

Replacement of the Methylene of Dihydrochalcones with Oxygen: Synthesis and Biological Evaluation of 2-Phenoxyacetophenones

Mahsa Ansari¹, Saeed Emami^{2,*},
Mohammad A. Khalilzadeh³, Malek T.
Maghsoodlou¹, Alireza Foroumadi⁴,
Mohammad A. Faramarzi⁵, Nasrin Samadi⁶
and Sussan K. Ardestani⁷

¹Department of Chemistry, University of Sistan and Baluchestan, P.O. Box 98135-674, Zahedan, Iran

²Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

³Department of Chemistry, Science and Research Branch, Islamic Azad University, Mazandaran, Iran

⁴Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

⁵Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran

⁶Department of Drug and Food Control, Faculty of Pharmacy and Pharmaceutical Quality Assurance Research Center, Tehran University of Medical Sciences, Tehran, Iran

⁷Institute of Biochemistry and Biophysics, Department of Biochemistry, University of Tehran, P.O. Box 13145-1384, Tehran, Iran

*Corresponding author: Saeed Emami, sd_emami@yahoo.com

With the aim of finding new bioactive compounds, a series of phenoxyacetophenone derivatives 2 were designed and synthesized as oxygen analogs of dihydrochalcones. Also, phenoxyacetophenones were converted to (Z)-oxime derivatives 3 and their geometry were characterized by ¹H-NMR spectroscopy. The *in vitro* antifungal activity of compounds 2 and 3 was evaluated against *Candida albicans*, *Candida glabrata*, *Saccharomyces cerevisiae*, and *Aspergillus niger* using micro-dilution method. In general, oxime derivative 3d containing 4-fluorophenoxy moiety showed comparable or more potent antifungal activity (MICs = 15.63–31.25 µg/mL) with respect to the reference drug fluconazole against all tested yeasts. In addition, the antileishmanial activity of title compounds was determined against promastigote form of *Leishmania major*. All compounds showed mild growth inhibitory activity against promastigotes. The most active compound was unsubstituted phenoxyacetophenone 2a (IC₅₀ = 80 µg/mL). To anticipate the

potential use as drugs, the target compounds were evaluated in their drug-like properties. The *in silico* values of molecular descriptors for bioactive compounds 2a and 3d revealed that these compounds are within the range set by Lipinski's 'Rule of 5' and show no violation of these rules. Moreover, bioactive compounds 2a and 3d are supposed to be non-mutagenic and non-tumorigenic, with no irritating or reproductive effects.

Key words: acetophenone derivatives, antifungal activity, antileishmanial activity, dihydrochalcones, oximes, promastigotes

Received 24 January 2012, revised 13 May 2012 and accepted for publication 5 June 2012

Dihydrochalcones, the reduced form of chalcones, are a subclass of the flavonoids that occur widely in natural products and are important intermediates for many pharmaceutical drugs (1). Dihydrochalcones have been reported to possess various biological activities and have received considerable attention as promising anti-inflammatory and cancer chemopreventive agents (2,3). Generally, the antifungal activity of flavonoids has been reported previously (4). Among their different classes, dihydrochalcones have been shown to possess promising antifungal activity (5–7).

Recently, a series of synthetic and naturally occurring chalcone-like compounds have been reported to have antileishmanial activity in a number of *in vitro* and *in vivo* assays. Hermoso *et al.* (8) described antileishmanial activities of dihydrochalcones from *Piper elongatum* and synthetic-related compounds against *Leishmania braziliensis*, *Leishmania tropica* and *Leishmania infantum*. Also, a number of chromeno dihydrochalcones, containing 2',2'-dimethyl benzopyran system, were tested against extracellular promastigotes of *Leishmania donovani* and intracellular amastigotes residing within murine macrophages (9,10).

Moreover, the results of previous studies support the fact that the pharmacophore contains two aromatic rings, and the propanone chain just functions as a spacer and that the ability to inhibit parasite growth apparently depends on the presence and ratio of lipophilic/hydrophilic substituents at both aromatic rings (8,11,12).

With these issues in mind and for finding new bioactive compounds, we replaced the methylene of propanone chain in dihydrochalcone scaffold with oxygen to design phenoxyacetophenones (Figure 1). Here, we report simple and convenient synthetic method for the

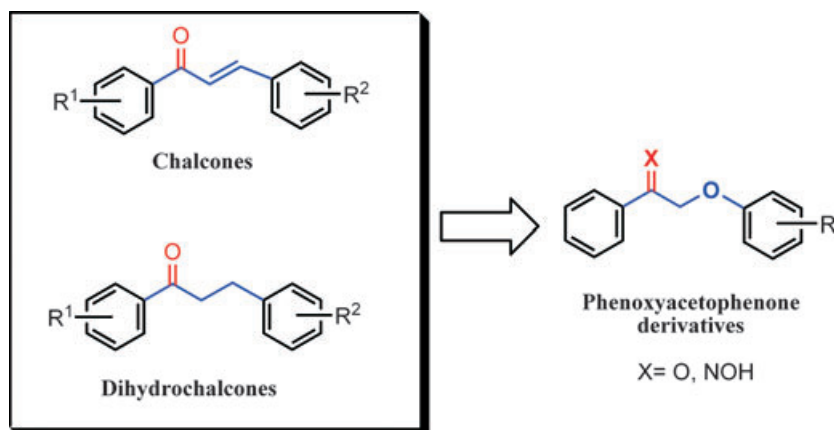


Figure 1: General structures of chalcones, dihydrochalcones, and designed compounds phenoxyacetophenone derivatives.

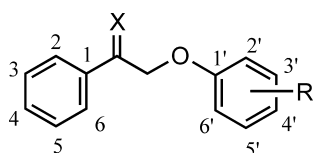


Figure 2: The atom numbering of compounds **2** and **3** for NMR spectral data assignments.

synthesis of phenoxyacetophenones **2** and phenoxyacetophenones oximes **3** and evaluation of their antifungal and antileishmanial activities.

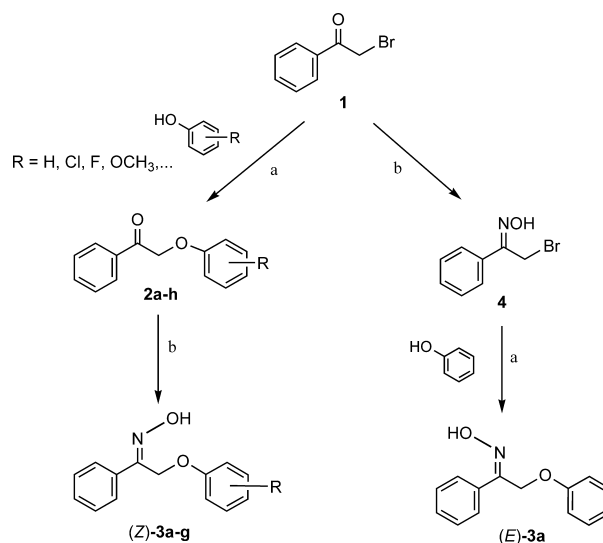
Experimental Section

Chemistry

All solvents and chemical reagents were purchased from Merck Co (Darmstadt, Germany), and used without further purification. α -Bromoacetophenone oxime **4** was prepared from α -bromoacetophenone **1** by using literature method (13). 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. The atom numbering of compounds **2** and **3** for NMR spectral data assignments was depicted in Figure 2. The IR spectra were obtained on a Perkin-Elmer FT-IR spectrophotometer (KBr disks). The progress of reactions and the purity of the compounds were checked by thin-layer chromatography (TLC) analysis on silica gel 60 F254 plates (Merck). The visualization of spots on TLC was performed with UV light (254 nm) or iodine. The synthetic route to phenoxyacetophenones **2** and their oximes **3** is presented in Scheme 1.

General procedure for the preparation of 2-phenoxy-1-phenylethanones (**2a-h**)

A suspension of appropriate phenol (6.18 mmol) and K_2CO_3 (1.493 g, 10.8 mmol) in dry DMF (27 mL) was stirred at room temperature for 10 min. Then, 2-bromoacetophenone (1.194 g, 6 mmol) was added portionwise at room temperature and the stirring was continued. After the completion of the reaction, water (25 mL) was



Scheme 1: Synthesis of compounds **2a-h** and **3a-g**. *Reagents and conditions:* (a) K_2CO_3 , DMF, rt; (b) hydroxylamine hydrochloride, MeOH, rt.

added and the mixture was refrigerated overnight. The precipitated solid was separated by filtration and washed with water (3×20 mL) and dried to give pure compound **2**.

2-Phenoxy-1-phenylethanone (**2a**)

Yellow solid; yield, 53%; m.p, 62–64 °C; IR (KBr, per cm), 3438, 3064, 1708, 1599, 1499, 1433, 1249, 1227, 1174, 975. 1H NMR (500 MHz, $CDCl_3$) δ : 5.27 (s, 2H, CH_2O), 6.95 (d, 2H, $^3J = 7.88$ Hz, H-2' and H-6' Ar), 6.99 (t, 1H, $^3J = 7.38$ Hz, H-4' Ar), 7.29 (t, 2H, $^3J = 7.06$ Hz, H-3' and H-5' Ar), 7.50 (t, 2H, $^3J = 7.90$ Hz, H-3 and H-5 Ph), 7.62 (t, 1H, $^3J = 7.42$ Hz, H-4 Ph), 8.00 (d, 2H, $^3J = 7.18$ Hz, H-2 and H-6 Ph).

2-(3-Chlorophenoxy)-1-phenylethanone (**2b**)

White solid; yield, 91%; m.p, 91–93 °C; IR (KBr, per cm), 1702, 1580, 1591, 1292, 1224, 1072, 977; 1H NMR (500 MHz, $CDCl_3$) δ : 5.26 (s, 2H, CH_2O), 6.83 (dd, 1H, $^3J = 8.69$ Hz, $^4J = 2.5$ Hz, H-6' Ar),

6.93 (t, 1H, $^4J = 2.17$ Hz, H-2' Ar), 6.96 (dt, 1H, $^3J = 7.91$ Hz, $^4J = 0.95$ Hz, H-4' Ar), 7.19 (t, 1H, $^3J = 8.14$ Hz, H-5' Ar), 7.50 (t, 2H, $^3J = 6.96$ Hz, H-3 and H-5 Ph), 7.62 (tt, 1H, $^3J = 7.46$ Hz, $^4J = 1.19$ Hz, H-4 Ph), 7.98 (dd, 2H, $^3J = 8.25$ Hz, $^4J = 0.98$ Hz, H-2 and H-6 Ph). ^{13}C NMR (125 MHz, CDCl_3) δ : 70.73, 113.22, 115.35, 121.89, 128.09, 128.94, 130.32, 134.07, 134.36, 134.99, 158.71, 193.81.

2-(3-Fluorophenoxy)-1-phenylethanone (2c)

White solid; yield, 66%; m.p. 82–84 °C; IR (KBr, per cm), 1708, 1612, 1489, 1428, 1286, 1146, 983. ^1H NMR (500 MHz, CDCl_3) δ : 5.26 (s, 2H, CH_2O), 6.64–6.74 (m, 3H, H-2', H-4' and H-6' Ar), 7.19–7.25 (m, 1H, H-5' Ar), 7.50 (t, 2H, $^3J = 7.5$ Hz, H-3 and H-5 Ph), 7.62 (t, 1H, $^3J = 7.4$ Hz, H-4 Ph), 7.98 (d, 2H, $^3J = 7.6$ Hz, H-2 and H-6 Ph).

2-(4-Fluorophenoxy)-1-phenylethanone (2d)

White solid; yield, 80%; m.p. 93–95 °C; IR (KBr, per cm), 2925, 1689, 1507, 1450, 1219, 1084, 832. ^1H NMR (500 MHz, CDCl_3) δ : 5.24 (s, 2H, CH_2O), 6.86–6.90 (m, 2H, H-2' and H-6' Ar), 6.96 (t, 2H, $^3J = 7.4$ Hz, H-3' and H-5' Ar), 7.49 (t, 2H, $^3J = 7.5$ Hz, H-3 and H-5 Ph), 7.61 (t, 1H, $^3J = 7.5$ Hz, H-4 Ph), 7.97 (d, 2H, $^3J = 7.4$ Hz, H-2 and H-6 Ph). MS (m/z, %): 230 (M^+ , 45), 105 (100), 77 (77), 51 (20).

2-(2-Methoxyphenoxy)-1-phenylethanone (2e)

White solid; yield, 71%; m.p. 97–99 °C; IR (KBr, per cm), 2920, 1687, 1507, 1470, 1255, 1211, 1130. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 3.78 (s, 3H, OCH_3), 5.54 (s, 2H, CH_2O), 6.80–7.05 (m, 4H, H-3', H-4', H-5' and H-6' Ar), 7.47–7.64 (m, 2H, H-3 and H-5 Ph), 7.65–7.76 (m, 1H, H-4 Ph), 8.02 (d, 2H, $^3J = 6.7$ Hz, H-2 and H-6 Ph). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ : 55.51, 70.68, 112.42, 113.65, 120.49, 121.31, 127.84, 128.78, 133.71, 134.41, 147.38, 148.93, 194.68.

2-(3-Methoxyphenoxy)-1-phenylethanone (2f)

White solid; yield, 68%; m.p. 96–98 °C; IR (KBr, per cm), 3002, 1697, 1582, 1499, 1193, 1158, 973. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 3.73 (s, 3H, OCH_3), 5.55 (s, 2H, CH_2O), 6.48–6.60 (m, 3H, H-2', H-4' and H-6' Ar), 7.17 (t, 1H, $^3J = 7.7$ Hz, H-5' Ar), 7.57 (t, 2H, $^3J = 6.85$ Hz, H-3 and H-5 Ph), 7.69 (t, 1H, $^3J = 6.85$ Hz, H-4 Ph), 8.03 (d, 2H, $^3J = 6.85$ Hz, H-2 and H-6 Ph). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ : 55.08, 70.09, 101.00, 106.48, 106.82, 127.84, 128.79, 129.86, 133.74, 134.38, 159.11, 160.41, 194.50.

2-(4-Methoxyphenoxy)-1-phenylethanone (2g)

White solid; yield, 78%; m.p. 94–96 °C; IR (KBr, per cm), 2833, 1691, 1507, 1448, 1216, 1187, 1029, 973. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 3.69 (s, 3H, OCH_3), 5.49 (s, 2H, CH_2O), 6.85 (d, 2H, $^3J = 8.3$ Hz, H-3' and H-5' Ar), 6.91 (d, 2H, $^3J = 8.1$ Hz, H-2' and H-6' Ar), 7.57 (t, 2H, $^3J = 7.0$ Hz, H-3 and H-5 Ph), 7.62–7.76 (m, 1H, H-4 Ph), 8.02 (d, 2H, $^3J = 7.1$ Hz, H-2 and H-6 Ph). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ : 55.32, 70.64, 114.47, 115.49, 127.81, 128.78, 133.69, 134.44, 151.90, 153.55, 194.89. MS (m/z, %): 242 (M^+ , 100), 123 (46), 105 (99), 91 (32), 77 (94), 51 (22).

2-(3,4,5-Trimethoxyphenoxy)-1-phenylethanone (2h)

White solid; yield, 65%; m.p. 102–104 °C; IR (KBr, per cm), 2990, 1697, 1598, 1466, 1423, 1203, 1126, 1012. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 3.58 (s, 3H, 4'-OMe), 3.75 (s, 6H, 3'-OMe and 5'-OMe), 5.53 (s, 2H, CH_2O), 6.33 (s, 2H, H-2' and H-6' Ar), 7.57 (t, 2H, $^3J = 7$ Hz, H-3 and H-5 Ph), 7.69 (t, 1H, $^3J = 7$ Hz, H-4 Ph), 8.03 (d, 2H, $^3J = 7$ Hz, H-2 and H-6 Ph). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ : 55.87, 60.06, 70.45, 92.81, 127.85, 128.77, 131.88, 133.69, 134.50, 153.26, 154.45, 194.54. MS (m/z, %): 302 (M^+ , 97), 183 (41), 105 (100), 77 (49).

General procedure for the preparation of 2-phenoxy-1-phenylethanone (Z)-oxime derivatives [(Z)-3a-g]

To a solution of compound **2** (1 mmol.) in MeOH (4 mL), hydroxylamine hydrochloride (277 mg, 4 mmol.) was added. The mixture was stirred at room temperature for 2 days. After completing the reaction, water (20 mL) was added and allowed to stay in refrigerator overnight. The precipitated solid was separated by filtration and washed with water (3 \times 5 mL) and dried to give pure compound (Z)-**3**.

2-Phenoxy-1-phenylethanone oxime [(Z)-3a]

White solid; yield, 52%; m.p. 86–88 °C; IR (KBr, per cm), 3790, 3435, 1600, 1498, 1459, 1308, 1263, 1213, 1049, 1086, 942. ^1H NMR (500 MHz, CDCl_3) δ : 5.29 (s, 2H, CH_2O), 6.94 (d, 2H, $^3J = 7.93$ Hz, H-2' and H-6' Ph), 6.96 (t, 1H, $^3J = 7.34$ Hz, H-4' Ph), 7.27 (t, 2H, $^3J = 8.09$ Hz, H-3' and H-5' Ph), 7.36–7.39 (m, 3H, H-3, H-4 and H-5 Ph), 7.66 (dd, 2H, $^3J = 7.82$ Hz, $^4J = 1.81$ Hz, H-2 and H-6 Ph), 8.98 (br s, 1H, NOH).

2-(3-Chlorophenoxy)-1-phenylethanone oxime [(Z)-3b]

White solid; yield, 91%; m.p. 74–76 °C; IR (KBr, per cm), 3445, 1644, 1596, 1477, 1296, 1054, 765. ^1H NMR (500 MHz, CDCl_3) δ : 5.25 (s, 2H, CH_2O), 6.8 (dd, 1H, $^3J = 8.41$, $^4J = 1.7$ Hz, H-6' Ar), 6.91–6.95 (m, 2H, H-2' and H-4' Ar), 7.17 (t, 1H, $^3J = 8.12$ Hz, H-5' Ar), 7.34–7.38 (m, 3H, H-3, H-4 and H-5 Ph), 7.62 (dd, 2H, $^3J = 7.01$, $^4J = 2.11$ Hz, H-2 and H-6 Ph).

2-(3-Fluorophenoxy)-1-phenylethanone oxime [(Z)-3c]

Light yellow; yield, 73%; m.p. 88–90 °C, IR (KBr, per cm) 3236, 2938, 1608, 1596, 1489, 1292, 1265, 1138, 1051, 912. ^1H NMR (500 MHz, CDCl_3) δ : 5.25 (s, 2H, CH_2O), 6.62–6.71 (m, 3H, H-2', H-4' and H-6' Ar), 7.15–7.21 (m, 1H, H-5' Ar), 7.33–7.43 (m, 3H, H-3, H-4 and H-5 Ph), 7.63 (dd, 2H, $^3J = 7.94$, $^4J = 2.12$ Hz, H-2 and H-6 Ph).

2-(4-Fluorophenoxy)-1-phenylethanone oxime [(Z)-3d]

White solid; yield, 87%; m.p. 74–76 °C; IR (KBr, per cm) 3251, 1595, 1474, 1506, 1310, 1219, 1029, 1011, 934. ^1H NMR (500 MHz,

CDCl₃) δ : 5.24 (s, 2H, CH₂O), 6.83–6.87 (m, 2H, H-2' and H-6' Ar), 6.90–6.98 (m, 2H, H-3' and H-5' Ar), 7.32–7.45 (m, 3H, H-3, H-4, and H-5 Ph), 7.59–7.63 (m, 2H, H-2 and H-6 Ph). MS (m/z, %): 245 (M⁺, 100), 230 (21), 125 (22), 103 (100), 91 (86), 77 (90).

2-(2-Methoxyphenoxy)-1-phenylethanone oxime [(Z)-3e]

White solid; yield, 81%; m.p., 90–92 °C; IR (KBr, per cm) 3244, 2919, 1592, 1507, 1464, 1261, 1185, 1128, 743. ¹H NMR (500 MHz, DMSO-*d*₆) δ : 3.67 (s, 3H, OCH₃), 5.20 (s, 2H, CH₂O), 6.85–7.05 (m, 4H, Ar), 7.26–7.48 (m, 3H, H-3, H-4 and H-5 Ph), 7.64–7.72 (m, 2H, H-2 and H-6 Ph), 11.85 (br s, 1H, NOH). ¹³C NMR (125 MHz, DMSO-*d*₆) δ : 55.49, 59.31, 112.44, 113.38, 120.67, 121.48, 126.33, 128.21, 128.80, 134.37, 147.49, 149.08, 152.86.

2-(4-Methoxyphenoxy)-1-phenylethanone oxime [(Z)-3f]

White solid; yield, 88%; m.p., 87–89 °C; IR (KBr, per cm) 3379, 2950, 1625, 1450, 1510, 1236, 1210, 1044, 714. ¹H NMR (500 MHz, DMSO-*d*₆) δ : 3.67 (s, 3H, OCH₃), 5.18 (s, 2H, CH₂O), 6.83 (d, 2H, ³J = 8.1 Hz, H-3' and H-5' Ar), 6.85 (d, 2H, ³J = 8.1 Hz, H-2' and H-6' Ar), 7.26–7.45 (m, 3H, H-3, H-4 and H-5 Ph), 7.55–7.78 (m, 2H, H-2 and H-6 Ph), 11.85 (br s, 1H, NOH). MS (m/z, %): 257 (M⁺, 79), 123 (100), 109 (25), 95 (45), 77 (46).

2-(3,4,5-Trimethoxyphenoxy)-1-phenylethanone oxime [(Z)-3g]

Brown solid; yield, 64%; m.p., 106–108 °C; IR (KBr, per cm) 3420, 2939, 1597, 1465, 1227, 1129, 1002, 945. ¹H NMR (500 MHz, DMSO-*d*₆) δ : 3.56 (s, 3H, 4'-OMe), 3.72 (s, 6H, 3'-OMe and 5'-OMe), 5.22 (s, 2H, CH₂O), 6.28 (s, 2H, H-2' and H-6' Ar), 7.30–7.46 (m, 3H, H-3, H-4 and H-5 Ph), 7.66 (m, 2H, H-2 and H-6 Ph), 11.97 (br s, 1H, NOH). MS (m/z, %): 317 (M⁺, 25), 302 (100), 287 (53), 183 (73), 168 (32), 151 (25), 119 (47), 105 (60), 91 (68), 77 (36).

The preparation of 2-phenoxy-1-phenylethanone (E)-oxime [(E)-3a]

2-Bromoacetophenone oxime (**4**, 214 mg, 1.0 mmol) was added portionwise to a suspension of phenol (97 mg, 1.03 mmol) and K₂CO₃ (248 mg, 1.8 mmol) in dry DMF (5 mL) at room temperature and the reaction mixture was stirred for 3 days. After completing the reaction, water (20 mL) was added and the precipitated solid was separated by filtration. The crude product was mixed with %5 NaOH and extracted with CHCl₃ (3 × 20 mL). The organic layer was washed with water and dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give pure product (E)-**3a**. White solid; yield, 52%; m.p., 80–82 °C; IR (KBr, per cm) 3696, 3385, 1594, 1487, 1443, 1284, 1251, 1112, 1018, 954. ¹H NMR (500 MHz, CDCl₃) δ : 4.91 (s, 2H, CH₂O), 6.94 (d, 2H, ³J = 8.56 Hz, H-2' and H-6' Ph), 6.97 (t, 1H, ³J = 7.33 Hz, H-4' Ph), 7.27 (t, 2H, ³J = 7.64 Hz, H-3' and H-5' Ph), 7.40–7.45 (m, 3H, H-3, H-4 and H-5 Ph), 7.61 (dd, 2H, ³J = 7.82 Hz, ⁴J = 1.39 Hz, H-2 and H-6 Ph), 8.29 (br s, 1H, NOH).

Antifungal activity assay

The antifungal activity of phenoxyacetophenones **2** and oximes derivatives **3** was evaluated against *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 90030, *Saccharomyces cerevisiae* NCYC 694, *S. cerevisiae* ATCC 9763 and *Aspergillus niger* ATCC 16404. For all fungi, the susceptibilities were determined by micro-dilution method using 96 U-shaped wells plates (14). Briefly, twofold serial dilution of the compounds was prepared by mixing 100 μ L of stock solution of each compounds (in DMSO) with Sabouraud dextrose broth (100 μ L) in ten wells. Then, aliquot of 100 μ L of fungal suspension (1 × 10³ CFU/mL) was added to each well to reach the final inoculum size of 0.5 × 10³ CFU/mL. After incubation at 35 °C, the plates were tested for the absence or presence of visible growth in comparison with that of control well. The MIC value was defined as lowest concentration of the compound at which there was no visible growth. The MIC values of test compounds were determined at 24 and 48 h for *Candida* and *Saccharomyces* strains, and 48 and 72 h for *A. niger*.

Antileishmanial activity assay

The antipromastigote activity of compounds **2** and **3** was evaluated by direct counting and MTT assay according to the literature method (15). The percentage of inhibition of compounds against promastigote form of *Leishmania major* (vaccine strain MRHO/IR/75/ER, obtained from Pasteur institute, Tehran, Iran) was determined at 75 μ g/mL concentration. Briefly, promastigotes (2 × 10⁶/mL) from early logarithmic phase of growth were cultured in 96-well plastic plates, containing 75 μ g/mL of compounds and phenol red-free RPMI 1640 medium, supplemented with 10% of FBS, 2 mM glutamine, pH ~7.2, and antibiotics, in a volume of 200 μ L. After 24 h of incubation at 25 °C, the media was renewed with 100 μ g/well of MTT solution (0.5 mg/mL) and plates were further incubated for 4 h at 37 °C. The plates were centrifuged (400 g, 5 min) and the pellets were dissolved in DMSO (200 μ L). The absorbance of sample was determined as the optical density at 492 nm in an ELISA plate reader. Two or more independent experiments in triplicate were performed for each compound.

Results and Discussion

Chemistry

The synthetic route to phenoxyacetophenones **2** and their oximes **3** is presented in Scheme 1. Phenoxyacetophenones **2** was prepared by the reaction of α -bromoacetophenone **1** with appropriate phenols in the presence of K₂CO₃ in dry DMF. Compounds **2** were predominantly converted to related (Z)-oximes **3** using excess hydroxylamine hydrochloride in methanol. This reaction was carried out at room temperature and mild acidic conditions provided by hydrochloride salt of hydroxylamine. No refluxing or using a base-like pyridine is required. The alternative route to oxime was attempted by employing α -bromoacetophenone oxime **4**. Thus, α -bromoacetophenone **1** was firstly converted to α -bromoacetophenone oxime **4** in the presence of excess HONH₂.HCl in methanol at room temperature (13). α -Bromoacetophenone oxime **4** was reacted with phenol in the presence of K₂CO₃ in dry DMF to give (E)-oxime **3a**.

The geometry of the oxime derivatives **3a-g** was assigned by ^1H NMR spectroscopy. The chemical shifts of the methylene protons for the (*E*)- and (*Z*)-isomers of oxime derivatives **3a-g** have significant deviations. For example, compound (*E*)-**3a** showed a signal at 4.91 ppm, while (*Z*)-**3a** exhibited the corresponding signal at a lower field, 5.29 ppm. Previous studies on oxime derivatives have been established that proximity to the oxygen of the oxime in the α -*syn* configuration will deshield the proton and cause a downfield shift in the signals of related protons (16–20). Thus, the protons of methylene that is *syn* to the oxime moiety shifted downfield in (*Z*)-isomers.

Biological activity

The antifungal activity of compounds **2** and **3** was evaluated against *C. albicans*, *C. glabrata*, *S. cerevisiae*, and *A. niger*. The MIC values of compounds **2** and **3** were determined at 24 and 48 h for *Candida* and *Saccharomyces* strains, and 48 and 72 h for *A. niger* by comparing with fluconazole as reference drug (Table 1). The MIC values of the test compounds against *C. albicans* indicate that oxime **3d** possesses a remarkable activity with MIC values of 31.25 $\mu\text{g}/\text{mL}$. Its activity was 2–4 times more than the standard drug fluconazole. Also, compounds **2a**, **3b**, and **3e** showed weak growth inhibition against *C. albicans*. Furthermore, compounds **3b** and **3d** with MIC values of 15.63–31.25 $\mu\text{g}/\text{mL}$ showed good activity against *C. glabrata* comparable or better than that of fluconazole. Although, compounds **2a**, **2b**, **2e**, **2f**, **2h**, **3e**, and **3e** exhibited some inhibitory activity against *Saccharomyces* strains, but only the activity of compound **3d** was respectable. Its activity

was equipotent to fluconazole with MIC values of 15.63–31.25 $\mu\text{g}/\text{mL}$.

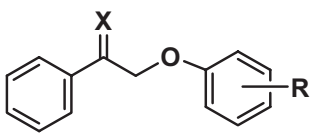
In general, oxime derivative **3d** containing 4-fluorophenoxy moiety showed good activity against all tested strain with the exception of activity against *A. niger*. In fact, the results of antifungal evaluation of test compounds indicated that compound **3d** showed comparable or more potent antifungal activity with respect to the reference drug fluconazole against all tested yeasts. In contrast, ketone counterpart of **3d** (compound **2d**) showed no inhibitory activity against these fungi. Thus, it could be concluded that the oximation of 4-fluorophenoxyacetophenone improves antifungal activity. Moreover, by comparing the activities of oximes **3a** and **3d**, it revealed that the 4-fluoro-substitution has a positive effect on antifungal potency.

The antipromastigote activity of phenoxyacetophenones **2** and their oximes **3** was evaluated against *L. major*. The percentage of inhibition for each compound was expressed in mean values and presented in Table 2. All compounds showed remarkable inhibition against promastigotes in comparison with glucantime as a reference drug. The most active compounds were unsubstituted phenoxyacetophenone **2a** with percentage of inhibition equal to 48% followed by 4-fluoro analog **2d** with 42 percent of inhibitory activity. A survey on the inhibitory activity of test compounds against promastigotes revealed that substitution on the different position of phenoxy moiety or oximation of ketone group could not improve the antileishmanial activity. Also, by comparing the inhibitory activity of (*Z*)- and (*E*)-stereoisomers of **3a**, it could be concluded that the geometry of oxime does not have a significant effect on inhibitory activity against promastigotes.

Table 1: Minimum inhibitory concentrations (MICs, $\mu\text{g}/\text{mL}$) of synthetic compounds **2a-h** and **3a-g** against different strains of fungi

Compound	X	R	<i>Candida albicans</i> ATCC 10231		<i>Candida glabrata</i> ATCC 90030		<i>Saccharomyces cerevisiae</i> NCYC 694		<i>Saccharomyces cerevisiae</i> ATCC 9763		<i>Aspergillus niger</i> ATCC 16404	
			24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	48 h	72 h
2a	O	H	125	>125	>125	>125	125	>125	125	>125	>125	>125
2b	O	3-Cl	>125	>125	>125	>125	125	>125	125	>125	>125	>125
2c	O	3-F	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
2d	O	4-F	>125	>125	>125	>125	>125	>125	15.63	>125	>125	>125
2e	O	2-OMe	>125	>125	>125	>125	125	>125	>125	>125	>125	>125
2f	O	3-OMe	>125	>125	>125	>125	125	>125	>125	>125	>125	>125
2g	O	4-OMe	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
2h	O	3,4,5-(OMe) ₃	>125	>125	>125	>125	125	>125	125	125	>125	>125
(<i>Z</i>)- 3a	NOH	H	>125	>125	>125	>125	>125	>125	31.25	>125	>125	>125
(<i>Z</i>)- 3b	NOH	3-Cl	125	>125	15.63	31.25	>125	>125	>125	>125	>125	>125
(<i>Z</i>)- 3c	NOH	3-F	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
(<i>Z</i>)- 3d	NOH	4-F	31.25	31.25	31.25	31.25	31.25	62.5	15.63	31.25	>125	>125
(<i>Z</i>)- 3e	NOH	2-OMe	125	>125	>125	>125	125	>125	125	>125	>125	>125
(<i>Z</i>)- 3f	NOH	4-OMe	>125	>125	>125	>125	125	>125	125	>125	>125	>125
(<i>Z</i>)- 3g	NOH	3,4,5-(OMe) ₃	>125	>125	>125	>125	>125	>125	125	125	>125	>125
Fluconazole			62.5	125	31.25	62.5	15.63	31.25	15.63	31.25	–	–

The significance of bold values in comparison with control group was less than 0.05 ($P < 0.05$).

Table 2: Antipromastigote activity of compounds **2a–h** and **3a–f**


Compounds	X	R	% Inhibition (75 $\mu\text{g}/\text{mL}$)
2a	O	H	48 ^a
2b	O	3-Cl	22
2c	O	3-F	38
2d	O	4-F	42
2e	O	2-OMe	34
2f	O	3-OMe	30
2g	O	4-OMe	30
2h	O	3,4,5-(OMe) ₃	33
(<i>E</i>)- 3a	NOH	H	37
(<i>Z</i>)- 3a	NOH	H	33
(<i>Z</i>)- 3b	NOH	3-Cl	27
(<i>Z</i>)- 3c	NOH	3-F	35
(<i>Z</i>)- 3d	NOH	4-F	37
(<i>Z</i>)- 3e	NOH	2-OMe	30
(<i>Z</i>)- 3f	NOH	4-OMe	20
Glucantime			50 ^b

^aThe IC₅₀ value of compound **2a** was 80 $\mu\text{g}/\text{mL}$.

^bGlucantime was tested at the dose of 25 $\mu\text{g}/\text{mL}$.

In silico study

Toxicity studies were traditionally conducted during the drug development; however, it has been a major cause of drug candidate attrition during preclinical and clinical phases. Thus, the early knowledge about the toxicity of lead compounds allows us to successfully achieving to the drugs (21). On the other hand, appropriate drug-like properties of lead compounds also allow us to convert them into successful drugs. Lipinski (22) introduced the term 'drug-

like' based on the structural properties that affect the physicochemical properties of solubility and permeability and their effect on drug absorption.

To anticipate the potential use as drugs, the compounds described here are evaluated in their drug-like properties – for example, mutagenicity, tumorigenicity, irritating and reproductive effects, *cLogP* (calculated *n*-octanol/water partition coefficient), *LogS* (solubility), and MW (molecular weight) – using *in silico* approaches provided by Osiris Properties Explorer tools^a (Table 3). Also, from this online properties explorer, the descriptors' drug-likeness and drug score were estimated for target compounds. According to these predictive tools, bioactive compounds **2a** and **3d** are supposed to be non-mutagenic and non-tumorigenic, with no irritating or reproductive effects (Table 3). Moreover, compound **2a** showed satisfactory drug-likeness and drug score, according to the classification of the Osiris Properties Explorer tools.

On the other hand, a set of simple molecular descriptors including *logP*, molecular weight (MW), number of hydrogen bond acceptors (HBA), and number of hydrogen bond donors (HBD) were used by Lipinski in formulating his 'Rule of 5'. Accordingly, the 'Rule of 5' defines four simple physicochemical parameter ranges (MW \leq 500, *Log P* \leq 5, HBD \leq 5, HBA \leq 10) for oral absorption (22). Molecules violating more than one of these rules may have problems with bioavailability. The values of molecular descriptors for bioactive compounds **2a** and **3d** reveal that these compounds are within the range set by Lipinski's 'Rule of 5' (low molecular weight, favorable *cLogP*, favorable hydrogen bond-donating and accepting capabilities) and show no violation of these rules.

Conclusion

In summary, to find new bioactive compounds, we designed a series of phenoxyacetophenone derivatives **2** and their related oximes

Table 3: Predicted toxicity risks and drug-like properties estimated by Osiris Property Explorer tools

Compound	Toxicity risks ^a				Drug-like properties				
	M	T	I	R	<i>cLogP</i>	<i>LogS</i>	MW	Drug-likeness	Drug score
2a	(–)	(–)	(–)	(–)	2.84	–3.25	212	0.89	0.74
2b	(–)	(–)	(–)	(–)	3.45	–3.99	246	0.22	0.6
2c	(–)	(–)	(–)	(±)	2.9	–3.56	230	–2.45	0.36
2d	(–)	(–)	(–)	(–)	2.9	–3.56	230	–0.34	0.59
2e	(–)	(–)	(–)	(–)	2.73	–3.27	242	1.51	0.78
2f	(–)	(–)	(±)	(+)	2.73	–3.27	242	0.09	0.32
2g	(–)	(–)	(–)	(–)	2.73	–3.27	242	0.88	0.74
2h	(–)	(–)	(–)	(–)	2.52	–3.3	302	2.4	0.81
(<i>Z</i>)- 3a	(–)	(–)	(–)	(–)	3.07	–3.62	227	0.06	0.63
(<i>Z</i>)- 3b	(–)	(–)	(–)	(–)	3.69	–4.36	261	–0.35	0.51
(<i>Z</i>)- 3c	(–)	(–)	(–)	(±)	3.13	–3.94	245	–3.04	0.33
(<i>Z</i>)- 3d	(–)	(–)	(–)	(–)	3.13	–3.94	245	–0.87	0.51
(<i>Z</i>)- 3e	(–)	(–)	(–)	(–)	2.97	–3.64	257	1.02	0.71
(<i>Z</i>)- 3f	(–)	(–)	(–)	(–)	2.97	–3.64	257	0.36	0.65
(<i>Z</i>)- 3g	(–)	(–)	(–)	(–)	2.76	–3.68	317	1.92	0.76

^aRanked according to: (–) no bad effect, (±) medium bad effect, (+) bad effect.

M, mutagenic effect; T, tumorigenic effect; I, irritating effect; R, reproductive effect.

3 as analogs of dihydrochalcones. The *in vitro* antifungal evaluation of target compounds revealed the oxime derivative **3d** containing 4-fluorophenoxy moiety possessing comparable or more potent antifungal activity with respect to the reference drug fluconazole against all tested yeasts. Moreover, all compounds showed remarkable growth inhibitory activity against promastigotes of *L. major* in MTT assay. The most active compound was unsubstituted phenoxyacetophenone **2a**. *In silico* molecular properties calculations and SAR study confirmed that compounds **2a** and **3d** are promising lead compounds for further development without great risk of toxicity in diverse fields of antileishmanial and antifungal activities.

Acknowledgments

This work was supported by a grant from the Research Council of Mazandaran University of Medical Sciences, Sari, Iran.

References

- Jung E.M., Lee Y.R. (2008) First concise total syntheses of biologically interesting nicolaioidesin C, crinatusinC1 and crinatusin C2. *Bull Korean Chem Soc*;29:1199–1204.
- Szliszka E., Czuba Z.P., Mazur B., Paradysz A., Krol W. (2010) Chalcones and dihydrochalcones augment TRAIL-mediated apoptosis in prostate cancer cells. *Molecules*;15:5336–5353.
- Briot A., Baehr C., Brouillard R., Wagner A., Mioskowski C. (2004) Concise synthesis of dihydrochalcones via palladium-catalyzed coupling of aryl halides and 1-aryl-2-propen-1-ols. *J Org Chem*;69:1374–1377.
- Pan S., Mukherjee B., Ganguly A., Mitra S.R., Bhattacharya A. (1985) Antifungal activity of some naturally occurring flavonoids. *Z Pflanzenkr Pflanzenschutz*;92:392–395.
- Okunade A.L., Hufford C.D., Clark A.L., Lentz D. (1997) Antimicrobial properties of the constituents of *Piper aduncum*. *Phytother Res*;11:142–144.
- Mustafa K.A., Kjaergaard H.G., Perry N.B., Weavers R.T. (2003) Hydrogen-bonded rotamers of 2',4',6'-trihydroxy-3'-formyldihydrochalcone, an intermediate in the synthesis of a dihydrochalcone from *Leptospermum recurvum*. *Tetrahedron*;59:6113–6120.
- Miles D.H., Rosa de Medeiros J.M., Chittawong V., Hedin P.A., Swithenbank C., Lidert Z. (1991) 3'-Formyl-2',4',6'-trihydroxydihydrochalcone from *Psidium acutangulum*. *Phytochemistry*;30:1131–1132.
- Hermoso A., Jimenez I.A., Mamani Z.A., Bazzocchi I.L., Pinero J.E., Ravelo A.G., Valladares B. (2003) Antileishmanial activities of dihydrochalcones from *Piper elongatum* and synthetic related compounds. Structural requirements for activity. *Bioorg Med Chem*;11:3975–3980.
- Narender T., Shweta, Gupta S. (2004) A convenient and biogenetic type synthesis of few naturally occurring chromenodihydrochalcones and their *in vitro* antileishmanial activity. *Bioorg Med Chem Lett*;14:3913–3916.
- Kayser O., Kiderlen A.F., Folkens U., Kolodziej H. (1998) Antileishmanial activity of aurones. *Planta Med*;65:316–319.
- Nielsen S.F., Kharazmi A., Christensen S.B. (1998) Modifications of the α,β -double bond in chalcones only marginally affect the antiprotozoal activities. *Bioorg Med Chem*;6:937–945.
- Kayser O., Kiderlen A.F. (2001) *In vitro* leishmanicidal activity of naturally occurring chalcones. *Phytother Res*;15:148–152.
- Emami S., Foroumadi A. (2007) Theoretical study on Z/E selectivity in the oximation of α -haloacetophenones. *Asian J Chem*;19:4727–4732.
- National Committee for Clinical Laboratory Standards (2006) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M27-A2. Wayne, PA, USA: National Committee for Clinical Laboratory Standards.
- Dutta A., Bandyopadhyay S., Mandal C., Chatterjee M. (2005) Development of a modified MTT assay for screening antimonial resistant field isolates of Indian visceral leishmaniasis. *Parasitol Int*;54:119–122.
- Emami S., Falahati M., Banifatemi A., Shafiee A. (2004) Stereoselective synthesis and antifungal activity of (Z)-trans-3-azoly-2-methylchromanone oxime ethers. *Bioorg Med Chem*;12:5881–5889.
- Emami S., Falahati M., Banifatemi A., Amanlou M., Shafiee A. (2004) (E)- and (Z)-1,2,4-Triazolylchromanone oxime ethers as conformationally constrained antifungals. *Bioorg Med Chem*;12:3971–3976.
- Emami S., Shafiee A. (2001) Stereoselective syntheses of (E)- and (Z)-2,3-dihydro-3-(1,2,4-triazolyl)-4H-1-benzopyran-4-one oxime ethers. *Heterocycles*;55:2059–2074.
- Letafat B., Emami S., Mohammadhosseini N., Faramarzi M.A., Samadi N., Shafiee A., Foroumadi A. (2007) Synthesis and antibacterial activity of new N-(2-(thiophen-3-yl)ethyl) piperazinyl quinolones. *Chem Pharm Bull*;55:894–898.
- Foroumadi A., Mohammadhosseini N., Emami S., Letafat B., Faramarzi M.A., Samadi N., Shafiee A. (2007) Synthesis and antibacterial activity of new 7-piperazinyl-quinolones containing a functionalized 2-(furan-3-yl)ethyl moiety. *Arch Pharm*;340:47–52.
- Cerecetto H., González M. (2010) Synthetic medicinal chemistry in Chagas' disease: compounds at the final stage of "hit-to-lead" phase. *Pharmaceuticals*;3:810–838.
- Lipinski C.A. (2004) Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol*;1:337–341.

Note

^a<http://www.organic-chemistry.org/prog/peo/>