Full Paper

Synthesis and Antioxidant Capacities of Some New Benzimidazole Derivatives

Gülgün Ayhan-Kılcıgil[1], Canan Kuş[1], Elçin D. Özdamar[2], Benay Can-Eke[2], and Mümtaz İşcan[2]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, Tandoğan, Ankara, Turkey
[2] Department of Toxicology, Faculty of Pharmacy, Ankara University, Tandoğan, Ankara, Turkey

In this study, we prepared some new oxadiazolyl benzimidazole derivatives and investigated their antioxidant properties by determination of microsomal NADPH-dependent inhibition of lipid peroxidation levels (LP assay) and microsomal ethoxyresorufin *O*-deethylase activity (EROD assay). Some of these compounds **20, 23** had slightly inhibitory effects (28%) on the lipid peroxidation levels at 10^{-3} M concentration lower than standard BHT (65%). 5-[2-(Phenyl)-benzimidazol-1-yl-methyl]-2-mercapto-[1,3,4]-oxadiazole **16** was found to be more active than caffeine on the ethoxyresorufin *O*-deethylase activity with an IC_{50} value of 2.0 6 10^{-4} M.

Keywords: Antioxidant activity / Benzimidazoles / Oxadiazoles

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Introduction

Many reports indicate that benzimidazoles have a variety of biological activities such as antimicrobial, antitubercular, anticancer, antihelmintic, antiallergic, and antioxidant. In our previous papers, we have already reported some antioxidant benzimidazole derivatives (Fig. 1). Furthermore, we have found that some compounds show potent antioxidant properties in various *in-vitro* systems. In this study, we synthesized some new compounds by modifying the triazole / thiadiazole ring to oxadiazole either at the N_1 position or at the C_5 position of the benzimidazole ring [1 – 5].

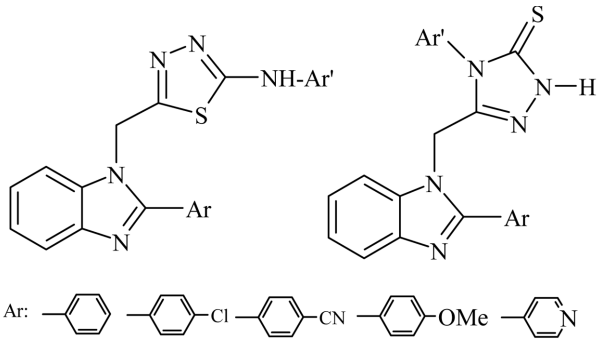


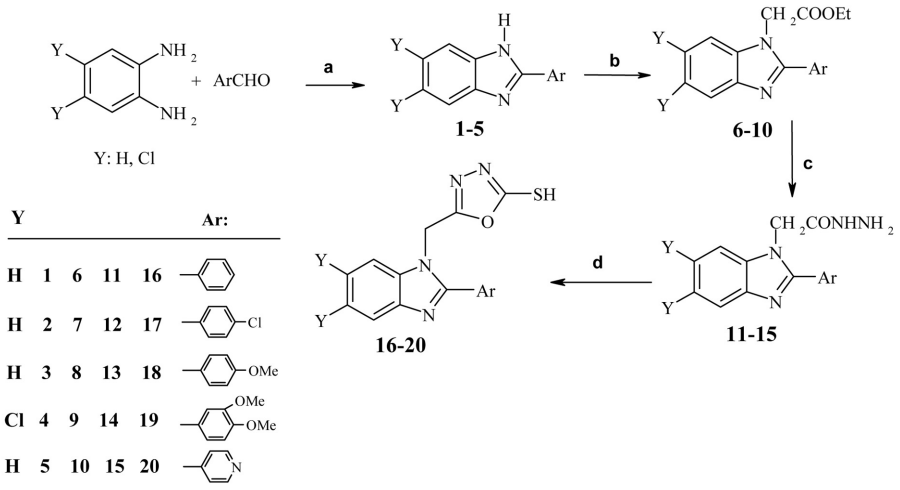
Figure 1. Structures of previously synthesized benzimidazole compounds.

Results and discussion

For the synthesis of the target compounds (**16 – 20, 23**) the reaction sequences outlined in Scheme 1 and Scheme 2, are the following. 1*H*-Benzimidazole derivatives **1 – 5** were prepared via oxidative condensation of *o*-phenylenediamine/4,5-dichloro-1,2-phenylenediamine, appropriate benzaldehyde derivatives, and sodium metabisulfite [6]. Treatment of 2-phenyl/substituted phenyl/pyridinyl-1*H*-benzimidazole with ethyl chloroacetate in KOH/DMSO

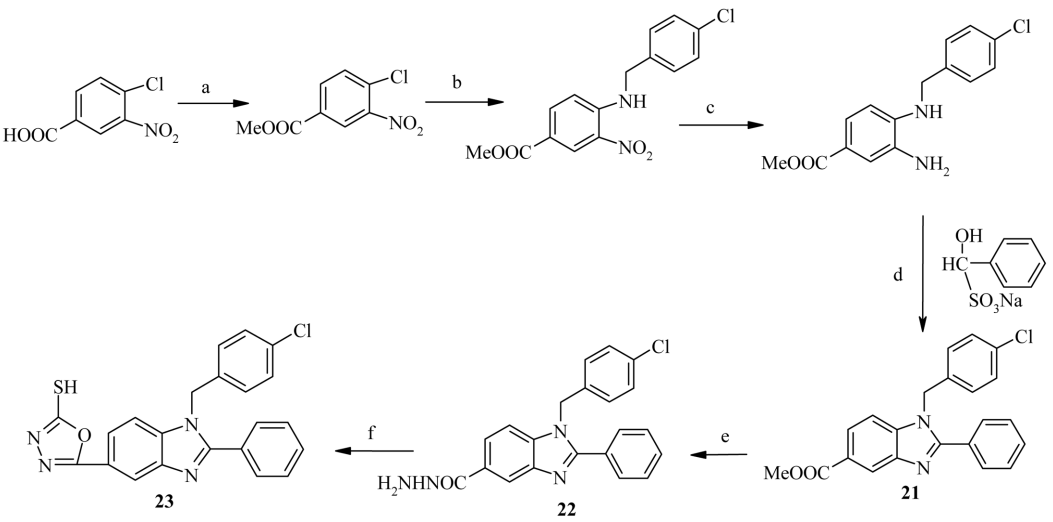
Correspondence: Gülgün Ayhan-Kılcıgil, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandoğan, Ankara, Turkey.
E-mail: kilcigil@pharmacy.ankara.edu.tr
Fax: +90 312 213-1081

Abbreviations: lipid peroxidation levels (LP assay); ethoxyresorufin *O*-deethylase activity (EROD assay); thiobarbituric acid reactant substances (TBARS)



Reagents: (a) $Na_2S_2O_3$ adduct of the related benzaldehyde or pyridinecarboxaldehyde/DMF; (b) Ethyl chloroacetate/KOH; (c) Hydrazine/EtOH; (d) CS_2/KOH.

Scheme 1. Synthesis of compounds **1**–**20**.



Reagents: (a) MeOH/H^+; (b) *p*-Chlorobenzylamine; (c) H_2, Pd/C; (d) $Na_2S_2O_3$ adduct of benzaldehyde/DMF; (e) Hydrazine/EtOH; (f) CS_2/KOH.

Scheme 2. Synthesis of compounds **21**–**23.**

gave the *N*-alkylated product, (2-aryl-benzimidazol-1-yl)-acetic acid ethyl esters **6**–**10** [7]. Hydrazine hydrate and the related esters **6**–**10** in ethanol were refluxed for 4 h to give the desired hydrazide compounds, (2-aryl-benzimidazol-1-yl)-acetic acid hydrazides **11**–**15**, in the range of 81-94% yields [8]. Cyclization of **11**–**15** with CS_2 and KOH [9] resulted to the formation of 5-[2-(phenyl/substituted phenyl/pyridinyl)-benzimidazol-1-yl-methyl]-2-mercapto-[1,3,4]-oxadiazoles **16**–**20**.

5-[1-(4-Chlorobenzyl)-2-phenyl-benzimidazol-1-yl-methyl]-2-mercapto-[1,3,4]-oxadiazole **23** was also obtained in the analogous way, starting from 3-nitro-4-chlorobenzoic acid. Nucleophilic displacement of the chlorine atom of methyl 3-nitro-4-chlorobenzoate by reaction with *p*-chlorobenzylamine in DMF gave methyl 4-(*p*-chlorobenzylamino)-3-nitro-benzoate. Its reduction with H_2 and Pd/C produced a 3-amino derivative. Condensation of methy 3-amino-4-(*p*-chlorobenzylamino)benzoate with the $Na_2S_2O_5$ adduct of benzaldehyde gave **21**. Subsequent reaction steps were carried out by the same route as **16**–**20** (Scheme 2).

We synthesized and analyzed the antioxidant properties of six new benzimidazole derivatives carrying 5-mercapto oxadiazole ring at the N-1 or C-5 position of benzi-

Table 1. The *in-vitro* effects of some compounds on liver LP levels[a].

Compounds[b]	Protein (nmol/mg)	% of Control
16	16.57 ± 0.18	102
17	16.57 ± 0.07	102
18	15.69 ± 0.05	97
19	17.00 ± 1.30	105
20	11.78 ± 0.85	72
23	11.78 ± 0.73	72
Control[c]	16.25 ± 1.45	100
BHT	5.68 ± 0.22	35

[a] Each value represents the mean ± S.D. of 2 – 4 independent experiments.
[b] Concentration in incubation medium 10^{-3} M.
[c] Dimethylsulfoxide only, control for compounds and BHT.

Table 2. The *in-vitro* effects of some compounds and caffeine on EROD activity in the liver[a].

Compounds	EROD ((pmol/mg)/min)	% of Control
16	4.31 ± 0.36	10
17	25.05 ± 1.80	60
18	17.87 ± 0.90	43
19	16.61 ± 0.73	40
20	27.41 ± 2.10	66
23	24.14 ± 1.91	58
Control[b]	41.53 ± 0.99	100
Caffeine	8.31 ± 0.42	20

[a] Each value represents the mean ± S.D. of 2 – 4 independent experiments.
[b] Dimethylsulfoxide only, control for compounds and caffeine.

midazole ring. The *in-vitro* effects of compounds **16 – 20, 23** and BHT on rat liver lipid peroxidation (LP) levels are shown in Table 1. BHT being a classical antioxidant compound was used as a positive control for comparison with benzimidazoles. Both compound **20** and **23** showed slightly inhibitory effects on LP levels at 10^{-3} M (28%). The inhibitory effect of BHT on LP was found to be approximately two-fold stronger (65%) than these compounds **20**, **23** (Table 1).

The *in-vitro* effects of compounds **16 – 20, 23** and caffeine on ethoxyresorufin *O*-deethylase activity (EROD) are shown in Table 2. As shown in Table 2, these compounds displayed various degrees on EROD, with decreasing activity in the following order: **20 > 17 > 23 > 18 ≅ 19 > 16**. Among them, 2-(non-substituted phenyl) benzimidazole derivative **16** was chosen for further experiments with 90% inhibition value at 10^{-3} M concentration. Compound **16** inhibited EROD activity with a 2.0×10^{-4} IC_{50} value while IC_{50} value of caffeine was 5.2×10^{-4}.

According to these results, the compounds bearing the oxadiazole ring at the N-1 position instead of C-5 position of benzimidazole ring exhibited good activities. Among the compounds bearing the oxadiazole ring at the N-1 position, the best activity was observed with compound **16** which has a non-substituted phenyl at the C-2 position of the benzimidazole ring. In addition, it appears that compounds **18** and **19** contain electron-donating substituents which allowed us to obtain the good EROD profile compared to compounds bearing electron-accepting groups (compound **17** and **20**).

The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined with Büchi SMP-20 (Büchi Labortechnik, Flawil, Switzerland) and Electrothermal 9100 capillary melting point apparatus (Electrothermal, Essex, U.K.) and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were measured with a Varian Mercury 400, 400 MHz instrument (Varian Inc., Palo Alto, CA, USA) using TMS internal standard and DMSO-d_6, coupling constants (*J*) are reported in Hertz. All chemical shifts were reported as δ (ppm) values. ESMS were obtained with a Waters ZQ Micromass LC-MS spectrometer (Waters Corporation, Milford, MA, USA) with Positive Electrospray Ionization Method. Elemental analyses (C, H, N, S) were determined on a Leco CHNS 932 instrument (Leco-932, St.Joseph, MI, USA), and were within ± 0.4% of the theoretical values. All instrumental analyses were performed at Ankara University, Faculty of Pharmacy. The chemical reagents used in synthesis were purchased from Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany) or Fluka (Germany). BHT and caffeine were obtained from Sigma (Sigma-Aldrich). Analytical thin layer chromatography was performed with Merck precoated TLC plates and spots were visualized with ultraviolet light. Compounds **1 – 3, 5 – 8, 10 – 13**, and **15** were synthesized according to the literature [1, 2, 4]. Methyl-[3-amino-4-(*p*-chlorobenzylamino)]benzoate (mp: 105°C) was synthesized according to the literature [10].

*5,6-Dichloro-2-[(3,4-dimethoxy)phenyl]-1H-benzimidazole **4***

The mixture of 4,5-dichloro-*o*-phenylendiamine (1 mmol) and sodium metabisulfite adduct of 3,4-dimethoxybenzaldehyde (1.25 mmol) in DMF were heated at 110°C for 5 h. Water was added, solid product was collected by filtration, washed with water, and crystallized from EtOH. Mp: 269°C, $C_{15}H_{12}Cl_2N_2O_2$, MS (ESI+) m/z (%) 323 (100) [M+H], 325 (100) [M+2], 327 (34) [M+4], ^{1}H-NMR (DMSO-d_6) δ: 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 7.16 (s, 1H, H-6′), 7.65 – 7.82 (m, 3H, H-4,7,5′), 7.89 (s, 1H, H-2′), 13.01 (s, 1H, NH).

*Ethyl 5,6-dichloro-2-(3,4-dimethoxyphenyl)-1H-benzimidazole acetate **9***

Dimethylsulfoxide (15 mL) was added to potassium hydroxide (1.5 g, 0.027 mol; crushed pellets) and the mixture was stirred for 15 min. 5,6-Dichloro-2-[3,4-dimethoxyphenyl]-1*H*-benzimidazole (6.7 mmol) was added and then the mixture was stirred 2 h.

Ethylchloroacetate (3 mL, 0.027 mol) was added and the mixture was cooled briefly and stirred for further 2 h. Water was added and the mixture was extracted with ether. Ether layers were washed with water, dried, and solvent and excess of ethylchloroacetate was removed under reduced pressure. The residue was recrystallized from ethanol and gave the desired ester compound **9**.

$C_{19}H_{18}Cl_2N_2O_4$, mp: 207–208°C, MS (ESI+) m/z (%) 409 (100) [M+H], 411 (64) [M+2], 413 (42) [M+4], ^{1}H-NMR (DMSO-d_6) δ: 1.13 (t, 3H, -H_2C-CH_3), 3.80 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.12 (q, 2H, -H_2C-CH_3), 5.35 (s, 2H, -N-CH_2), 7.16 (d, 1H, J_o = 8.2 Hz, H-5′), 7.28 (d, 1H, J_o = 8.2 Hz; H-2′), 7.3 (s, 1H, H-6′), 7.99 (s, 1H, H-4), 8.24 (s, 1H, H-7).

Methyl 1-(p-chlorobenzyl)-2-phenyl-1H-benzimidazole-5-acetate ***21***

The mixture of methyl-[3-amino-4-(*p*-chlorobenzylamino)]benzoate (1 mmol) and sodium metabisulfite adduct of benzaldehyde (1.25 mmol) in DMF were heated at 110°C for 5 h. Water was added, solid product was collected by filtration, washed with water and crystallized from MeOH. $C_{22}H_{17}ClN_2O_2$, mp: 144–146°C, MS (ESI+) m/z (%) 377 (100) [M+H], 379 (42) [M+2], ^{1}H-NMR (DMSO-d_6) δ: 3.84 (s, 3H, OCH_3), 5.62 (s, 2H, CH_2), 6.98–7.90 (m, 11H, Ar-H), 8.32 (d, 1H, J_m = 1.17 Hz, H-4).

General procedure for the preparation of ***14*** *and* ***22***

Hydrazine hydrate (4 mL) and related esters **9**, **21** (1.5 mmol) in ethanol (5 mL) were refluxed for 4 h. The reaction mixture was cooled and poured into water. The crude product was filtered off and recrystallized from ethanol to give the desired hydrazide compounds **14**, **22**.

5,6-Dichloro-2-(3,4-dimethoxyphenyl)-1H-benzimidazole acetic acid hydrazide **14**

$C_{17}H_{16}Cl_2N_4O_3$, mp: 265–266°C, MS (ESI+) m/z (%) 395 (100) [M+H], 397 (66) [M+2], 399 (12) [M+4], ^{1}H-NMR (DMSO-d_6) δ: 3.78 s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.4 (brs, 2H, NH_2), 4.87 (s, 2H, -CH_2), 7.10 (d, 1H, J_o = 8.0 Hz, H-5'), 7.31–7.35 (m, 2H-H-2‘,6′), 7.83 (s, 1H, H-4), 7.93 (s, 1H, H-7), 9.54 (s, 1H, NH).

1-(p-Chlorobenzyl)-2-phenyl-1H-benzimidazole-5-acetic acid hydrazide ***22***

$C_{21}H_{17}ClN_4O$, mp: 230–233°C, MS (ESI+) m/z (%) 377 (100) [M+H], 379 (36) [M+2], ^{1}H-NMR (DMSO-d_6) δ: 4.50 (s, 2H, NH_2), 5.62 (s, 2H, CH_2), 7.00 (d, 2H, J_o = 8.21 Hz, H-3″,5″), 7.35 (d, 2H, J_o = 8.21 Hz, H-2″,6″), 7.54–7.78 (m, 7H, H-6,7,2′,3′,4′,5′,6′), 8.22 (s, 1H, H-4), 9.78 (s, 1H, NH).

General procedure for the preparation of ***16–20*** *and* ***23***

Corresponding hydrazide compounds (0.4 mmol) and CS_2 (31 mg, 0.4 mmol) were added to a solution of KOH (22.4 mg, 0.4 mmol) in 1 mL of H_2O and 1 mL of ethanol. The reaction mixture was refluxed for 3 h. After evaporating under reduced pressure, a solid was obtained. This was dissolved in water and acidified with conc. HCl. The precipitate was filtered, washed with water, and recrystallized from ethanol.

5-(2-Phenyl-benzimidazol-1-yl-methyl)-2-mercapto-[1,3,4]-oxadiazole ***16***

Mp: 228°C, MS (ESI+) m/z (%) 309 (100) [M+H], ^{1}H-NMR (DMSO-d_6) δ 5.7 (s, 2H, -CH_2), 7.25–7.80 (m, 9H, Ar-H), Anal. ($C_{16}H_{12}N_4OS$ 6 0.25 C_2S 6 0.2 H_2O) C, H, N, S.

5-[2-(4-Chlorophenyl)-benzimidazol-1-yl-methyl]-2-mercapto-[1,3,4]-oxadiazole ***17***

Mp: 277°C dec., MS (ESI+) m/z (%) 343 (100) [M+H], 345 (48) [M+2], ^{1}H-NMR (DMSO-d_6) δ 5.72 (s, 2H, CH_2), 7.29–7.37 (m, 2H, H-5,6), 7.65–7.75 (m, 4H, H-2′,6′,4,7), 7.82 (d, 2H, J_o = 8.6 Hz, H-3′,5′), Anal ($C_{16}H_{11}ClN_4OS$ 6 0.7 H_2O) C, H, N, S.

5-[2-(4-Methoxyphenyl)-benzimidazol-1-yl-methyl]-2-mercapto-[1,3,4]-oxadiazole ***18***

Mp: 245–247°C, MS (ESI+) m/z (%) 339 (100) [M+H], ^{1}H-NMR (DMSO-d_6) δ 3,85 (s, 3H, -OCH_3), 5.68 (s, 2H, -CH_2), 7.14 (d, 2H, J_o = 8.99 Hz, H-3′,5′), 7.27–7.33 (m, 2H, H-5,6), 7.63–7.71 (m, 2H, H-4,7), 7.74 (d, 2H, J_o = 8.99 Hz, H-2′,6′). ^{13}C-NMR (DMSO-d_6) 56, 111, 115, 119, 122, 123.3, 123.5, 131, 136, 142, 153, 159, 161, 178. Anal ($C_{17}H_{14}N_4O_2S$) C, H, N, S.

5-[5,6-Dichloro-2-(3,4-dimethoxy)phenyl-benzimidazol-1-yl-methyl]-2-mercapto-[1,3,4]-oxadiazole ***19***

Mp: 250°C, MS (ESI+) m/z (%) 437 (100) [M+H], 439 (56) [M+2], 441 (14) [M+4], ^{1}H-NMR (DMSO-d_6) δ 3.81 (s, 3H, -OCH_3), 3.83 (s,3H, -OCH_3), 5.69 (s, 2H, -CH_2), 7.13 (d, 1H, J_o = 8.2 Hz, H-5′), 7.29–7.32 (m, 2H, H-2′,6′), 7.99 (s, 1H, H-4), 8.09 (s, 1H, H-7), Anal ($C_{18}H_{14}Cl_2N_4O_3S$ 6 0.1 H_2O) C, H, N, S.

5-[2-(4-Pyridinyl)-benzimidazol-1-yl-methyl]-2-mercapto-[1,3,4]-oxadiazole ***20***

Mp: 236°C bubl., ESI (+) [M+H] 310, ^{1}H-NMR (DMSO-d_6) δ 5.79 (s, 2H, -CH_2), 7.33–7.42 (m, 2H, H-5,6), 7.74 (d, 1H, J_o = 7.42 Hz, H-4), 7.79 (d, 1H, J_o = 7.42 Hz, H-7), 7.82 (dd, 2H, J_o = 4.69 Hz, J_m = 1.56 Hz, H-3′,5′), 8.79 (d, 2H, J_o = 5.86 Hz, H-2′,6′), Anal ($C_{15}H_{11}N_5OS$ 6 0.6 H_2O) C, H, N, S.

5-[1-(4-Chlorobenzyl)-2-phenyl-benzimidazol-1-yl-methyl]-2-mercapto-[1,3,4]-oxadiazole ***23***

Mp: 323°C, MS (ESI+) m/z (%) 419 (100) [M+H], 421 (43) [M+2], ^{1}H-NMR (DMSO-d_6) δ 5.65 (s, 2H, -CH_2), 7.00 (d, 2H, J_o = 8.59 Hz, H-3″,5″), 7.35 (d, 2H, J_o = 8.60 Hz, H-2″,6″), 7.54–7.56 (m, 3H, H-3′,4′,5′), 7.69–7.80 (m, 4H, H-6,7,2′,6′), 8.19 (s, 1H, H-4), Anal ($C_{22}H_{15}ClN_4OS$ 6 0.7 H_2O) C, H, N, S.

Biological evaluation – Antioxidant activity studies

Lipid peroxidation assay

Male albino Wistar rats (200–225 g) were used in the experiments. Animals were fed with standard laboratory rat chow and tab water *ad libitum*. The animals were starved for 24 h prior to sacrifice and then killed by decapitation under anesthesia. The livers were removed immediately and washed in ice-cold water and the microsomes were prepared as described previously [11].

NADPH-dependent lipid peroxidation (LP) was determined using the optimum conditions as determined and described previously [11]. NADPH-dependency was measured spectrophotometrically by estimation of thiobarbituric acid reactant substan-

ces (TBARS). Amounts of TBARS were expressed in terms of nmol malondialdehyde (MDA)/mg protein. The assay was essentially derived from the methods of Wills [12, 13] as modified by Bishayee [14]. A typical optimized assay mixture contained 0.2 nM Fe^{++}, 90 mM KCl, 62.5 mM potassium-phosphate buffer, pH 7.4, NADPH generating system consisting of 0.25 mM $NADP^+$, 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 1.0 U glucose-6-phosphate dehydrogenase, and 14.2 mM potassium phosphate buffer, pH 7.8, and 0.2 mg microsomal protein in a final volume of 1.0 mL.

EROD assay

7-Ethoxyresorufin-*O*-deethylase (EROD) activity was measured by the spectrofluorometric method of Burke *et al.* [15]. A typical optimized assay mixture contained 1.0 mM ethoxyresorufin, 100 mM Tris-HCl buffer, pH 7.8, NADPH generating system consisting of 0.25 mM $NADP^+$, 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 1.0 U glucose-6-phosphate dehydrogenase, and 14.2 mM potassium phosphate buffer, pH 7.8, and 0.2 mg liver microsomal protein in a final volume of 1.0 mL. EROD activity of compound **16** and caffeine was expressed as IC_{50} which determined from a calibration curve.

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