

Synthesis and Antioxidant Properties of Some Novel Benzimidazole Derivatives on Lipid Peroxidation in the Rat Liver

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Some benzimidazole derivatives namely 1-[(substituted thiocarbamoylhydrazine carbonyl) methyl]-2-phenyl-1*H*-benzimidazoles (**1a-13a**), *N*-[(2-phenylbenzimidazol-1-yl methyl)-[1,3,4]-thiadiazole-2-yl]-substituted phenyl amines (**1b-13b**) and 5-(2-phenyl benzimidazol-1-yl-methyl)-4-substituted phenyl-4*H*-1,2,4-triazole-3-thiones (**1c-13c**) were synthesized, and their *in vitro* effects on the rat liver microsomal NADPH-dependent lipid peroxidation (LP) levels were determined. The most active compound **10a** caused an 84% inhibition of LP at 10⁻³ M, which is better than that of butylated hydroxytoluene (BHT) (65%).

Key words: Thiosemicarbazides, Thiadiazolylmethylbenzimidazoles, Triazolylbenzimidazoles, Antioxidant

INTRODUCTION

In recent years, there has been increasing interest in the role played by oxygen-derived free radicals such as the superoxide (O₂⁻), nitric oxide (NO[•]), hydroxyl (OH[•]) and peroxy (RO₂[•]) radicals in human diseases including atherosclerosis, rheumatoid arthritis and carcinogenesis (Rice-Evans and Diplock, 1991). Peroxyl radicals are formed during lipid oxidation chain reactions. Lipid peroxidation may be initiated by any species that possesses sufficient reactivity to abstract a hydrogen atom from a polyunsaturated fatty acid side chain in membrane lipids (Kappus, 1987). For free radicals to have any deleterious effect on the cellular components they must circumvent a host of antioxidant defenses that are aimed at scavenging specific species including enzymatic systems (superoxide dismutase, catalase) and both aqueous (glutathione-GSH and ascorbate) and non-aqueous scavengers (vitamin E). It is an imbalance between free radical production and antioxidant protection that characterizes many diseases states including autoimmune diseases (Griffiths *et al.*, 1998). Drugs possessing antioxidant and free radical scavenging properties have been considered for the prevention and/or treatment of

diseases that are directly related to the lack of an antioxidant capacity of the organisms.

It is well known that benzimidazoles exhibit antimicrobial (Abdel-Rahman *et al.*, 1983; Soliman *et al.*, 1984; Coburn *et al.*, 1987; Habib *et al.*, 1989; Kılıçgil *et al.*, 1999; Göker *et al.*, 2002), antitubercular (Khairnar *et al.*, 1980), anticancer (Kruse *et al.*, 1989; Islam *et al.*, 1991), antihelminthic (Habernickel, 1992), antiallergic (Fukuda *et al.*, 1984a; Fukuda *et al.* 1984b; Nakano *et al.*, 1999; Nakano *et al.*, 2000) and antioxidant (Can-Eke *et al.*, 1998) activities. In addition, the triazoles display anti-inflammatory (Boschelli *et al.*, 1993), antimicrobial (Shams El-Dine and Hazzaa, 1974; Habib *et al.*, 1989; Tsotinis *et al.*, 1997), antiviral (Witkowski *et al.*, 1973), and antioxidant (Andreadou *et al.*, 2002) activities. Incorporating the thiadiazoles, triazoles as well as their open-chain counterparts thiosemicarbazides in the 1st position of a benzimidazole ring is believed to yield more potent antioxidant compounds. Accordingly, the aim of this study was to synthesize compounds containing benzimidazoles and thiadiazoles and triazoles in addition to their open-chain analogs, thiosemicarbazides, in one frame and to determine their antioxidant properties.

MATERIALS AND METHODS

Chemicals

The melting points were determined using a Büchi

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SMP-20 melting point apparatus and are uncorrected. The IR spectra were recorded on a Jasco FT/IR 420 spectrometer from potassium bromide discs. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were measured on a Bruker GmbH DPX-400 400 MHz spectrometer using TMS as the internal standard and $\text{DMSO-}d_6$. All the chemical shifts were reported as δ (ppm) values. EIMS were obtained with a VG Platform II, Micromass spectrometer with an ionization energy maintained at 70 eV and FAB MS spectrum was taken on a Micromass ZSpec Mass Spectrometer by TUBITAK (Instrumental Analyse Lab., Gebze). Elemental analyses (C, H, N) were determined on a Leco CHNS 932 instrument (St. Joseph, USA), and were within $\pm 0.4\%$ of the theoretical values. All the instrumental analysis were performed at Scientific and Technical Research Council of Turkey. The chemical reagents used were purchased from E. Merck (Darmstadt, FRG) and Aldrich (Milwaukee, USA). 2-Phenyl-1*H*-benzimidazole (m.p. 290°C; Lit Jerchel *et al.*, 1954, m.p. 291°C), (2-phenyl-benzimidazol-1-yl)-acetic acid ethyl ester (m.p. 109°C; Lit Jerchel *et al.*, 1954, m.p. 110-111°C) and (2-phenylbenzimidazol-1-yl)-acetic acid hydrazide (m.p. 204°C; Lit Jerchel *et al.*, 1954, m.p. 203-204°C) were prepared in our laboratory.

Chemistry

Thiosemicarbazides (1-[(substituted thiocarbamoylhydrazinecarbonyl)methyl] 2-phenyl-1*H*-benzimidazoles) (1a-13a)

0.54 g acid hydrazide (2.03 mmol) in absolute ethanol

(20 mL) and appropriate isothiocyanate (3.05 mmol) were heated under reflux for 30 min. The precipitate formed was cooled, filtered and recrystallized from ethanol.

[5-(2-Phenylbenzimidazol-1-yl methyl)-[1,3,4]-thiadiazole-2-yl]-substituted phenyl amines (1b-13b)

The appropriate thiosemicarbazide **1a-13a** (3.4 mmol) in 10 mL ice-cold concentrated sulfuric acid was stirred for 10 minutes, then left to stand for another 10 minutes 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.

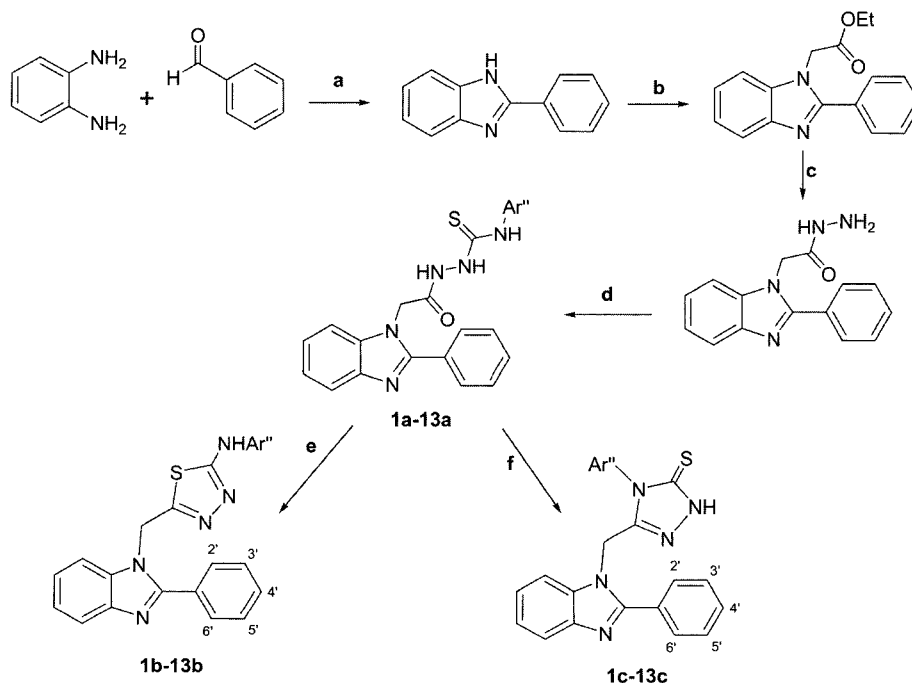
5-(2-Phenylbenzimidazol-1-yl methyl)-4-substituted phenyl-2,4-dihydro-[1,2,4]-triazole-3-thiones (1c-13c)

The appropriate thiosemicarbazide (3.4 mmol) **1a-13a** in 10 mL 1 N sodium hydroxide was refluxed for one hour. The reaction mixture was cooled and then acidified to pH 6 with 1 N hydrochloric acid. The precipitate was filtered, washed with water and recrystallized from ethanol.

Biological evaluation

Assay of lipid peroxidation

Male albino Wistar rats (200-225 g) were used in the experiments. The animals were fed with standard laboratory rat chow and tap water *ad libitum*. The animals were fasted for 24 h prior to sacrifice by decapitation under anesthesia. The livers were removed immedi-



Scheme 1. Synthetic route for the preparation of compounds **1a-13a**, **1b-13b**, and **1c-13c**.

a: $\text{Na}_2\text{S}_2\text{O}_5$, b: $\text{ClCH}_2\text{COOEt/KOH-DMSO}$, c: $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O/EtOH}$, d: appropriate phenylisothiocyanate, e: H_2SO_4 , f: NaOH .

ately and washed in ice-cold water and the microsomes were prepared, as described previously (Isacan *et al.*, 1984).

NADPH-dependent LP was determined using the optimum conditions determined and described previously

(Isacan *et al.*, 1984). NADPH-dependent LP was measured spectrophotometrically by estimated the thiobarbituric acid reactant substances (TBARS). The amounts of TBARS are expressed in terms of nmol malondialdehyde (MDA)/mg protein. The assay was essentially derived from the

Table I. Physical and spectral data of compounds **1a-13a**

No	Formulas	M.p (°C)	Yield (%)	¹ H-NMR data (δ ppm)	Mass data (70 eV)
1a	C ₂₂ H ₁₉ N ₅ OS	192-194	80	4.96 (s, 2H, CH ₂), 7.09-7.28 (m, 5H, NH-phenyl), 7.34-7.58 (m, 7H, H-5,6, phenyl), 7.76-7.79 (m, 2H, H-4,7), 9.43 (s, 2H, CONHNH), 10.45 (s, 1H, CSNH)	266 (M-ArNCS) (15.30), 207 (52.08), 129 (21.99), 103 (46.53), 77 (100), 70 (88.89), 58 (96.30)
2a	C ₂₃ H ₂₁ N ₅ OS	195-197	96	2.31 (s, 3H, CH ₃), 5.04 (s, 2H, CH ₂), 7.17 (d, 2H, J _o =8.15 Hz, H-3'', 5''), 7.26-7.33 (m, 4H, H-5, 6, 2'', 6''), 7.54-7.59 (m, 3H, H-3', 4', 5'), 7.72 (m, 2H, H-4, 7), 7.79-7.81 (m, 2H, H-2', 6'), 9.68 (br s, 2H, CONHNH), 10.55 (br s, 1H, CSNH)	266 (M-ArNCS) (2.45), 207 (15.95), 129(89.66), 42 (100)
3a	C ₂₃ H ₂₁ N ₅ OS	178-180	83	2.32 (s, 3H, CH ₃), 5.03 (s, 2H, CH ₂), 7.02 (s, 1H, H-2''), 7.23-7.59 (m, 8H, H-5, 6, 3', 4', 5', 4'', 5'', 6''), 7.70-7.80 (m, 4H, H-4, 7, 2', 6'), 9.74 (br s, 2H, CONHNH), 10.58 (br s, 1H, CSNH)	266 (M-ArNCS) (2.02), 207 (9.21), 129 (74.19), 103 (66.13), 77 (75.27), 59 (82.80), 42 (100)
4a	C ₂₃ H ₂₁ N ₅ OS	163-165 (bubl)	98	2.16 (s, 3H, CH ₃), 5.03 (s, 2H, CH ₂), 7.21-7.79 (13H, Ar-H), 9.53 (s, 1H, NH), 9.71 (br s, 1H, NH), 10.55 (br s, 1H, CSNH)	415 (M ⁺) (2.32), 266 (2.59), 232 (2.59), 207 (10.53), 129 (100), 103 (13.68), 77 (39.78), 59 (12.26)
5a	C ₂₂ H ₁₈ FN ₅ OS	188-190	65	5.03 (s, 2H, CH ₂), 7.22 (t, 2H, J _o = 8.80 Hz, H-3'', 5''), 7.27-7.32 (m, 2H, H-5, 6), 7.41-7.45 (m, 2H, H-2'', 6''), 7.52-7.58 (m, 3H, H-3', 4', 5'), 7.70-7.74 (m, 2H, H-4, 7), 7.79-7.80 (m, 2H, H-2', 6'), 9.82 (s, 2H, CONHNH), 10.53 (s, 1H, CSNH)	232 (5.23), 207 (4.88), 194 (9.15), 165 (12.96), 129 (20.53), 103 (27.24), 77 (21.34), 59 (29.27), 44 (100)
6a	C ₂₂ H ₁₈ FN ₅ OS	184-186	88	5.04 (s, 2H, CH ₂), 7.23-7.79 (m, 13H, Ar-H), 9.93 (s, 2H, CONHNH), 10.53 (s, 1H, CSNH)	208 (1.71), 207 (12.38), 129 (20.63), 103 (44.42), 77 (62.14), 70 (92.23), 58 (89.32), 44 (100)
7a	C ₂₂ H ₁₈ FN ₅ OS	185-187	72	5.02 (s, 2H, CH ₂), 7.20-7.79 (m, 13H, Ar-H), 9.61 (s, 1H, NH), 9.96 (s, 1H, NH), 10.63 (s, 1H, CSNH)	419(M ⁺) (4.06), 297 (3.52), 232 (4.06), 207 (4.45), 194 (4.79), 165 (3.86), 129 (6.32), 103 (10.77), 77 (29.52), 47 (100)
8a	C ₂₂ H ₁₈ ClN ₅ OS	192-194 (bubl)	71	5.03 (s, 2H, CH ₂), 7.26-7.32 (m, 2H, H-5,6), 7.42-7.51 (dd, 4H, J=8.63 Hz, H-2'', 3'', 5'', 6''), 7.56-7.58 (m, 3H, H-3', 4', 5'), 7.70-7.78 (m, 4H, H-4, 7, 2', 6'), 9.90 (s, 2H, CONHNH), 10.56 (s, 1H, CSNH)	435 (M ⁺) (0.15), 391 (3.02), 193 (10.42), 149 (96.15), 129 (11.22), 104 (9.21), 95 (9.13), 59 (100)
9a	C ₂₂ H ₁₈ ClN ₅ OS	178-179 (bubl)	67	5.05 (s, 2H, CH ₂), 7.25-7.79 (m, 13 H, Ar-H), 9.90 (br s, 2H, CONHNH), 10.6 (br s, 1H, CSNH)	266 (M-ArNCS) (2.30), 207 (9.12), 194 (2.28), 129 (8.39), 111 (10.03), 103 (16.72), 77 (30.67), 70 (100), 58 (56.40), 44 (87.79)
10a	C ₂₂ H ₁₈ ClN ₅ OS	175	86	4.95 (s, 2H, CH ₂), 7.08 (t, 1H, J _o =7.63 Hz, H-4''), 7.19-7.23 (m, 3H, H-5, 6, 6''), 7.33 (d, 1H, J _o =7.81 Hz, H-3''), 7.42-7.47 (m, 3H, H-3', 4', 5'), 7.69-7.71 (m, 2H, H-2', 6'), 7.78-7.98 (m, 3H, H-4, 7, 5''), 8.98 (s, 1H, NH), 9.30 (s, 1H, NH), 10.46 (s, 1H, CSNH)	266 (M-ArNCS) (15.73), 232 (15.72), 207 (80.65), 194 (50.00), 166 (24.90), 151 (9.78), 129 (72.98), 103 (60.89), 77 (100)
11a	C ₂₂ H ₁₈ BrN ₅ OS	193-195 (bubl)	66	5.03 (s, 2H, CH ₂), 7.25-7.32 (m, 2H, H-5,6), 7.44 (d, 2H, J _{2,3''} = J _{6,5''} = 8.36 Hz, H-2'', 6''), 7.52-7.59 (m, 5H, H-3', 4', 5', 3'', 5''), 7.69-7.78 (m, 4H, H-4, 7, 2', 6'), 9.89 (s, 2H, CONHNH), 10.57 (s, 1H, CSNH)	480(M ⁺) (4.39), 207 (6.90), 129 (9.42), 103 (4.32), 70 (88.57) 58 (69.71), 44 (100)
12a	C ₂₂ H ₁₈ BrN ₅ OS	179-182 (bubl)	82	5.04 (s, 2H, CH ₂), 7.26-7.78 (m, 13 H, Ar-H), 9.95 (s, 2H, CONHNH), 10.57 (s, 1H, CSNH)	266 (M-ArNCS) (6.81), 215 (16.57), 207 (71.54), 194 (14.33), 129 (7.93), 103 (18.39), 77 (39.43), 70 (96.75), 58 (64.23), 44 (100)
13a	C ₂₂ H ₁₈ BrN ₅ OS	178-180 (bubl)	98	5.04 (s, 2H, CH ₂), 7.25-7.78 (m, 13H, Ar-H), 9.64 (s, 1H, NH), 9.92 (s, 1H, NH), 10.60 (s, 1H, CSNH)	266 (M-ArNCS) (7.58), 207 (53.33), 129 (31.83), 103 (50.00), 77 (90.00), 70 (90.00), 59 (100), 58 (93.33)

methods reported by Wills (Wills,1966; Wills, 1969) and modified by Bishayee (Bishayee and Balasubramanian, 1971). A typical optimized assay mixture contained 0.2 nM Fe²⁺, 90 mM KCl, 62.5 mM potassium-phosphate

Table II. Physical and spectral data of compounds **1b-13b**

No	Formulas	M.p (°C)	Yield (%)	¹ H-NMR data (δ ppm)	Mass data (70 eV)
1b	C ₂₂ H ₁₇ N ₅ S	237-239	68	5.24 (s, 2H, CH ₂), 6.98-7.71 (m, 14H, Ar-H), 13.0 (s, 1H, NH)	292 (M-NHC ₆ H ₅) (11.49), 233 (48.85), 219 (20.55), 207 (11.06), 129 (20.26), 92 (10.63), 77 (100), 58 (51.72)
2b	C ₂₃ H ₁₉ N ₅ S	253-255	66	2.23 (s, 3H, CH ₃), 5.83 (s, 2H, CH ₂), 7.11 (d, 2H, J _o =8.33 Hz, H-3", 5"), 7.29-7.32 (m, 2H, H-5, 6), 7.41 (d, 2H, J _o =8.33 Hz, H-2", 6"), 7.59-7.61 (m, 3H, H-3', 4', 5'), 7.68-7.73 (m, 2H, H-4,7), 7.85-7.87 (m, 2H, H-2', 6'), 10.15 (br s, 1H, NH)	397 (M ⁺) (36.5), 291(25.3), 263(16.5), 232(34), 218(29), 204(17), 194(100), 163(18.8), 131(48), 129(25.5), 103(28), 91(94), 77(46)
3b	C ₂₃ H ₁₉ N ₅ S	197-199	7	2.24 (s, 1H, CH ₃), 5.62 (s, 2H, CH ₂), 6.73 (d, 1H, J _o =7.58 Hz, H-4"), 7.08 (t, 1H, J _o =7.73 Hz, H-5"), 7.20-7.29 (m, 4H, H-5, 6, 2", 6"), 7.46-7.52 (m, 7H, H-5,6, phenyl), 7.74-7.76 (m, 2H, H-4,7), 9.75 (br s, 1H, NH)	397 (M ⁺) (2.66), 291 (5.97), 265 (4.92), 250 (5.20), 232 (32.79), 219 (7.68), 194 (24.08), 163 (22.95), 102 (56.97), 91 (100), 77 (79.51)
4b	C ₂₃ H ₁₉ N ₅ S	225-226	42	2.20 (s, 3H, CH ₃), 5.81 (s, 2H, CH ₂), 6.98-7.87 (13H, Ar-H), 9.43 (br s, 1H, NH)	397 (M ⁺) (2.67), 263 (5.84), 251 (4.59), 232 (26.18), 207 (12.56), 194 (19.08), 131 (40.28), 104 (56.40), 91 (98.58), 77 (100), 58 (68.72)
5b	C ₂₂ H ₁₆ FN ₅ S	223-225	93	5.85 (s, 2H, CH ₂), 7.15 (t, 2H, H-3", 5"), 7.29-7.34 (m, 2H, H-5, 6), 7.55-7.60 (m, 5H, H-3', 4', 5', 2", 6"), 7.68-7.75 (m, 2H, H-4, 7), 7.85-7.87 (m, 2H, H-2', 6'), 10.25 (br s, 1H, NH)	401 (M ⁺) (6.96), 297 (30.63), 232 (33.13), 194 (55.83), 103 (69.17), 95 (38.13), 77 (71.67), 52 (100)
6b	C ₂₂ H ₁₆ FN ₅ S	233-235	34	5.87 (s, 2H, CH ₂), 6.80 (dd, 1H, J _{4',F} = 8.34 Hz, J _{4',5'} = 7.47 Hz, H-4"), 7.20 (dd, 1H, J _{2',F} = 8.16 Hz, J _m = 1.90 Hz, H-2"), 7.29-7.34 (m, 3H, H-5, 6, 6"), 7.57-7.60 (m, 4H, H-3', 4', 5', 5"), 7.69-7.75 (m, 2H, H-2', 6'), 7.84-7.87 (m, 2H, H-4,7), 10.20 (br s, 1H, NH)	401 (M ⁺) (2.36), 297 (2.95), 264 (14.73), 251 (21.23), 233 (30.48), 219 (49.66), 154 (53.77), 136 (42.47), 103 (71.58), 95 (80.82), 77 (55.82), 58 (100)
7b	C ₂₂ H ₁₆ FN ₅ S	238-240	65	5.86 (s, 2H, CH ₂), 7.02-8.24 (m, 13H, Ar-H), 10.14 (br s, 1H, NH)	401(M ⁺) (2.48), 291(72.89), 263 (34.04), 251 (38.55), 232 (51.81), 219 (100), 194 (17.32), 129 (29.82), 103 (37.50), 77 (40.96), 58 (93.37)
8b	C ₂₂ H ₁₆ ClN ₅ S	258-260	63	5.66 (s, 2H, CH ₂), 7.15 (d, 2H, H-3", 5"), 7.22-7.24 (m, 2H, H-5, 6), 7.49-7.51 (m, 5H, H-3', 4', 5', 2", 6"), 7.66-7.68 (m, 2H, H-4, 7), 7.75-7.77 (m, 2H, 2', 6'), 9.59 (br s, 1H, NH)	418 (M ⁺) (1.52), 232 (9.98), 194 (100), 129 (3.23), 103 (8.85), 76 (11.81), 63 (24.31), 58 (16.93)
9b	C ₂₂ H ₁₆ ClN ₅ S	228-230	87	5.87 (s, 2H, CH ₂), 7.02 (dt, 1H, J _o = 7.41 Hz, J _m = 1.75 Hz, H-4"), 7.28-7.37 (m, 4H, H-5, 6, 2", 6"), 7.58-7.61 (m, 3H, H-3', 4', 5'), 7.69-7.75 (m, 2H, H-2', 6'), 7.82-7.87 (m, 2H, H-4,7), 10.9 (br s, 1H, NH)	417 (M ⁺) (15.58), 419 (M+2) (5.90), 232(11.66) 194(100), 129 (5.60), 103 (29.10), 77(33.21), 58(27.99)
10b	C ₂₂ H ₁₆ ClN ₅ S	215-218	65	5.85 (s, 2H, CH ₂), 7.04-7.86 (m, 13H, Ar-H), 9.87 (br s, 1H, NH)	417 (M ⁺) (12.12), 419 (M+2) (4.17), 381 (2.40), 232 (50.0), 194 (100), 129 (25.61), 111 (44.82), 103 (60.98), 77 (90.24), 63 (76.83), 51(78.05)
11b	C ₂₂ H ₁₆ BrN ₅ S	266-268	53	5.86 (s, 2H, CH ₂), 7.29-7.32 (m, 2H, H-5, 6), 7.48 (d, 2H, J _{3',2'} =J _{5',6'} = 9.05 Hz, H-3", 5"), 7.53 (d, 2H, J _{2',3'} =J _{6',5'} = 9.05 Hz, H-2", 6"), 7.58-7.60 (m, 3H, H-3', 4', 5'), 7.68-7.74 (m, 2H, H-4,7), 7.84-7.87 (m, 2H, H-2', 6'), 10.46 (br s, 1H, NH)	359 (13.61), 291 (42.08), 264 (31.31), 251 (15.84), 232 (31.68), 219 (28.09), 196 (11.26), 166 (17.95), 129 (39.60), 103 (52.48), 77 (40.10), 58 (100)
12b	C ₂₂ H ₁₆ BrN ₅ S	251-253	71	5.87 (s, 2H, CH ₂), 7.15 (d, 1H, J _o =7.80 Hz, H-4"), 7.24-7.39 (m, 4H, H-5, 6, 2", 6"), 7.57-7.61 (m, 3H, H-3', 4', 5'), 7.68-7.75 (m, 2H, H-2', 6'), 7.84-7.98 (m, 3H, H-4, 7, 5"), 10.47 (br s, 1H, NH)	461 (M ⁺) (4.34), 463 (M+2) (4.48), 232 (9.71), 194 (100), 129 (11.70), 103 (63.83), 77 (45.21), 63 (66.67), 50 (86.52)
13b	C ₂₂ H ₁₆ BrN ₅ S	215-218	29	5.84 (s, 2H, CH ₂), 7.02-7.86 (m, 13H, Ar-H), 9.62 (br s, 1H, NH)	461 (M ⁺) (3.88), 463 (M+2) (3.43), 291 (2.78), 265 (2.83), 232 (33.52), 207 (21.02), 194 (55.30), 166 (24.81), 129 (17.61), 103 (83.33), 77 (60.61), 63 (77.27), 50 (100)

buffer, pH 7.4, a NADPH generating system consisting of 0.25 mM NADP⁺, 2.5 mM MgCl₂, 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 of the microsomal protein in a final volume of 1.0 mL.

RESULTS AND DISCUSSION

The reaction sequences for the synthesis of the target compounds is outlined in the Scheme 1, as follows: 2-Phenyl-1*H*-benzimidazole was prepared *via* the oxidative condensation of *o*-phenylenediamine, benzaldehyde and

Table III. Physical and spectral data of compounds **1c-13c**

No	Formulas	M.p (°C)	Yield (%)	¹ H-NMR data (δ ppm)	Mass data (70 eV)
1c	C ₂₂ H ₁₇ N ₅ S	267-269	67	5.61 (s, 2H, CH ₂), 6.80-7.76 (m, 14H, Ar-H), 9.7 (s, 1H, NH)	383 (M ⁺) (5.56), 306 (1.56), 232 (4.44), 207 (2.78), 77 (100)
2c	C ₂₃ H ₁₉ N ₅ S	284-285	57	2.33 (s, 3H, CH ₃), 5.44 (s, 2H, CH ₂), 6.97 (d, 2H, J _o =8.26 Hz, H-3", 5"), 7.15 (d, 2H, J _o =8.09 Hz, H-2", 6"), 7.24-7.27 (m, 2H, H-5, 6), 7.44 (m, 7H, H-4, 7, phenyl), 13.75 (br s, 1H, NH)	397 (M ⁺) (74.31), 399 (M+2) (2.64), 320 (1.73), 203 (71.96), 194 (100), 116 (9.42), 91 (34.55), 77 (57.64)
3c	C ₂₃ H ₁₉ N ₅ S	238-240	66	2.20 (s, 3H, CH ₃), 5.46 (s, 2H, CH ₂), 5.76 (s, 1H, H-2"), 6.82 (d, 1H, J _o =7.12 Hz, H-4"), 7.21-7.26 (m, 4H, H-5, 6, 5", 6"), 7.39-7.607 (m, 7 H, H-4,7, phenyl), 13.84 (br s, 1H, NH)	397 (M ⁺) (6.52), 293 (1.88), 234 (7.74), 217 (16.60), 203 (71.96), 194 (100), 129 (15.71), 103 (27.67), 91 (35.41), 77 (54.13)
4c	C ₂₃ H ₁₉ N ₅ S	282-284	44	1.50 (s, 3H, CH ₃), 5.38 (dd, 2H, CH ₂), 7.05 (d, 1H, J _o =7.80 Hz, H-3"), 7.10 (t, 1H, J _o =7.3 Hz, H-5"), 7.23-7.26 (m, 2H, H-5,6), 7.31 (dd, 1H, J _o =7.48 Hz, J _m =1.23 Hz, H-4"), 7.48-7.53 (m, 6H, H-4, 7, 3', 4', 5', 6"), 7.66-7.68 (m, 2H, H-2', 6"), 13.44 (br s, 1H, NH) ¹³ C-NMR: 17.4, 31.5, 111.8, 120.1, 123.2, 123.6, 128.0, 128.7, 129.4, 129.9, 130.1, 130.7, 131.1, 131.9, 132.3, 136.6, 136.8, 143.4, 148.3, 153.9, 169.1	397 (M ⁺) (8.5), 294 (70.3), 232 (100), 207 (52.47), 194 (66.36), 129 (62.96), 103 (39.20), 91 (61.73), 77 (83.95), 59 (65.74)
5c	C ₂₂ H ₁₆ FN ₅ S	272-273	82	5.37 (s, 2H, CH ₂), 6.82-6.84 (m, 2H, H-3", 5"), 6.90-6.94 (m, 2H, H-2", 6"), 7.18-7.24 (m, 2H, H-5,6), 7.29-7.65 (m, 7H, H-4,7, phenyl), 13.82 (br s, 1H, NH)	401 (M ⁺) (0.35), 194 (94.19), 103 (37.34), 77 (38.17), 63 (100)
6c	C ₂₂ H ₁₆ FN ₅ S	239-242	27	5.51 (s, 2H, CH ₂), 6.92-7.66 (m, 13 H, Ar-H), 13.27 (br s, 1H, NH)	401 (M ⁺) (1.59), 324 (3.66), 232 (21.65), 207 (89.69), 194 (100), 129 (29.64), 103 (50.0), 95 (52.58), 77 (55.41)
7c	C ₂₂ H ₁₆ FN ₅ S	279-281	49	5.47 (dd, 2H, CH ₂), 7.19-7.67 (m, 13 H, Ar-H), 13.98 (br s, 1H, NH)	308 (M-Ar") (0.33), 207 (4.69), 129 (23.30), 103 (38.64), 77 (50.85), 59 (100)
8c	C ₂₂ H ₁₆ ClN ₅ S	247-249	14	5.51 (s, 2H, CH ₂), 7.11 (dd, 2H, J _o =8.67 Hz, J _m =1.99 Hz, H-3", 5"), 7.25-7.27 (m, 2H, H-5, 6), 7.39 (dd, 2H, J _o =8.67 Hz, J _m =1.99 Hz, H-2", 6"), 7.51-7.67 (m, 7 H, H-4,7, phenyl), 13.95 (br s, 1H, NH)	417 (M ⁺) (35.56), 304 (1.95), 232 (9.44), 194 (86.20), 129 (5.81), 103 (13.34), 77 (14.18), 63 (17.95), 28 (100)
9c	C ₂₂ H ₁₆ ClN ₅ S	250	66	5.52 (s, 2H, CH ₂), 7.00-7.66 (m, 13 H, Ar-H), 13.92 (br s, 1H, NH)	417 (M ⁺) (3.65), 419 (M+2) (1.64), 223 (23.59), 194 (96.48), 129 (23.06), 103 (55.63), 77 (100), 59 (70.42)
10c	C ₂₂ H ₁₆ ClN ₅ S	310-312	48	5.29 (dd, 2H, CH ₂), 6.73-7.63 (m, 13 H, Ar-H), 13.80 (br s, 1H, NH)	417 (M ⁺) (6.64), 419 (M+2) (1.93), 381 (45.76), 232 (61.58), 207 (16.95), 149 (100), 129 (18.22), 103 (33.05), 77 (64.41), 59 (48.59)
11c	C ₂₂ H ₁₆ BrN ₅ S	256-258	48	5.51 (s, 2H, CH ₂), 7.03 (d, 2H, J _{3',2'} =J _{5',6'} =8.55 Hz, H-3", 5"), 7.25-7.27 (m, 2H, H-5,6), 7.43-7.68 (m, 9 H, H-4, 7, 2", 6", phenyl), 13.83 (br s, 1H, NH)	461 (M ⁺) (14.49), 463 (M+2) (12.56), 266 (1.66), 193 (100), 103 (10.0), 77 (42.05), 63 (36.41), 50 (19.36)
12c	C ₂₂ H ₁₆ BrN ₅ S	256-258	46	5.51 (s, 2H, CH ₂), 7.04-7.66 (m, 13 H, Ar-H), 13.87 (br s, 1H, NH)	461 (M ⁺) (11.34), 463 (M+2) (12.05), 266 (13.48), 232 (4.60), 194 (100), 129 (16.34), 103 (28.93), 77 (45.71), 63 (27.50), 50 (32.86)
13c	C ₂₂ H ₁₆ BrN ₅ S	280-282	38	5.44 (s, 2H, CH ₂), 7.24-7.71 (m, 13H, Ar-H), 13.68 (br s, 1H, NH)	382 (M-Br) (2.78), 244 (85.92), 192 (15.78), 149 (30.22), 134 (33.50), 103 (100), 77 (97.57), 63 (52.91), 50 (69.90) FAB : 461 (M ⁺) (95), 463 (M+1) (100)

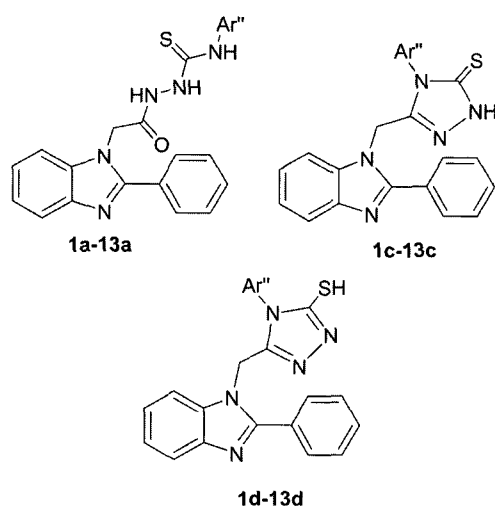


Fig. 1. Structures of **1a-13a**, **1c-13c**, and **1d-13d**

sodium metabisulfite (Ridley *et al.*, 1965). Treatment of 2-phenyl-1*H*-benzimidazole with ethyl chloroacetate in KOH/DMSO gave the *N*-alkylated product, (2-phenylbenzimidazol-1-yl)-acetic acid ethyl ester (Heaney and Ley, 1973). The hydrazine hydrate and the ester in ethanol was refluxed for 4 h to give the desired hydrazide compound, (2-phenylbenzimidazol-1-yl)-acetic acid hydrazide, in a 94% yield (Smith, 1949). The thiosemicarbazides (**1a-13a**) (Scheme 1) were obtained from a reaction of acid hydrazide with aryl isothiocyanates in ethanol (Siatra-Papastaikoudi *et al.*, 1995). Cyclization of compounds **1a-13a** with sulfuric acid or sodium hydroxide (Shams El-Dine and Hazzaa, 1974; Tsoinis *et al.*, 1997; Varvaresou *et al.*, 1998) resulted in the formation of 1-(5-substituted amino-1,3,4-thiadiazole-2-yl)benzimidazoles (**1b-13b**) and 1-(4-substituted-4*H*-1,2,4-triazole-5-thion-3-yl)benzimidazoles (**1c-13c**), respectively. Some of the physico-chemical properties and spectral data of the compounds are given in Tables I-III.

Although two types of tautomers (compounds **1c-13c** and **1d-13d**) could be expected from the cyclisation of compounds **1a-13a** under alkaline conditions, compounds **1c-13c** (thione form) were observed. This was demonstrated in the IR spectra by the presence of the two absorption maxima at 1316-1328 and 1272-1294 cm^{-1} belonging to the C=S group (Dziewońska, 1967). Moreover, the ^{13}C -NMR spectra of compound **4c** exhibited signals at 169.1 ppm, which were characteristic of the C=S bond. The conversions were monitored by the disappearance of C=O strong stretching bands of thiosemicarbazides at 1675-1680 cm^{-1} and the appearance of the medium bands at 1566-1608 cm^{-1} for the C=N stretching band in the IR spectra of the compounds **1c-13c** (Fig. 1).

The NADPH-dependent lipid peroxidation inhibition

Table IV. Effects of the compounds on the liver LP levels ^a *in vitro*

Compound ^b	Ar ^{''}	LP (nmol MDA/mg protein)	Percent of control
Control ^c		16.25 ± 1.45	100
1a	Phenyl	6.88 ± 0.25	42
1b	Phenyl	12.67 ± 0.72	78
1c	Phenyl	9.38 ± 0.08	58
2a	4-tolyl	8.24 ± 3.13	51
2b	4-tolyl	13.35 ± 2.33	82
2c	4-tolyl	8.36 ± 0.08	51
3a	3-tolyl	6.03 ± 0.64	37
3b	3-tolyl	14.60 ± 1.20	90
3c	3-tolyl	10.17 ± 0.40	63
4a	2-tolyl	8.30 ± 0.80	51
4b	2-tolyl	14.89 ± 3.21	92
4c	2-tolyl	9.72 ± 0.56	60
5a	4-fluorophenyl	7.10 ± 0.57	44
5b	4-fluorophenyl	11.88 ± 0.08	73
5c	4-fluorophenyl	8.35 ± 0.08	51
6a	3-fluorophenyl	5.97 ± 0.24	37
6b	3-fluorophenyl	15.63 ± 0.08	96
6c	3-fluorophenyl	7.22 ± 0.73	44
7a	2-fluorophenyl	3.70 ± 1.68	23
7b	2-fluorophenyl	11.54 ± 1.21	71
7c	2-fluorophenyl	11.42 ± 2.0	70
8a	4-chlorophenyl	7.44 ± 3.29	46
8b	4-chlorophenyl	14.60 ± 4.10	90
8c	4-chlorophenyl	10.34 ± 0.66	64
9a	3-chlorophenyl	3.53 ± 1.60	22
9b	3-chlorophenyl	15.06 ± 2.32	93
9c	3-chlorophenyl	7.11 ± 2.17	44
10a	2-chlorophenyl	2.68 ± 0.88	16
10b	2-chlorophenyl	10.91 ± 0.81	67
10c	2-chlorophenyl	10.46 ± 0.32	64
11a	4-bromophenyl	3.07 ± 0.01	19
11b	4-bromophenyl	16.02 ± 2.73	99
11c	4-bromophenyl	5.74 ± 0.24	35
12a	3-bromophenyl	3.52 ± 0.33	22
12b	3-bromophenyl	18.98 ± 2.73	117
12c	3-bromophenyl	6.20 ± 1.21	38
13a	2-bromophenyl	3.07 ± 0.02	19
13b	2-bromophenyl	32.61 ± 3.05	201
13c	2-bromophenyl	8.70 ± 0.88	54
BHT		5.68 ± 0.22	35

^aEach value represents the mean ± S.D. of 2-4 independent experiments

^bConcentration in incubation medium (M), 10^{-3}

^cDimethylsulfoxide only, control for compounds

produced by all new compounds in the rat liver microsomes was examined by measuring the formation of the 2-thiobarbituric acid reactive substances for their antioxidant capacity. As can be seen from Table IV, the thiosemicarbazides, **1a-13a**, exhibited stronger inhibitory effects on the LP levels than that of the thiadiazole **1b-13b**, and the triazole counterparts **1c-13c**. All of the thiosemicarbazides produced a >50% inhibition of the LP levels at concentrations of 10^{-3} M in the liver (Table IV). Moreover, some of them, compounds **7a**, **9a**, and **11a-13a**, which bore the *o*-fluoro, *m*-, and *o*-chloro and *p*-, *m*-, and *o*-bromo phenyl groups, respectively, as Ar" substituents demonstrated a higher activity than that of the butylated hydroxytoluene (BHT) used as a positive control. The most active compound is **10a**, which causes an 84% inhibition of LP at this concentration. This inhibition is better than that of the BHT. Compounds **3a** and **6a** caused a 63% inhibition of LP at concentrations of 10^{-3} M, which is comparable to that obtained from BHT. In these three series of compounds, the thiosemicarbazides particularly those bearing an *o*-halogen substituent at the Ar ring were the most active compounds. For thiadiazole compounds, **1b-11b** showed rather limited inhibition of lipid peroxidation but compounds **12b** and **13b** enhanced LP by 17% and 101%, respectively. In the triazole series, compounds **11c** and **12c** had a stronger inhibitory effects on the liver LP levels, by approximately 65% and 62%, respectively, than the others, which is similar to that of BHT (65%). Thus, this feature of the synthesized compounds particularly the thiosemicarbazide derivatives are likely to render promising antioxidant compounds.

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