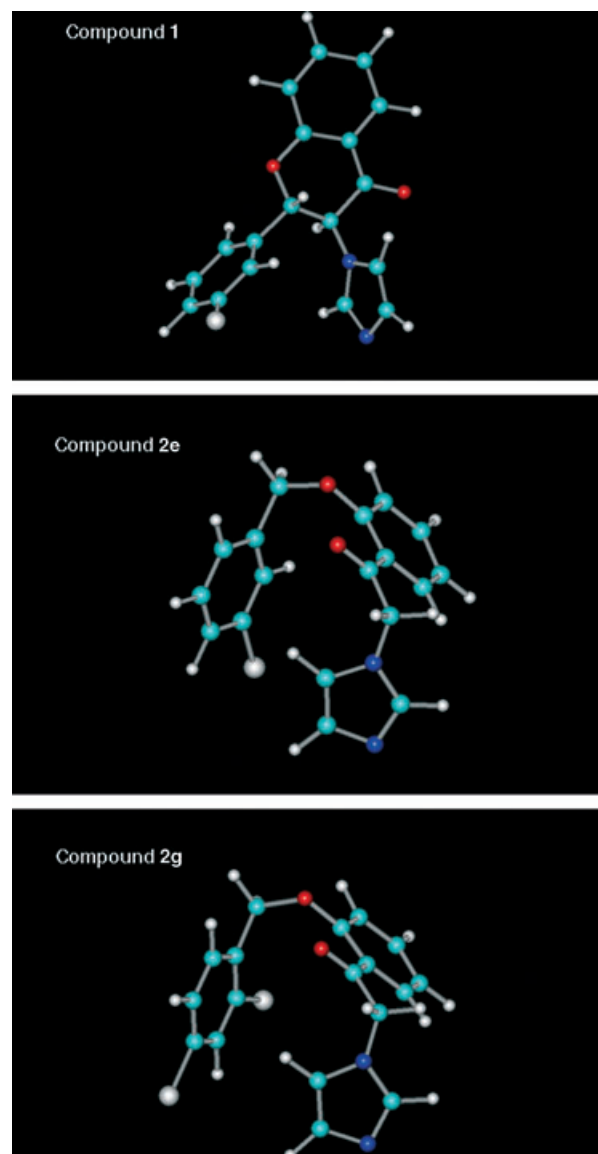

Figure 3: Lowest energy conformers of the compounds **1**, **2e** and **2g**.

Table 3: Drug-likeness properties of selected compounds **2c**, **2g** and **2h** predicted by Osiris Property Explorer tool

Compound	LogS	CLogP	MW	Toxicity risks[a]				Drug-likeness	Drug-score
				M	T	I	R		
2c	−3.07	2.06	310	(−)	(−)	(−)	(±)	0.74	0.58
2g	−4.23	3.23	360	(−)	(−)	(−)	(−)	4.49	0.72
2h	−4.23	3.23	360	(−)	(−)	(−)	(−)	3.74	0.71

M, mutagenic effect; T, tumorigenic effect; I, irritating effect; R, reproductive effect.
[a]Ranked according to: (−), no bad effect; (±), medium bad effect; (+), bad effect.

within HyperChem and bond angles, and the lengths were optimized with the Polak–Ribiere algorithm and RMS gradient of 0.01 kcal/Å mol. Using the minimized structure, a general procedure of multi-conformational search about all rotatable bonds described in Figure 2 was conducted using the conformational search routine with HyperChem. This consists of studying the energy variation according to the variation in the dihedral angle by rotational increments of 5°. Torsion angles were defined by clockwise rotation around the appropriate bonds, and molecular geometries were obtained after the lowest molecular energy minimization. Each of the valid conformations derived from the conformational search was individually minimized using molecular mechanics force field and applying the Polak–Ribiere algorithm. The global minimum-energy conformation and those conformations within ~6 kcal/mol of the minimum-energy conformation were stored.

A proposed pharmacophore model of azole antifungals includes the N-3 (N-4) atom of the azole ring, the phenyl ring attached to C-2, and another aromatic ring, as found in compounds **1**, **2e**, and **2g**. It is known that the flavanone skeleton in compound **1** may exist without strain in half-chair conformation. On the basis of this fact, compound **1** should adopt a conformation in which the 2-phenyl and 3-imidazolyl groups are diequatorial. The global minimum-energy conformations of compounds **1**, **2e**, and **2g** are depicted in Figure 3. The geometry of the global minimum-energy conformations of compounds **2e** and **2g** does not accurately mimic the geometry of the most active conformationally restricted imidazolylflavanone **1** as defined by the position between the N-3 (N-4) atom of the azole ring and the centroids of two different phenyl rings. In addition, the same situation exists with regard to the distance defined by the position between the centroids of the two phenyl rings. Thus, the comparison of the global minimum-energy conformations of compounds could rationalize the significant differences observed in antifungal activity between analogs if it is postulated that the global minimum-energy conformation of rigid compound **1** is representative of the optimum bioactive conformation for this series of azole antifungals. However, the antifungal activity improvement of 2,4-dichloro-compound **2g** with respect to 3-chloro-compound **2e** might be the result of steric and/or electronic effects rather than conformational differences.

In silico calculation of drug-like properties
The concepts of drug-likeness and druggability have drawn considerable attention for improving the lead compounds into the

drug candidates with reduced mammalian toxicity (15,16). Several approaches have been proposed for predicting compound's drug-likeness partially based on structural fragmentation and physicochemical parameters such as MW (molecular weight), CLogP (calculated *n*-octanol/water partition coefficient), and log S (solubility) (17). In this study, online Osiris property explorer was used for the prediction of drug toxicity and drug-likeness of selected compounds **2c**, **2g**, and **2h**. The Osiris calculations for toxicity and drug-likeness are fragment-based approach. A positive drug-likeness value (0.1–10) indicates that a molecule contains predominantly fragments that are frequently present in traded drugs.[a]

The predicted toxicity risks including mutagenicity, tumorigenicity, irritating, and reproductive effects of compounds **2c**, **2g**, and **2h** is presented in Table 3. The results indicate that all compounds are supposed to be non-mutagenic, non-tumorigenic, with no irritating effects. Among them, compound **2c** exhibited medium risk of reproductive effect. Furthermore, these compounds had positive drug-likeness values (0.74–4.49), and the fragments of the most active compound **2g** have a more contribution for drug like activity (drug-likeness = 4.49). The drug-score measured using Osiris explorer can be used to judge the compound's overall potential to qualify for a drug. This score is calculated based on the combination of toxicity risks, drug-likeness, and some physicochemical parameters such as CLog P, log S, and MW.[a] As noted in Table 3, compounds **2g** and **2h** showed good drug-score (≥0.71) that revealed their potential as safe lead compounds.

Conclusion

Previously, we described new *trans*-3-imidazolylflavanones, which consisted of a *N*-(phenethyl)azole scaffold as the common pharmacophore of azole antifungals. Among the synthesized compounds, 3′-chloro-analog of 3-imidazolylflavanone (compound **1**) exhibited better profile of antifungal activity against strains of fungi tested. In this work, we introduced imdazole and triazole derivatives of the 1-[(2-benzyloxy)phenyl]-2-(azol-1-yl)ethanone **2** and **3**, which can be considered as flexible analogs of 3-imidazolylflavanone **1** resulted from C2–C3 disconnection and containing benzyloxyphenyl scaffold of croconazole. By comparing the antifungal activity profiles of flexible compounds **2** and **3** with that of rigid analog **1**, it can be inferred that lower susceptibilities (higher MICs) were observed with flexible compounds. Meanwhile, the most direct analog of **1** (compound **2e**) was proved to be among the least active com-

pounds. The conformational study revealed that the geometry of the global minimum-energy conformations of compounds **2e** and **2g** does not accurately mimic the geometry of the most active conformationally restricted imidazolylflavanone **1** as defined by the position between pharmacophoric points.

Acknowledgments

References

1. Marr K.A. (2008) Fungal infections in hematopoietic stem cell transplant recipients. Med Mycol;46:293–302.
2. Neely M.N., Ghannoum M.A. (2000) The exciting future of antifungal therapy. Eur J Clin Microbiol Infect Dis;19:897–914.
3. Maertens J.A. (2004) History of the development of azole derivatives. Clin Microbiol Infect;10(Suppl. 1):1–10.
4. Bolouri F., Moghadami Tabrizi N., Davari Tanha F., Niroomand N., Azmoodeh A., Emami S., Falahati M., Sharifynia S., Safavi M., Shasiee A., Foroumadi A. (2009) Effectiveness of fluconazole for suppressive maintenance therapy in patients with RVVC: a randomized placebo-controlled study. Iran J Pharm Res;8:307–313.
5. Emami S., Behdad M., Foroumadi A., Falahati M., Lotfali E., Sharifynia S. (2009) Design of conformationally constrained azole antifungals: Efficient synthesis and antifungal activity of *trans*-3-imidazolylflavanones. Chem Biol Drug Des;73:388–395.
6. Ogata M., Matsumoto H., Shimizu S., Kida S., Shiro M., Tawara K. (1987) Synthesis and antifungal activity of new 1-vinylimidazoles. J Med Chem;30:1348–1354.
7. Sobel J.D., Vazquez J.A. (2006) Contemporary Diagnosis and Management of Fungal Infections, 2nd edn. Newtown, PA: Handbooks in Health Care Company Publishers.
8. Emami S., Foroumadi A., Falahati M., Lotfali E., Rajabalian S., E-brahimi S.-A., Farahyar S., Shafiee A. (2008) 2-Hydroxyphenacyl azoles and related azolium derivatives as antifungal agents. Bioorg Med Chem Lett;18:141–146.
9. National Committee for Clinical Laboratory Standards (2006) Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard M27-A2, Wayne, Pennsylvania, USA.
10. Espinel-Ingroff A., Fothergill A., Peter J., Rinaldi M.G., Walsh T.J. (2002) Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for *Aspergillus* spp.: NCCLS collaborative study. J Clin Microbiol;40:3204–3208.
11. Bryan J.D., Goldberg A.A., Wragg A.H. (1960) Synthesis of the 7-chloro-derivatives of chromone, flavone, and isoflavone. J Chem Soc;1279–1281.
12. King L.C., Ostrum G.K. (1964) Selective bromination with copper (II) bromide. J Org Chem;29:3459–3461.
13. Emami S., Shafiee A. (2001) Stereoselective syntheses of (*E*)- and (*Z*)-2,3-dihydro-3-(1,2,4-triazolyl)-4*H*-1-benzopyran-4-one oxime ethers. Heterocycles;55:2059–2074.
14. Rossello A., Bertini S., Lapucci A., Macchia M., Martinelli A., Rapposelli S., Herreros E., Macchia B. (2002) Synthesis, antifungal activity, and molecular modeling studies of new inverted oxime ethers of oxiconazole. J Med Chem;45:4903–4912.
15. Proudfoot J.R. (2002) Drugs, leads, and drug-likeness: an analysis of some recently launched drugs. Bioorg Med Chem Lett;12:1647–1650.
16. Keller T.H., Pichota A., Yin Z. (2006) A practical view of 'druggability'. Curr Opin Chem Biol;10:357–361.
17. Lipinski C.A. (2004) Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol;1:337–341.

Note

[a][Online] available at: http://www.organic-chemistry.org/prog/peo/