Journal of Enzyme Inhibition and Medicinal Chemistry, December 2007; 22(6): 696–701

Synthesis, antifungal and antioxidant screening of some novel benzimidazole derivatives

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(Received 18 July 2006; in final form 29 September 2006)

Abstract

Some novel benzimidazole derivatives were synthesized and their *in vitro* effects on rat liver microsomal NADPH-dependent lipid peroxidation (LP) level, ethoxyresorufin *O*-deethylase (EROD) and antifungal activities were determined. A significant decrease in male rat liver microsomal LP level was noted by compounds **4c** (52%), **4e** (58%) and **4h** (43%) at 10^{-3} M concentration. Compounds **4c** (100.0%), **4h** (100.0%)**,** **5c** (98.0%) and **5h** (100.0%) inhibited the microsomal ethoxyresorufin *O*-deethylase (EROD) enzyme activity better than that of the specific inhibitor **caffeine** (85%). Among these compounds, only compounds **4b** and **4h** exhibited moderate activity against *C.albicans* whereas the others had weak effects.

Keywords: *Thiosemicarbazides, thiadiazolylmethylbenzimidazoles, triazolylmethylbenzimidazoles, antimicrobial activity, antioxidant activity, lipid peroxidation*

Introduction

In recent years, there has been an increasing interest in molecular oxygen derived free radicals such as superoxide ($O_2^{-\cdot}$), hydroxyl ($OH^{\cdot}$), and peroxyl ($ROO^{\cdot}$) radicals, and hydrogen peroxide (H_2O_2) which are products of normal aerobic metabolic processes. Free radicals have been implicated in a variety of human diseases, ranging from atherosclerosis, to cancer, to neurodegenerative disorders [1,2]. These molecules are unstable and highly reactive and can damage cells by chemical chain reactions such as lipid peroxidation. On the other hand, all living organisms contain antioxidant enzymes systems and the major action of antioxidant enzymes systems/antioxidants in the cell is to prevent damage due to free radicals.

In addition, reactive oxygen species are produced by different mechanisms such as cytochrome P 450s (CYPs). CYPs are a superfamily of enzymes involved in the oxidation of numerous xenobiotics. For example, CYP1A1/2 activates polycylic aromatic hydrocarbons to their ultimate, mutagenic or carcinogenic metabolites. Moreover, CYP1A1/2, which catalyzes ethoxyresorufin *O*-deethylase (EROD) activity, is effective in producing reactive oxygen species [3].

Ketoconazole, a well known azole antifungal drug, was found to inhibit lipid peroxidation in both microsomal and liposomal systems. In addition, it was observed that the fungistatic effect of ketoconazole on *Candida* species was associated with its membrane stabilizing effects as indicated by inhibition of lipid peroxidation [4].

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ISSN 1475-6366 print/ISSN 1475-6374 online © 2007 Informa UK Ltd.
DOI: 10.1080/14756360701228558

Previously, we reported the synthesis, antioxidant [5–7] and antimicrobial [8,9] activities of benzimidazole derivatives and some of them have proved promising antioxidants *in vitro*.

As part of our ongoing research program on benzimidazoles we prepared a new series of benzimidazoles and determined their *in vitro* antifungal activities and antioxidant effects by measuring NADPH-dependent lipid peroxidation (LP) level and 7-ethoxyresorufin *O*-deethylase (EROD) activity from rat liver were.

Materials and methods

Chemistry

Melting points were determined with an Electrothermal (Electrothermal Eng. Ltd., Essex, UK) and a Büchi SMP-20 (Büchi Laboratoriumstechnik, Flawil, Switzerland) melting point apparatus and are uncorrected. IR spectra were recorded on a Jasco FT/IR 420 spectrometer (Jasco Corp., Tokyo, Japan) as potassium bromide discs. ^{1}H NMR spectra were measured with a Varian Mercury 400, 400 MHz instrument (California, USA) using TMS as internal standard and DMSO-d_6. All chemical shifts were reported as δ (ppm) values. ESMS were obtained with a Waters ZQ Micromass LC-MS spectrometer (Milford, USA) with Positive Electrospray Ionization method. Elemental analyses (C, H, and N) were determined on a Leco CHNS 932 instrument (St.Joseph, USA), and were within 0.4% of the theoretical values. ^{1}H NMR and Mass analyses were performed at Ankara University, Faculty of Pharmacy. Elemental analyses were performed at Scientific and Technical Research Council of Turkey. ATCC strains of the fungi were obtained from the culture collection of Refik Saydam Health Institution of Health Ministry, Ankara, Turkey. The chemical reagents used in synthesis were from E. Merck (Darmstadt, FRG) and Aldrich (Milwaukee, USA).

*General procedure for the preparation of the thiosemicarbazides (1-(substituted thiocarbamoyl-hydrazinecarbonyl) methyl 2-(4-cyanophenyl)-1H-benzimidazoles) (**4a-4ı**)*. 0.54 g Acid hydrazide (2.03 mmol) in absolute ethanol (20 mL) and appropriate isothiocyanate (3.05 mmol) were heated under reflux for 1 h. Precipitate formed was cooled, filtered and recrystallized from ethanol or isopropanol.

*General procedure for the preparation of the [5-(2-(4-cyanophenyl) benzimidazol-1-yl methyl)-[1,3,4]-thiadiazole-2-yl-substituted phenyl amines (**5a,5c,5e, 5f, 5h**)*. Appropriate thiosemicarbazide **4a, 4c, 4e, 4f, 4h** (3.4 mmol) in 10 mL ice-cold concentrated sulfuric acid was stirred for 10 min, and then left for a further 10 min at room temperature. The resulting solution was poured slowly into ice-cold water, made alkaline to pH 8 with aqueous ammonia and the precipitated product was filtered, washed with water and crystallized from ethanol.

Biological evaluation

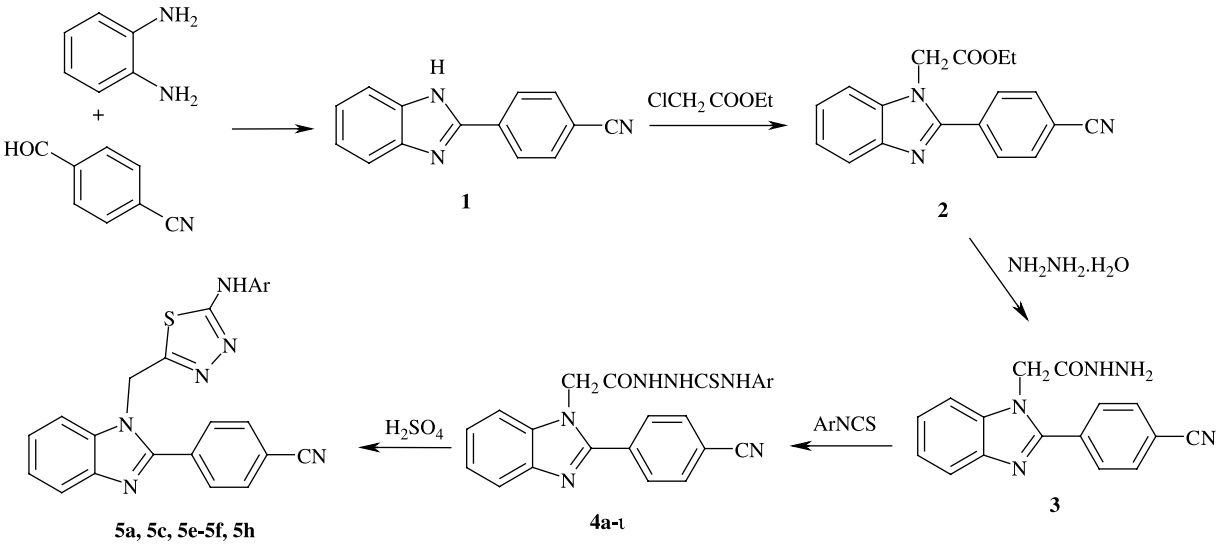
Antioxidant activity studies

Lipid peroxidation assay. Male albino Wistar rats (200–225 g) were used in the experiments. Animals were fed with standard laboratory rat chow and tap water *ad libitum*. The animals were starved for 24 h prior to sacrifice and then killed by decapitation under anaesthesia. The livers were removed immediately and washed in ice-cold water and the microsomes were prepared as described previously [10].

NADPH-dependent LP was determined using the optimum conditions determined and described previously [10]. NADPH-dependent LP was measured spectrophotometrically by estimation of thiobarbituric acid reactant substances (TBARS). Amounts of TBARS were expressed in terms of nmol malondialdehyde (MDA)/mg protein. The assay was essentially derived from the methods of Wills [11,12] as modified by Bishayee [13]. 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. [14]. 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.

Antifungal activity assay. Some of the compounds were tested for their antifungal activities by the agar diffusion method [15] using *C. albicans* ATCC 10231 and *C. krusei* ATCC 6258 as test organisms. A paper disc (8mm in diameter) was soaked in a 200 μg/ml solution of the test compounds in dimethylsulphoxide and placed on an agar plate containing fungi cells, which was incubated at 37°C



Scheme 1. Synthetic route for the preparation of the compounds.

for 24 h. The diameter of the growth inhibition zone around the paper disc was measured.

Results and discussion

For the synthesis of the target compounds the reaction sequences outlined in Scheme 1, were followed. 2-(4-Cyanophenyl)-1*H*-benzimidazole (M.P:265°C, Lit. M.P: 262.5°C [16]) was prepared via oxidative condensation of *o*-phenylenediamine, 4-cyanobenzaldehyde and sodium metabisulfite [17]. Treatment of 2-(4-cyanophenyl)-1*H*-benzimidazole with ethyl chloroacetate in KOH/DMSO gave the *N*-alkylated product, (2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid ethyl ester (2) [M.P:152°C, IR cm^{-1}: 2229; ^1H NMR 1.1 (t, 3H, CH_3), 4.09 (q, 2H, CH_2), 7.29-7.33 (m, 2H, H-5,6), 7.64 (d, 1H, H-7), 7.74 (d, 1H, H-4), 7.92 (d, 2H, H-3′,5′), 8.03 (d, 2H, H-2′,6′); Mass m/e 307 (M + H)]. Hydrazine hydrate and the ester (**2**) in ethanol were refluxed for 4 h to give the desired hydrazide compound, (2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid hydrazide (**3**), in 86% yield [M.P:277°C, IR cm^{-1}:2226; ^1H NMR 4.37 (br s, 1H, NH_2), 4.87 (s, 1H, -CH_2), 7.26-7.33 (m, 2H, H-5,6), 7.51 (d, 1H, H-7), 7.72 (d, 1H, H-4), 8.00-8.07 (m, 4H, H-2′,3′,5′,6′); Mass m/e 293 (M + H)]. The thiosemicarbazides (Scheme 1) were obtained upon reaction of the acid hydrazide with aryl isothiocyanates in ethanol. Cyclization with sulfuric acid resulted in the formation of 1-(5-substituted amino-1,3,4-thiadiazole-2-yl)benzimidazoles. Some physico-chemical properties and spectral data of the compounds are given in Table I.

In the IR spectra —C≡N stretching bonds were observed at 2222–2239 cm^{-1}. The conversion of thiosemicarbazides to thiadiazoles was monitored by the disappearance of C=O strong stretching bands at 1674–1732 cm^{-1} and the appearance of medium bands at 1592–1695 cm^{-1} for C=N stretching.

All of the compounds showed lower antifungal activity potencies than the control fluconazole against *C.albicans* and *C.krusei* (Table II). Among the investigated compounds **4b**, **4h** against *C.albicans* and **4b** and **5h** against *C.krusei* showed good activity. Compound **4h** exhibited the best activity against *C.albicans* which was close to fluconazole.

The NADPH-dependent lipid peroxidation inhibition produced by compounds in the rat liver microsomes was examined by measuring the formation of the 2-thiobarbituric acid reactive substances for their antioxidant capacity. A significant decrease in rat liver microsomal LP levels was determined for compounds **4c**, **4e** and **4h** at 10^{-3} M concentrations (Table III). The most active compound was found to be **4e** causing 58% inhibition of LP at a concentration of 10^{-3}M. The inhibition achieved by **4e** was very close to that obtained for butylated hydroxytoluene (BHT) (65%). Compounds **4c** and **4h** exhibited moderately inhibition (52% and 43%) of LP at 10^{-3}M. Compound **4a** (15%) showed rather limited inhibition of lipid peroxidation but compounds **4d** and **4f** enhanced LP by 16% and 86%, respectively.

The *in vitro* effect of compounds and caffeine on liver microsomal EROD activity are shown in Table IV. All the tested compounds except compound **4d** (75%) showed significant inhibition (62%–100.0%) of EROD activity. Compounds **4c, 4h, 4ı, 5c** and **5h** decreased liver EROD activity by 100.0%, 100.0%, 90%, 98.0% and 100.0%, respectively, better than the specific inhibitor caffeine (85%). Compound **4g** caused 81% inhibition which was close to caffeine. Significant inhibitory activities were also observed for

Table I. Physical and spectral data for the compounds.

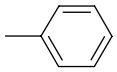
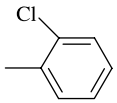
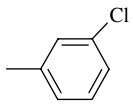
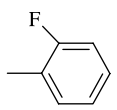
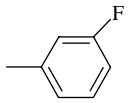
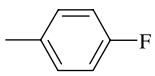
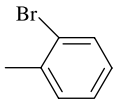
No	Ar	Formulas Calc. %C %H %N %S	Formulas Found %C %H %N %S	M.p (°C)	Yield (%)	^{1}H NMR data (ppm)	ES +
4a		$C_{23}H_{18}N_6OS.1.2H_2O$	61.64 4.58 18.75 7.15	61.61 4.56 18.74 7.14	164	48	5.07 (s, 2H, -CH_2), 7.31-8.04 (m, 13H, aromatic protons), 9.77, 10.37, 10.59 (s, 3H, CO*NHNH*CS*NH*)	427
4b		$C_{23}H_{17}ClN_6OS$	59.93 3.71 18.23 6.95	59.65 3.98 18.57 6.99	272	30	5.06 (s, 2H, -CH_2), 7.30-8.06 (m, 12H, aromatic protons), 9.61, 9.91, 10.63 (s, 3H, CO*NHNH*CS*NH*)	461
4c		$C_{23}H_{17}ClN_6OS.\ 0.8\ H_2O$	58.11 3.94 17.67 6.74	58.05 3.91 17.57 6.74	275	25	5.05 (s, 2H, -CH_2), 7.16-7.99 (m, 12H, aromatic protons), 10.43, 10.59, 11.20 (s, 3H, CO*NHNH*CS*NH*)	461
4d		$C_{23}H_{17}FN_6OS.\ 0.5\ (CH_3)_2CHOH$	62.01 4.46 17.71 6.75	61.96 4.42 17.74 6.74	190	20	5.05 (s, 2H, -CH_2), 7.21-8.01 (m, 12H, aromatic protons), 9.58, 9.94, 10.66 (s, 3H, CO*NHNH*CS*NH*)	445
4e		$C_{23}H_{17}FN_6OS.\ 1.0\ H_2O$	59.72 4.14 18.17 6.93	59.67 4.10 18.16 6.91	270	34	5.07 (s, 2H, -CH_2), 7.26-8.02 (m, 12H, aromatic protons), 9.92, 10.53, 10.76 (s, 3H, CO*NHNH*CS*NH*)	445
4f		$C_{23}H_{17}FN_6OS.\ 0.2\ (CH_3)_2CHOH$	62.09 4.11 18.40 7.02	62.03 4.60 18.43 7.02	257	19	5.04 (s, 2H, -CH_2), 7.16-8.03 (m, 12H, aromatic protons), 9.78, 9.89, 10.57 (s, 3H, CO*NHNH*CS*NH*)	445
4g		$C_{23}H_{17}BrN_6OS$	54.65 3.39 16.62 6.34	54.72 3.51 16.67 6.43	237	15	5.07 (s, 2H, -CH_2), 7.25-8.01 (m, 12H, aromatic protons), 9.81, 9.93, 11.22 (s, 3H, CO*NHNH*CS*NH*)	505, 507

Table I – *continued*

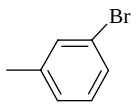
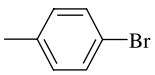
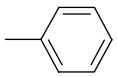
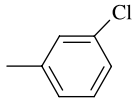
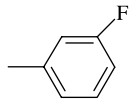
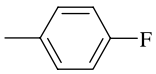
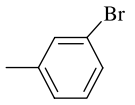
No	Ar	Formulas Calc. %C %H %N %S	Formulas Found %C %H %N %S	M.p (°C)	Yield (%)	^{1}H NMR data (ppm)	ES +
4h		$C_{23}H_{17}BrN_6OS.\ 1.5\ H_2O$	51.88 3.78 15.78 6.02	51.91 3.50 15.47 6.07	212	32	5.07 (s, 2H, -CH_2), 7.31-8.04 (m, 12H, aromatic protons), 9.61, 9.90, 10.63 (s, 3H, CO*NHNH*CS*NH*), ^{13}*C: 46, 111, 113, 119, 120, 121, 123.1, 123.3, 123.7, 123.9, 127.9, 128.4, 130.6, 130.7, 133.3, 135, 137, 141, 143, 152, 167*	505, 507
4i		$C_{23}H_{17}BrN_6OS$	54.65 3.39 16.62 6.34	54.68 3.20 16.45 6.78	185	20	5.07 (s, 2H, -CH_2), 7.30-8.07 (m, 12H, aromatic protons), 9.79, 9.88, 10.61 (s, 3H, CO*NHNH*CS*NH*), ^{13}*C:46, 111, 113, 119, 120, 123.3, 123.9, 130, 131, 133, 134, 137, 139, 142, 152, 167*	505, 507
5a		$C_{23}H_{16}N_6S.\ 0.2H_2O$	67.03 4.01 20.39 7.78	67.09 3.92 20.28 8.01	249	35	5.88 (s, 2H, -CH_2), 7.26-8.05 (m, 13H, aromatic protons), 10.31 (s, 1H, NH), ^{13}*C:43, 111, 113, 118, 119, 120, 122, 123, 124, 129, 130, 133, 134, 136, 140, 143, 151, 155, 165*	409
5c		$C_{23}H_{15}ClN_6S$	62.36 3.41 18.97 7.23	62.12 3.71 18.72 7.50	240	17	5.90 (s, 2H, -CH_2), 7.00-8.05 (m, 12H, aromatic protons), 10.53 (s, 1H, NH), ^{13}*C: 43, 111, 113, 116, 117, 119, 120, 122, 123, 124, 130, 131, 133, 134, 136, 142, 143, 151, 155, 165*	443
5e		$C_{23}H_{15}FN_6S.\ 0.5H_2O$	63.43 3.70 19.29 7.36	63.37 3.71 19.15 7.31	258	13	5.93 (s, 2H, -CH_2), 7.21-8.08 (m, 12H, aromatic protons), 10.58 (s, 1H, NH)	427
5f		$C_{23}H_{15}FN_6S.\ 0.5H_2O$	63.43 3.70 19.29 7.36	63.36 3.71 19.10 7.30	273	21	5.87 (s, 2H, -CH_2), 7.10-8.05 (m, 12H, aromatic protons), 10.33 (s, 1H, NH)	427
5h		$C_{23}H_{15}BrN_6S.\ 0.7H_2O$			254	10	5.90 (s, 2H, -CH_2), 7.13-8.05 (m, 12H, aromatic protons), 10.53 (s, 1H, NH)	487, 489

Table II. The *in vitro* antifungal activity of the compounds.

Compounds	Growth inhibition zone diameter (mm)*	
	C.albicans	*C.krusei*
4a	10	12
4b	15	15
4c	12	12
4d	10	9
4e	12	11
4f	10	9
4h	17	11
5a	10	10
5c	11	10
5e	8	13
5f	8	12
5h	11	15
Fluconazole	19	20

*: weak: <10, moderate:10–17, significant: >17.

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

Compounds[b]	nmol/mg protein	% of control
4a	13.81 ± 0.62	85
4c	7.80 ± 0.30	48
4d	18.85 ± 0.82	116
4e	6.82 ± 0.25	42
4f	30.23 ± 0.87	186
4h	9.27 ± 0.43	57
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 all of the tested compounds and BHT.

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

Compounds	EROD pmol/mg per min	% of control
4a	14.89 ± 018	36
4c	0.00 ± 0.00	0.0
4d	31. 21 ± 0.08	75
4e	13.76 ± 0.11	33
4f	15.69 ± 0.18	38
4g	8.01 ± 0.05	19
4h	0.00 ± 0.00	0.0
4ı	4.3 ± 0.02	10
5a	14.53 ± 0.05	35
5c	0.84 ± 0.04	2.0
5h	0.00 ± 0.00	0.0
Control[b]	41.53 ± 0.9898	100
Caffeine	6.40 ± 0.36	15

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

compounds **4a** (64%), **4e** (67%), **4f** (62%) and **5a** (65%). In this series of compounds, those bearing a m-chloro/bromo substituent in the Ar ring in either the thiosemicarbazide or thiadiazole series were the most active compounds.

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