Med Chem Res (2013) 22:3897–3904
DOI 10.1007/s00044-012-0391-5

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and cytotoxic activities of new 2,4,5-triarylimidazoles

A. Zarghi · S. Arfaei · F. H. Shirazi

Received: 8 July 2012 / Accepted: 4 December 2012 / Published online: 15 December 2012

Abstract A new group of 2,4,5-triarylimidazoles containing *N*,*N*-dimethylaminoethoxy or piperidinyl ethoxy group at the *para* position of the C-5 phenyl ring were synthesized and their cytotoxic activities were evaluated on three different breast cancer cell lines using MTT assay. The compounds contain various substituents at the *para* position of C-2 phenyl ring. Among the synthesized compounds, 4-(5-(4-(2-piperidin-1-yl)ethoxy)phenyl)-4-phenyl-1*H*-imidazol-2-yl)phenol (**11e**) and 1-2-(4-(2,4-diphenyl-1*H*-imidazol-5-yl)phenoxy)ethyl) piperidine (**11h**) with IC_{50}s of less than 0.1 μM on all three cell lines were the most potent cytotoxic compounds.

Keywords Synthesis · 2,4,5-Triarylimidazoles · Cytotoxic activity · MTT · Docking study

Introduction

Imidazole scaffold exists in many significant biomolecules including biotin, histamine, and amino acids such as histidine. Many compounds possessing significant biochemical and pharmacological roles contain imidazole nucleus. Members of this class of heterocycles are known to possess antibiotic (Brogden *et al*., 1978), anti-fungal (Di Santo *et al*., 2005), anti-inflammatory (Mano *et al*., 2003), COX-2 inhibitors (Zarghi *et al*., 2012), and cytotoxic activities (Narasimhan *et al*., 2011). Imidazole compounds have been an interesting source for researchers for more than a century and many of them are being used as chemotherapy compounds today. A wide variety of imidazole containing compounds having cytotoxic activities on different cancer cell lines with different mechanisms of action have been developed over years, such as inhibitors of B-Raf kinase **1**,**2** (Takle *et al*., 2006), combretastatin analogs **3**,**4** (Wang *et al*., 2002), and modulators of P-glycoprotein (P-gp)-mediated multidrug resistance (MDR) **5**,**6** (Newman *et al*., 2000; Robert and Jarry, 2003) shown in Fig. 1. Tamoxifen **7** was developed more than 30 years ago for the treatment of advanced breast cancer (Osborne, 1998). Tamoxifen, like other SERMs, binds to estrogen receptor (ER) and, in breast cancer cells, antagonizes the effect of estrogen on a variety of growth-regulatory genes (Dhingra, 1999). The predominant effect of tamoxifen and many other SERMs is cytostatic, by inducing a G1 cell cycle block, thereby slowing cell proliferation. Tamoxifen may also induce apoptosis, although this process has not always been easy to demonstrate in tumors (Ellis *et al*., 1997). Based upon the anti-cancer properties of imidazole compounds in Fig. 1 and anti-breast cancer effects of tamoxifen, new 2,4,5-triaryl-1*H*-imidazoles were designed, synthesized, and their cytotoxic activities were evaluated on three breast cancer cell lines. For designing the new imidazoles, the vicinal diaryl moiety and the basic side chain (*N*,*N*-dialkylamino ethoxyphenyl) was derived from the structure of tamoxifen, while the total 2,4,5-triarylimidazole resembles the other cytotoxic imidazole compounds shown in Fig. 1.

A. Zarghi (✉) · S. Arfaei
Department of Medicinal Chemistry,
School of Pharmacy, Shahid Beheshti University of Medical Sciences, P.O. Box 14155-6153, Tehran, Iran
e-mail: azarghi@yahoo.com

F. H. Shirazi
Department of Toxicology, School of Pharmacy,
Shahid Beheshti University of Medical Sciences, Tehran, Iran

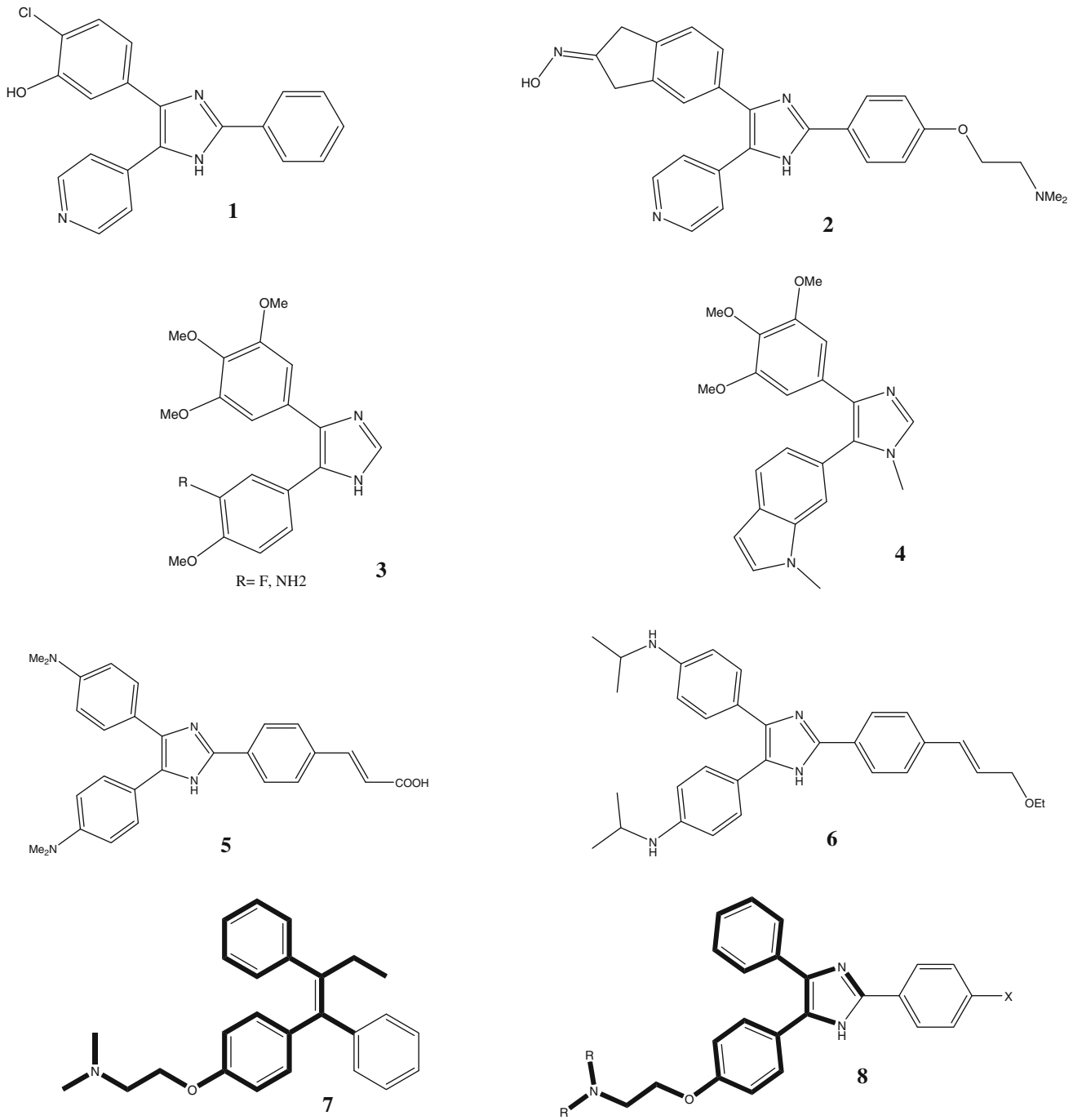
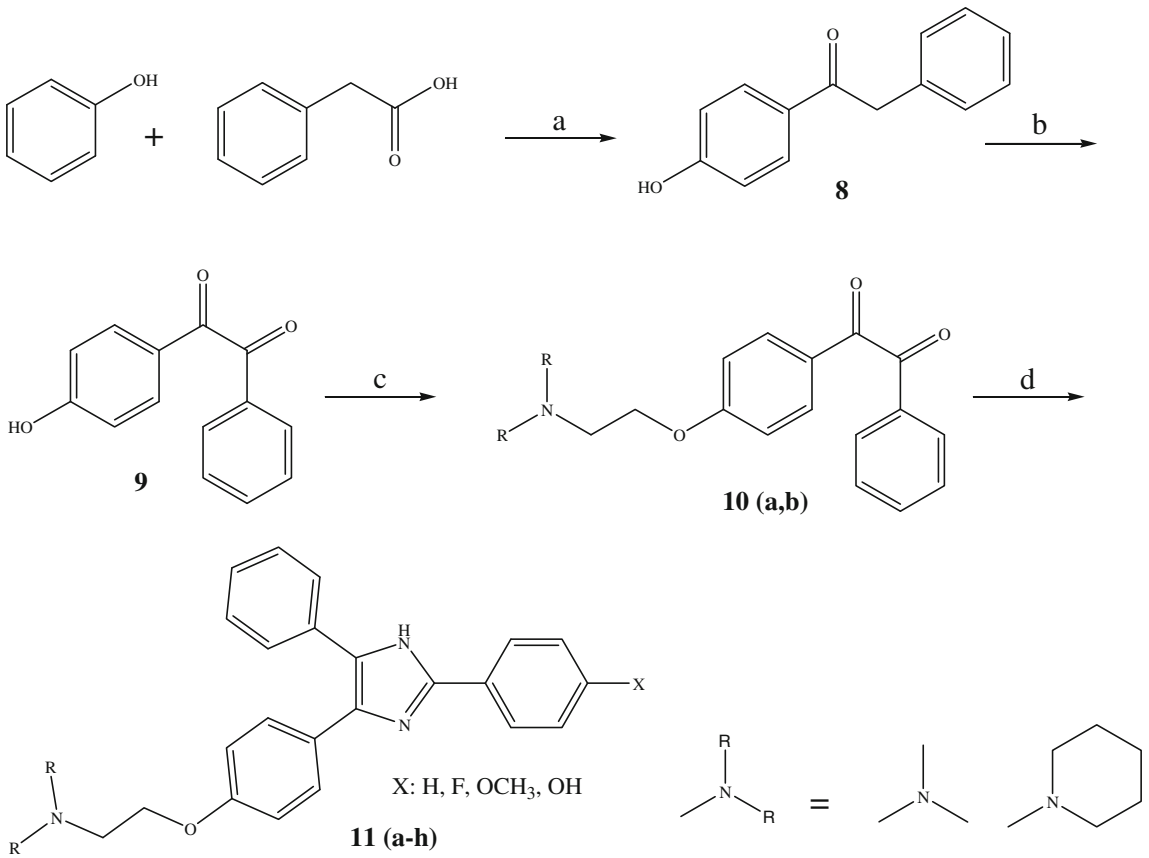


Fig. 1 Structures of imidazole compounds, tamoxifen (7) and our designed molecules (8)

Results and discussions

Chemistry

As shown in Scheme 1 the target 2,4,5-triaryl-1*H*-imidazole derivatives **11a**–**h** were synthesized via a one-pot microwave reaction between 1,2-diketone **10** and the appropriate aromatic aldehyde in the presence of ammonium acetate under microwave irradiation (Wolkenberg *et al*., 2004; Zarghi *et al*., 2012). Primarily 1-(4-hyroxyphenyl)-2-phenylethanone **8** was synthesized using Friedel–Crafts reaction between phenol and phenylacetic acid in the presence of Lewis acid $BF_3{\cdot}Et_2O$ (Wahala and Hase, 1991). Compound **8** was then oxidized to diketone **9** using selenium dioxide (Caturla *et al*., 2004). Diketone **9** undergone a nucleophilic substitution reaction with appropriate reagents (*N*-(2-chloroethyl)-*N*,*N*-dimethylammonium chloride or (*N*-(2-chloro ethyl)-piperidinium chloride) to add the basic side chain and



Scheme 1 Reagents and conditions: **a** $BF_3{\cdot}Et_2O$, 80 °C; **b** SeO_2, dioxane/H_2O, reflux; **c** *N*-(2-chloroethyl)-*N*,*N*-dimethylammonium chloride or *N*-(2-chloroethyl)-piperidinium chloride, K_2CO_3, acetonitrile, reflux; **d** Ar-CHO, NH_4OAc, AcOH, MW, 180 W, 10 min

was converted to diketone **10** (**a**, **b**) (Um et al., 2003). 1,2-Diketone **10** was used to synthesize the final 2,4,5-triaryl-1*H*-imidazoles **11a–h** via the multi-component microwave reaction. The purity of all products was determined by thin layer chromatography using several solvent systems of different polarity. All compounds were pure and stable. The compounds were characterized by ^{1}HNMR, MS, IR, and CHN analysis. The physical data of final synthesized derivatives were summarized in Table 1.

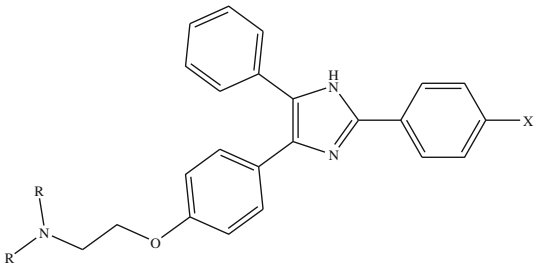
Cytotoxicity

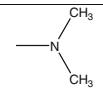
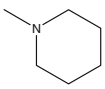
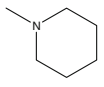
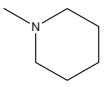
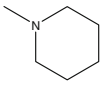
The cytotoxicity of compounds **11a–h** was determined on breast cancer cell lines using MTT assay (Mosmann, 1983). The MTT assay of the compounds was performed on three different breast cancer cell lines: MCF-7 and T47D (containing medium to high levels of estrogen receptors) and MDA-MB-231 (without estrogen receptors) (Siciliano *et al*., 1979; Dickstein *et al*., 1993; Keydar *et al*., 1979) to indicate that the anti-proliferative activities of designed compounds are mediated through hormone-dependent or hormone-independent mechanisms. The results of MTT assay of these compounds are shown in Table 2. According to these results, all compounds **11a–h** were cytotoxic against three different breast cancer cell lines. In addition, our results indicated that the synthesized compounds **11a–h** were more cytotoxic against T47D cells which contain high amounts of estrogen receptors in comparison with those of MCF7 and MDA-MB-231. This may be due to the ability of synthesized compounds to blockade estrogen receptors in breast cancer cell lines. However, our results showed that some compounds (**11d**, **11e**, and **11h**) had similar cytotoxic activities on MDA-MB-231 cell line as well which indicated that other anti-cancer mechanisms may be involved in addition to blockade estrogen receptors in breast cancer cell lines. Moreover, our results indicated that among the synthesized compounds, **11e** and **11h** containing piperidino basic side chain with IC_{50}s of less than 0.1 μM on all three cell lines had the highest cytotoxic effects and were the most potent compounds in this group.

Docking study

Regarding the resemblance between the structures of the designed 2,4,5-triaryl-1*H*-imidazoles **11a–h** and the structure of tamoxifen and obtained MTT assay results, it could be

Table 1 Physicochemical properties of compounds 2,4,5-triarylimidazoles **11a–h**



Compound	X		MP °C	Yield%	Molecular formula	Molecular weight
11a	OH		147–149	36	$C_{25}H_{25}N_3O_2$	399.5
11b	F		172–173	30	$C_{25}H_{24}N_3OF$	401.5
11c	OMe		159–160	46	$C_{26}H_{27}N_3O_2$	413.5
11d	H		150–151	35	$C_{25}H_{25}N_3O$	383.5
11e	OH		225–227	20	$C_{28}H_{29}N_3O_2$	439.5
11f	F		188–190	26	$C_{28}H_{28}N_3OF$	441.5
11g	OMe		118–120	30	$C_{29}H_{31}N_3O_2$	453.6
11h	H		117–118	34	$C_{28}H_{29}N_3O$	423.5

assumed that one of the mechanisms of cytotoxic activities of compounds **11a–h** on breast cancer cell lines might be through acting on estrogen receptors. Therefore, the orientation of 4-(5-(4-(2-(dimethylamino)ethoxy)phenyl)-4-phenyl-1*H*-imidazol-2-yl)phenol **11a** in the estrogen receptor α (ERα) active site was examined by a docking experiment (Fig. 2). This molecular modeling study shows that compound **11a** was well bound in the active site of ERα so that the N atom of the tertiary amino group of the basic side chain (dimethylaminophenoxy), which can be protonized in physiologic pH, is in the vicinity of the oxygen of carboxylate group of Asp^{351} (distance = 4.7 Å) and is capable of

Table 2 In vitro cytotoxicity data of compounds **11a–h** using MTT assay

Compound	MDA-MB-231 IC_{50} (μM)	T47D IC_{50} (μM)	MCF-7 IC_{50} (μM)
11a	82 ± 6	63 ± 3	70 ± 5
11b	76 ± 4	61 ± 3	82 ± 2
11c	53 ± 2	28 ± 2	42 ± 4
11d	58 ± 4	50 ± 3	53 ± 4
11e	<0.1	<0.1	<0.1
11f	>100	61 ± 4	78 ± 4
11g	71 ± 2	54 ± 2	65 ± 4
11h	<0.1	<0.1	<0.1
Tamoxifen	55.5 ± 2.7	12.1 ± 0.9	25.1 ± 1.5

Experimental values represent the average for two experiments performed in triplicate along with the standard deviation (SD) between the assay values

binding to this amino acid. The OH group at the *para* position of C-2 phenyl ring is in the vicinity of ASP^{394} and Glu^{353} so that the H atom of OH group is in a 4.3 Å distance from the O atom of carboxylate group in Glu^{353} and the O atom of the OH group is in a 3.0 Å distance from H atom of the NH group in Arg^{394} (Barrett *et al*., 2008). These data together with biological results may explain that one of the mechanisms of cytotoxic activities of compounds **11a–h** on breast cancer cell lines might be through acting on estrogen receptors.

Experimental

Materials

All reagents were purchased from the Aldrich (USA) or Merck (Germany) Chemical Company and were used without further purifications.

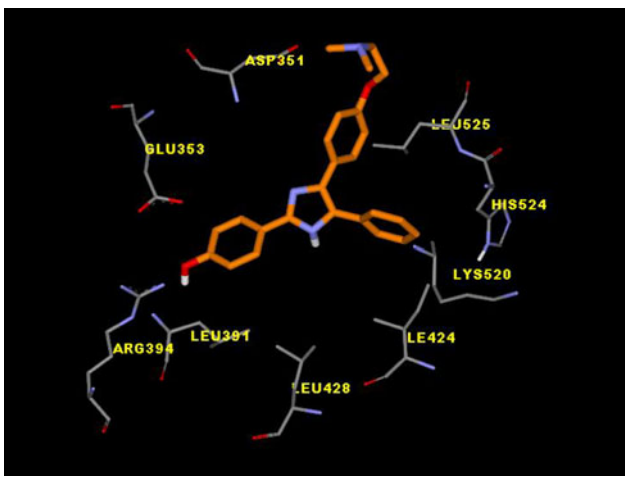


Fig. 2 Docking **11a** (*in orange*) in the active site of human ERα. Hydrogen atoms have been removed to improve clarity (Color figure online)

General

Melting points (mp) were determined using a Thomas Hoover melting point apparatus (Philadelphia, USA). Synthos 3000 microwave oven (Anton Paar, Austria) was used to synthesize the final compounds. Infrared spectra were acquired on a Perkin Elmer 1420 ratio recording spectrometer. A Bruker FT-500 MHz instrument (Brucker Biosciences, Germany) was used to acquire ^{1}HNMR spectra and chloroform-D was used as solvent. Coupling constant (*J*) values are estimated in hertz (Hz) and spin multiples are given as s (singlet), d (double), t (triplet), q (quartet), m (multiplet), and br (broad). The mass spectral measurements were performed on an 6410 Agilent LCMS triple quadrupole mass spectrometer (LCMS) with an electrospray ionization (ESI) interface. Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus (Perkin Elmer, Norwalk, CT, USA). The results of the elemental analyses (C, H, N) were within ± 0.4 % of the calculated amounts.

Preparation of 1-(4-hydroxyphenyl)-2-phenylethanone ***8***

Equimolar amounts of phenol and phenyl acetic acid were added to 40 ml of $BF_3{\cdot}Et_2O$ and stirred under Argon atmosphere in 0 °C for 15 min. The reaction temperature was then allowed to gradually reach the room temperature and subsequently the reaction mixture was refluxed in 75 °C for 20 h. After cooling, the reaction mixture was poured onto crushed ice, 20 ml of ethyl acetate was added and the organic phase was separated and washed primarily with water and afterward with saturated $NaHCO_3$ solution, dried with sodium sulfate and evaporated under vacuum. The oily residue was dissolved in DCM and precipitates formed by adding hexane drop wise. The precipitate was filtered and recrystallized in DCM to obtain white powder. Yield: 21 %; Mp: 148–150 °C; IR (KBr): υ (cm^{-1}) 3,340 (OH), 1,670 (C=O); MS *m/z* (%): 212.1 (M^+, 10), 181.0 (15), 165.0 (10), 152.0 (10), 120.9 (10), 93.1 (60).

Preparation of 1-(4-hydroxyphenyl)-2-phenylethan-1,2-dione ***9***

4 g (0.0188 mmol) of compound **8** was dissolved in 15 ml dioxane and added to a solution of 12 g SeO_2 in 50 ml dioxane and 10 ml water. The reaction mixture was refluxed for 24 h. After cooling, selenium was filtered off and the filtrate was poured onto crushed ice and extracted with ethyl acetate. The organic phase was washed with water and dried with sodium sulfate and evaporated under vacuum. The oily residue was dissolved in DCM and the precipitates were formed by adding hexane drop wise. The precipitate was filtered and recrystallized in DCM to obtain orange powder. Yield: 64 %; Mp: 127–129 °C; IR (KBr): υ (cm^{-1}) 3,385

(OH), 1,670, 1,645 (C=O); MS *m/z* (%): 226.1 (M^+, 10), 121.0 (100), 105.1 (75), 93.1 (70), 77.0 (85).

Preparation of 1-(4-(2-(piperidin-1-yl or dimethlamino) ethoxy)phenyl)-2-phenylethan-1,2-dione ***(10a, b)***

1 g (4.42) mmol of diketone **9** was dissolved in 40 ml acetonitrile and 5.8 g K_2CO_3 was added while stirring. Four equivalents of *N*-(2-chloroethyl)piperidinium chloride or *N*-(2-chloroethyl)-*N*,*N*-dimethylammonium chloride was added subsequently and the reaction mixture was refluxed for 24 h. The inorganic material was filtered off and the filtrate was evaporated under vacuum, dissolved in DCM and washed with brine. The organic phase was dried with sodium sulfate and evaporated. The oily residue was purified using column chromatography with a mobile phase of $CHCl_3$/*n*-hexane (9:1 v/v), to obtain a pure yellow viscous oil.

1-(4-(2-(Piperidin-1-yl)ethoxy)phenyl)-2-phenylethan-1, 2-dione ***10a***

Yield: 30 %; IR (CCl_4): υ (cm^{-1}) 1,715, 1,685 (C=O); LC–MS (ESI) *m/z* : 338.8 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 1.48–1.52 (m, 2H, piperidine CH_2), 1.65–1.69 (m, 4H, piperidine CH_2), 2.57–2.60 (m, 4H, piperidine CH_2), 2.86–2.88 (t, 2H, CH_2N, $J = 5.9$ Hz), 4.23–4.25 (t, 2H, OCH_2, $J = 5.9$ Hz), 6.99 (d, 2H, 1-phenyl H_3 and H_5, $J = 9.0$ Hz), 7.53–7.56 (m, 2H, 2-phenyl H_3 and H_5), 7.67–7.70 (t, 1H, 2-phenyl H_4, $J = 7.4$ Hz), 7.96 (d, 2H, 1-phenyl H_2 and H_6, $J = 9.0$ Hz), 8.00 (d, 2H, 2-phenyl H_2 and H_6, $J = 7.2$ Hz).

1-(4-(2-(Dimethlamino)ethoxy)phenyl)-2-phenylethan-1, 2-dione ***10b***

Yield: 32 %; IR (CCl_4): υ (cm^{-1}) 1,720, 1,680 (C=O); LC–MS (ESI) *m/z* : 298.9 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 2.39 (s, 6H, $N(CH_3)_2$), 2.80–2.82 (t, 2H, CH_2N, $J = 5.6$ Hz), 4.18–4.20 (t, 2H, OCH_2, $J = 5.6$ Hz), 7.03 (d, 2H, 1-phenyl H_3 and H_5, $J = 8.6$ Hz), 7.53–7.56 (m, 2H, 2-phenyl H_3 and H_5), 7.67–7.70 (t, 1H, 2-phenyl H_4, $J = 7.3$ Hz), 7.97 (d, 2H, 1-phenyl H_2 and H_6, $J = 8.6$ Hz), 8.01 (d, 2H, 2-phenyl H_2 and H_6, $J = 8.2$ Hz).

General procedure for preparation of 2,4,5-triaryl-1*H*-imidazoles **11a–h**

Equivalent amounts of diketone **10 (a** or **b)** and appropriate aromatic aldehyde along with 2 g ammonium acetate in 6 ml glacial acetic acid were placed in microwave reactor for 10 min, while the power was set at 180 W. After cooling, the solution was neutralized with aqueous ammonia in which the product precipitated immediately. The precipitate was filtered and washed with water and purified using column chromatography (Yields: 20–46 %).

4-(5-(4-(2-(Dimethylamino)ethoxy)phenyl)-4-phenyl-1H-imidazol-2-yl)phenol ***11a***

IR (KBr): υ (cm^{-1}) 3,500–2,500 (OH), 3,058 (NH); LC–MS (ESI) *m/z*: 400.8 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 2.27 (s, 6H, $N(CH_3)_2$), 2.66–2.68 (t, 2H, CH_2N, $J = 5.7$ Hz), 3.99–4.01 (t, 2H, OCH_2, $J = 5.7$ Hz), 6.77–6.79 (d, 4H, 2-phenyl H_3 and H_5 and 5-phenyl H_3 and H_5, $J = 8.7$ Hz), 7.12–7.15 (m, 1H, 4-phenyl H_4), 7.19–7.22 (m, 2H, 4-phenyl H_3 and H_5), 7.37 (d, 2H, 4-phenyl H_2 and H_6, $J = 8.6$ Hz), 7.47 (d, 2H, 5-phenyl H_2 and H_6, $J = 7.5$ Hz), 7.79 (d, 2H, 2-phenyl H_2 and H_6, $J = 8.6$ Hz), 9.32 (s, 1H, NH); Anal. Calcd. for. $C_{25}H_{25}N_3O_2$: C, 75.16; H, 6.31; N, 10.52. Found: C, 75.32; H, 6.65; N, 10.39.

2-(4-(2-(4-Fluorophenyl)-4-phenyl-1H-imidazol-5-yl)phenoxy)-N,N-dimethylethanamine ***11b***

IR (KBr) : υ (cm^{-1}) 3,050 (NH); LC–MS (ESI) *m/z* : 402.8 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 2.39 (s, 6H, $N(CH_3)_2$), 2.79–2.81 (t, 2H, CH_2N, $J = 5.4$ Hz), 4.12–4.14 (t, 2H, OCH_2, $J = 5.4$ Hz), 6.90 (d, 2H, 5-phenyl H_3 and H_5, $J = 8.0$ Hz), 7.12–7.15 (t, 2H, 2-phenyl H_3 and H_5), 7.29 (m, 1H, 4-phenyl H_4), 7.33–7.35 (m, 2H, 5-phneyl H_2 and H_6), 7.45 (m, 2H, 4-phenyl H_3 and H_5), 7.58 (m, 2H, 4-phenyl H_2 and H_6), 7.90–7.92 (t, 2H, 2-phenyl H_2 and H_6), 9.44 (s, 1H, NH). Anal. Calcd. for. $C_{25}H_{24}N_3OF$: C, 74.79; H, 6.03; N, 10.47. Found: C, 74.99; H, 6.25; N, 10.31.

2-(4-(2-(4-Methoxyphenyl)-4-phenyl-1H-imidazol-5-yl)phenoxy)-N,N-dimethylethanamine ***11c***

IR (KBr) : υ (cm^{-1}) 3,070 (NH); LC–MS (ESI) *m/z* : 414.8 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 2.37 (s, 6H, $N(CH_3)_2$), 2.77–2.80 (t, 2H, CH_2N, $J = 5.7$ Hz), 3.87 (s, 3H, OCH_3), 4.10–4.12 (t, 2H, OCH_2, $J = 5.7$ Hz), 6.88 (d, 2H, 5-phenyl H_3 and H_5, $J = 8.7$ Hz), 6.95 (d, 2H, 2-phenyl H_3 and H_5, $J = 8.7$ Hz), 7.25–7.28 (m, 1H, 4-phenyl H_4), 7.32–7.34 (m, 3H, 5-phenyl H_2 and H_6, and NH), 7.44 (d, 2H, 4-phenyl H_3 and H_5, $J = 7.6$ Hz), 7.56 (d, 2H, 4-phenyl H_2 and H_6, $J = 7.6$ Hz), 7.86 (d, 2H, 2-phenyl H_2 and H_6, $J = 8.7$ Hz); Anal. Calcd. for. $C_{26}H_{27}N_3O_2$: C, 75.52; H, 6.58; N, 10.16. Found: C, 75.66; H, 6.35; N, 10.22.

2-(4-(2,4-Diphenyl-1H-imidazol-5-yl)phenoxy)-N,N-dimethylethanamine ***11d***

IR (KBr) : υ (cm^{-1}) 3,065 (NH); LC–MS (ESI) *m/z* : 384.9 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 2.48 (s, 6H,

$N(CH_3)_2$), 2.89–2.91 (t, 2H, CH_2N, $J = 5.2$ Hz), 4.18–4.20 (t, 2H, OCH_2, $J = 5.2$ Hz), 6.91 (d, 2H, 5-phenyl H_3 and H_5, $J = 8.5$ Hz), 7.35–7.60 (m, 10H, 5-phenyl H_2 and H_6, 4-phenyl and 2-phenyl H_3–H_5), 7.97 (d, 2H, 2-phenyl H_2 and H_6, $J = 7.68$ Hz), 9.38 (s, 1H, NH); Anal. Calcd. for. $C_{25}H_{25}N_3O$: C, 78.30; H, 6.57; N, 10.96. Found: C, 78.61; H, 6.85; N, 11.12.

4-(5-(4-(2-Piperidin-1-yl)ethoxy)phenyl)-4-phenyl-1H-imidazol-2-yl)phenol ***11e***

IR (KBr): υ (cm^{-1}) 3,500–2,500 (OH), 3,056 (NH); LC–MS (ESI) *m/z* : 440.2 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 1.43–1.45 (m, 2H, piperidine CH_2), 1.60–1.64 (m, 4H, piperidine CH_2), 2.50–2.54 (m, 4H, piperidine CH_2), 2.78–2.81 (t, 2H, CH_2N, $J = 5.9$ Hz), 4.10–4.12 (t, 2H, OCH_2, $J = 5.9$ Hz), 6.80 (d, 2H, 2-phenyl H_3 and H_5, $J = 8.8$ Hz), 6.83 (d, 2H, 5-phenyl H_3 and H_5, $J = 8.7$ Hz), 7.19–7.21 (m, 1H, 4-phenyl H_4), 7.26–7.30 (m, 3H, 4-phenyl H_2 and H_6, NH), 7.42 (d, 2H, 4-phenyl H_3 and H_5, $J = 8.5$ Hz), 7.53 (d, 2H, 4-phenyl H_2 and H_6, $J = 7.4$ Hz), 7.81 (d, 2H, 5-phenyl H_2 and H_6, $J = 8.7$ Hz); Anal. Calcd. for. $C_{28}H_{29}N_3O_2$: C, 76.51; H, 6.65; N, 9.56. Found: C, 76.36; H, 6.45; N, 9.75.

1-(2-(4-(2-(4-Fluorophenyl)-4-phenyl-1H-imidazol-5-yl)phenoxy)ethyl)piperidine ***11f***

IR (KBr): υ (cm^{-1}) 3,050 (NH); LC–MS (ESI) *m/z*: 442.8 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 1.49–1.53 (m, 2H, piperidine CH_2), 1.65–1.69 (m, 4H, piperidine CH_2), 2.57–2.61 (m, 4H, piperidine CH_2), 2.85–2.87 (t, 2H, CH_2N, $J = 5.5$ Hz), 4.15–4.18 (t, 2H, OCH_2, $J = 5.5$ Hz), 6.90–6.94 (m, 2H, 5-phenyl H_3 and H_5), 7.15–7.18 (t, 2H, 2-phenyl H_3 and H_5, $J = 7.0$ Hz), 7.34–7.69 (m, 7H, 5-phenyl H_2 and H_6 and 4-phenyl), 7.91–7.94 (dd, 2H, 2-phenyl H_2 and H_6), 9.46 (s, 1H, NH); Anal. Calcd. for. $C_{28}H_{28}N_3OF$: C, 76.16; H, 6.39; N, 9.52. Found: C, 76.23; H, 6.54; N, 9.69.

1-(2-(4-(2-(4-Methoxyphenyl)-4-phenyl-1H-imidazol-5-yl)phenoxy)ethyl)piperidine ***11g***

IR (KBr): υ (cm^{-1}) 3,068 (NH); LC–MS (ESI) *m/z*: 454.7 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 1.48–1.51 (m, 2H, piperidine CH_2), 1.65–1.68 (m, 4H, piperidine CH_2), 2.55–2.58 (m, 4H, piperidine CH_2), 2.83–2.85 (t, 2H, CH_2N, $J = 5.5$ Hz), 3.90 (s, 3H, OCH_3), 4.16–4.18 (t, 2H, OCH2, $J = 5.5$ Hz), 6.79–6.80 (m, 2H, 5-phenyl H_3 and H_5), 7.00 (d, 2H, 2-phenyl H_3 and H_5, $J = 7.9$ Hz), 7.35–7.69 (m, 6H, 5-phenyl H_2 and H_6, 4-phenyl), 7.87 (d, 2H, 2-phenyl H_2 and H_6, $J = 8.0$ Hz), 9.31 (s, 1H, NH); Anal. Calcd. for. $C_{29}H_{31}N_3O_2$: C, 76.79; H, 6.89; N, 9.26. Found: C, 76.99; H, 7.02; N, 8.98.

1-2-(4-(2,4-Diphenyl-1H-imidazol-5-yl)phenoxy)ethyl) piperidine ***11h***

IR (KBr): υ (cm^{-1}) 3,078 (NH); LC–MS (ESI) *m/z* : 424.7 (M + 1) (100); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 1.55–1.58 (m, 2H, piperidine CH_2), 1.81–1.84 (m, 4H, piperidine CH_2), 2.78–2.83 (m, 4H, piperidine CH_2), 3.02–3.04 (m, 2H, CH_2N), 4.30–4.32 (m, 2H, OCH_2), 6.88 (d, 2H, 5-phenyl H_3 and H_5, $J = 7.5$ Hz), 7.37–7.59 (m, 10H, 5-phenyl H_2 and H_6, 4-phenyl and 2-phenyl H_3–H_5), 8.00 (d, 2H, 2-phenyl H_2 and H_6, $J = 7.6$ Hz), 8.88 (s, 1H, NH); Anal. Calcd. for. $C_{28}H_{29}N_3O$: C, 79.40; H, 6.90; N, 9.92. Found: C, 79.68; H, 6.77; N, 10.11.

Cytotoxicity studies

The anti-proliferative activities of the compounds were determined on three breast cancer cell lines using MTT assay. 1×10^4 cells/well were seeded in 198 µl RPMI (phenol red free) medium, supplemented with 10 % FBS in each well of 96-well micro culture plates and incubated for 24 h at 37 °C in a CO_2 incubator. 2 µl of the compounds, diluted to the desired concentrations in DMSO, were added to the wells with respective vehicle control. After 72 h of incubation, media were removed and to each well 20 µl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/ml) was added and the plates were further incubated for 4.5 h. Then, the supernatant from each well was carefully removed, formazan crystals were dissolved in 200 µl of DMSO and absorbance at 540 nm wavelength were recorded.

Molecular modeling (docking) studies

Docking studies were performed using Autodock Vina software (Trott and Olson, 2010). The coordinates of the X-ray crystal structure of the selective estrogen receptor modulator lasofoxifene bound to the human estrogen receptor α was obtained from the RCSB Protein Data Bank (2OUZ) and hydrogens were added, Kollman charge was calculated and non-polar hydrogens were deleted. The ligand molecule was constructed using the Hyperchem 8.0.3 and was energy minimized using AMBER MM force field, Polak–Ribier algorithm for 1,000 iterations reaching a convergence of 0.01 kcal/mol Å. A grid box of 24–24–24 Å with the central *X*–*Y*–*Z* coordinates of *X*: 30.6130 *Y*: −1.2140 *Z*: 27.6360 was built for calculation of the energy map. In the end of docking process, conformations having optimal docking energy were visualized using Viewer Lite 5.0 software, residues with atoms >7.5 Å from the docking box were removed for efficiency and the distances between atoms of ligand and amino acids in the active site were calculated. For docking validation, lasofoxifene was docked in the active

site of ERα with exactly similar conditions and the docked conformation having lowest docking energy was aligned with lasofoxifene in crystallography with ERα (2OUZ), using Pymol software and RMSD acquired was 1.74 Å showing that the docking method was valid (Ali *et al*., 2007).

Acknowledgments We like to thank Deputy of Research, School of Pharmacy, Shahid Beheshti University of Medical Sciences for financial support of this study as a part of PhD thesis of Sara Arfaei.

References

Ali HI, Ashida N, Nagamatsu T (2007) Antitumor studies. Part 3: design, synthesis, antitumor activity and molecular docking study of novel 2-methylthio-, 2-amino-, and 2-(*N*-substituted amino)-10-alkyl-2-deoxo-5-deazaflavins. Bioorg Med Chem 15:6336–6352

Barrett I, Meegan MJ, Hughes RB, Carr M, Knox AJS, Artemenko N, Golfis G, Zisterer DM, Lloyd DG (2008) Synthesis, biological evaluation, structural-activity relationship and docking study for a series of benzoxepin derived estrogen receptor modulators. Bioorg Med Chem 16:9554–9573

Brogden RN, Heel RC, Speight TM, Avery GS (1978) Metronidazole in anaerobic infections: a review of its activity, pharmacokinetics and therapeutic use. Drugs 16:387–417

Caturla F, Jimenez JM, Godessart N, Amat M, Cardenas A, Soca L, Beleta J, Ryder H, Crespo Ml (2004) Synthesis and biological evaluation of 2-phenylpyran-4-ones: a new class of orally active cyclooxygenase-2 inhibitors. J Med Chem 47:3874–3886

Dhingra K (1999) Antiestrogens: tamoxifen, SERMs and beyond. Invest New Drugs 17:285–311

Di Santo R, Tafi A, Costi R, Botta M, Artico M, Corelli F, Forte M, Caporuscio F, Angiolella L, Palamara AT (2005) Antifungal agents. 11. *N*-substituted derivatives of 1-[(aryl)(4-aryl-1*H*-pyrrol-3-yl)methyl]-1*H*-imidazole: synthesis, anti-candida activity, and QSAR studies. J Med Chem 48:5140–5153

Dickstein B, Valverius EM, Wosikowski K, Saceda M, Pearson JW, Martn MB, Mates SE (1993) Increased epidermal growth factor receptor in an estrogen-responsive, adriamycin-resistant MCF-7 cell line. J Cell Physiol 157:110–118

Ellis PA, Saccani-Jotti G, Clarke R, Johnston SRD, Anderson E, Howell A, A'Hern R, Salter J, Detre S, Nicholson R, Robertson J, Smith IE, Dowsett (1997) Induction of apoptosis by tamoxifen and ICI 182780 in primary breast cancer. Int J Cancer 72:608–613

Keydar I, Chen L, Karby S, Weiss FR, Delarea J, Radu M, Chaicik S, Brener HJ (1979) Establishment and characterization of a cell line of human breast carcinoma origin. Eur J Cancer 15:659–670

Mano T, Stevens RW, Ando K, Nakao K, Okumura Y, Sakakibara M, Okumura T, Tamura T, Miyamoto K (2003) Novel imidazole compounds as a new series of potent, orally active inhibitors of 5-lipoxygenase. Bioorg Med Chem 11:3879–3887

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Narasimhan B, Sharma D, Kumar P (2011) Biological importance of imidazole nucleus in the new millennium. Med Chem Res 20:1119–1140

Newman MJ, Rodarte JC, Benbatoul KD, Romano SJ, Zhang C, Krane S, Moran EJ, Uyeda RT, Dixon R, Guns ES, Mayer LD (2000) Discovery and characterization of OC144-093, a novel inhibitor of P-glycoprotein-mediated multidrug resistance. Cancer Res 60:2964–2972

Osborne CK (1998) Drug therapy: tamoxifen in the treatment of breast cancer. N Engl J Med 339:1609–1618

Robert J, Jarry C (2003) Multidrug resistance reversal agents. J Med Chem 46:4805–4817

Siciliano MJ, Barker PE, Cailleau R (1979) Mutually exclusive genetic signatures of human breast tumor cell lines with a common chromosomal marker. Cancer Res 39:919–922

Takle AK, Brown MJB, Davies S, Dean DK, Francis G, Gaiba A, Hird AW, King FD, Lovell PJ, Naylor A, Reith AD, Steadman JG, Wilson DM (2006) The identification of potent and selective imidazole-based inhibitors of B-Raf kinase. Bioorg Med Chem Lett 16:378–381

Trott O, Olson AJ (2010) AutoDock vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multi threading. J Comput Chem 31:455–461

Um SJ, Park SH, Park CH, Chang BH, Yoon JH, Sin HS (2003) Synthesis of novel quinoline carboxamide derivatives with estrogenic activity. Bull Korean Chem Soc 24:677–680

Wahala K, Hase TA (1991) Expedient synthesis of polyhydroxyflavonoids. J Chem Soc Perkin Trans 1 1:3005–3008

Wang L, Woods KW, Li Q, Barr KJ, McCroskey RW, Hannick SM, Gherke L, Credo RB, Hui YH, Marsh K, Warner R, Lee JY, Zielinsky-Mozng N, Frost D, Rosenberg SH, Sham HL (2002) Potent, orally active heterocycle-based combretastatin A-4 analogues: synthesis, structure—activity relationship, pharmacokinetics, and in vivo antitumor activity evaluation. J Med Chem 45:1697–1711

Wolkenberg SE, Wisnoski DD, Leister WH, Wang Y, Zhao Z, Lindsley CW (2004) Efficient synthesis of imidazoles from aldehydes and 1, 2-diketones using microwave irradiation. Org Lett 6:1453–1456

Zarghi A, Arfaei S, Ghodsi R (2012) Design and synthesis of new 2,4,5-triarylimidazole derivatives as selective cyclooxygenase (COX-2) inhibitors. Med Chem Res 21:1803–1810