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Research Letter

Synthesis and Evaluation of Antioxidant Properties of Novel 2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(2-arylmethylene amino) acetamides and 2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(4-oxo-2-aryl-thiazolidine-3-yl) acetamides-I

Gülgün Ayhan-Kılıcıgil[1*], Selen Gürkan[1], Tülay Çoban[2], Elçin Deniz Özdamar[2] and Benay Can-Eke[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ankara, 06100 Tandogan, Ankara, Turkey
[2]Department of Pharmaceutical Toxicology, Faculty of Pharmacy, University of Ankara, 06100 Tandogan, Ankara, Turkey
**Corresponding author: Gülgün Ayhan-Kılıcıgil, kilcigil@pharmacy.ankara.edu.tr*

Our approach was to synthesize and examine the antioxidant properties of some new 2-[2-(4-chlorophenyl)benzimidazole-1-yl]-N-(2-arylmethyleneamino) acetamide (1–18) and 2-[2-(4-chlorophenyl)benzimidazole-1-yl]-N-(4-oxo-2-aryl-thiazolidine-3-yl)acetamide (1t–18t) derivatives. Their *in vitro* effects on rat liver microsomal NADPH-dependent lipid peroxidation levels (LP assay) and microsomal ethoxyresorufin O-deethylase activities (EROD assay) were determined. The free radical scavenging properties of the compounds were also examined *in vitro* determining the interaction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The compounds showed significant effects in the above tests.

Key words: antioxidant activity, benzimidazole, methyleneamino acetamide, thiazolidine

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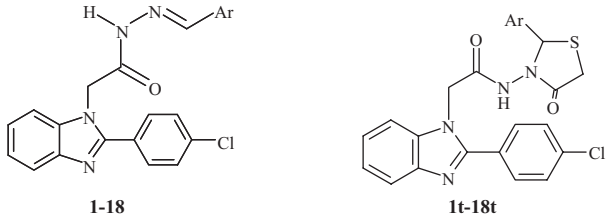
Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated in aerobic organisms as part of the normal physiological and metabolic processes or from exogenous factors and agents. These ROS are capable of damaging a wide range of essential macromolecules (membrane lipids, proteins, nucleic acids). The damages by ROS are directly or indirectly implicated in the pathogenesis of various disorders such as cardiovascular diseases, Alzheimer's and other neurodegenerative diseases, cancer, and atherosclerosis (1,2). The interest for the protective role of antioxidants has been growing over the past 15 years. Antioxidants are considered as potential drugs because of their ability to delay or prevent the oxidation of cellular oxidizable substrates by ROS/RNS. The human antioxidant protections include enzymes such as catalase, superoxide dismutase, and glutathione peroxidase as well as some water- and lipid-soluble antioxidant vitamins such as ascorbic acid (vitamin C) and α-tocopherol (vitamin E) (1–5).

It is known that lipid peroxidation is a free radical chain reaction that causes the degeneration of the cell membranes. Most products of lipid peroxidation are known to have mutagenic and/or carcinogenic properties. On the other hand, the antioxidants and antioxidant enzyme systems are major protective systems of organisms. Therefore, drugs possessing antioxidant and free radical scavenging properties are considered for preventing and/or treatment of such diseases that are directly related to the lack of antioxidant capacity of organism. Reactive oxygen species are produced by different mechanisms such as cytochrome P450 (CYP)-dependent enzymes that metabolize chemicals and endogenous substances to produce reactive oxygen species. In this system, CYP1A1/2 have to play important role in NADPH-dependent lipid peroxidation (LP). Thus, it is important to evaluate the effects of synthesized compounds on NADPH-dependent lipid peroxidation and CYP system (5). On the other hand, it is suggested that the DPPH assay is an accurate method with regard to measuring the antioxidant capacity of various compounds (6).

It is well known that benzimidazoles have a variety of biological activities such as antimicrobial (7–10), anticancer (11,12), antihelminthic (13), antiallergic (14,15), and antioxidant (16). In addition, the thiazolidinones display anticonvulsant (17), anticancer (18), antimicrobial (19), and antioxidant (20) activities.

Previously, we reported the synthesis, characterization, and antioxidant properties of some benzimidazole derivatives containing thiadiazole, triazole, and oxadiazole rings at the 1st position

(21–28). The aim of this work was to modify the structure of either the arylmethyleneamino (**1–18**) or aryl thiazolidine (**1t–18t**) acetamides and to continue searching for improved ROS and RNS scavengers.



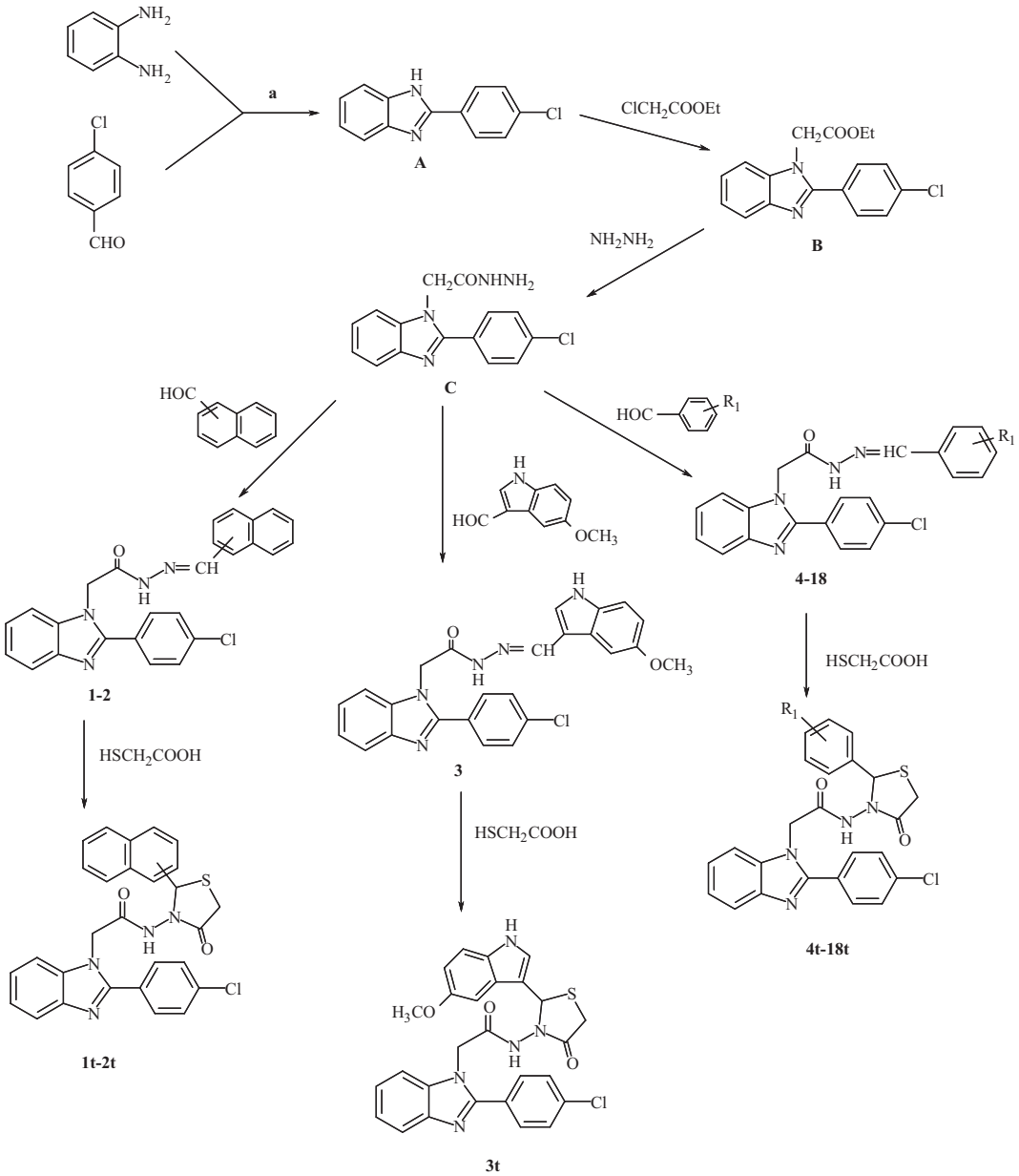
1-18 **1t-18t**

General formulas of the synthesized compounds

Material and Methods

Chemistry

Melting points were determined with a Thermo Scientific Electrothermal melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-spectra were measured with a Varian Mercury 400 MHz instrument using TMS internal standard and DMSO-d_6; coupling constants (J) are reported in Hertz. All chemical shifts were reported as δ (ppm) values. ES–MS were obtained with a Waters ZQ Micromass LC–MS spectrometer with positive electrospray ionization method. Elemental analyses (C, H, N, and S) were determined on a Leco CHNS 932 instrument and were within ±0.4% of the theoretical values. Compounds **A**, **B**, **C,** and 5-methoxy-indole-3-carboxaldehyde were previously prepared in our laboratory (23,29).



Scheme 1: Synthetic pathways to compounds **1–18** and **1t–18t.**

All instrumental analyses were performed at the Central Laboratory, Ankara University, Faculty of Pharmacy. The chemical reagents used in synthesis were purchased from E. Merck and Aldrich. Butylated hydroxy toluene (BHT) and caffeine were obtained from Sigma. Analytical thin-layer chromatography was performed with Merck precoated TLC plates, and spots were visualized with ultraviolet light.

General procedure for the preparation of (E)/(Z)-2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(2-arylmethylene amino) acetamides (1–18)

A mixture of acyl hydrazide (0.02 mol), corresponding aldehyde derivatives (0.02 mol), and ceric ammonium nitrate (0.05 mol) in ethanol (10 mL) was heated under reflux with stirring for 30 min. Water was added, and the precipitated product was filtered and crystallized from ethanol.

General procedure for the preparation of 2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(4-oxo-2-aryl-thiazolidine-3-yl) acetamides (1t–18t)

A mixture of the hydrazone **1–18** (0.1 mmol), thioglycolic acid (0.2 mmol), and anhydrous sodium acetate in dry toluene was refluxed for 10 h, and the mixture was then filtered while hot. The filtrate was evaporated under reduced pressure, and the obtained solid was crystallized from ethanol.

Antioxidant activity

Lipid peroxidation level

Male albino Wistar rats (200–225 g) used in the experiments were fed with standard laboratory rat chow and tap water *ad libitum*. The animals were starved for 24 h prior to kill and then killed by decapitation under anesthesia. The livers were removed immediately and washed in ice-cold distilled water, and the microsomes were prepared as described previously (30). NADPH-dependent LP was determined using the optimum conditions determined and described previously (30) and measured spectrophotometrically by estimation of thiobarbituric acid-reactive 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 (31,32) as modified by Bishayee (33). Lipid peroxidation was determined spectrophotometrically at 532 nm as the thiobarbituric acid-reactive material. Compounds inhibit the production of malondialdehyde, and therefore, the produced color after addition of thiobarbituric acid is less intensive. A typical optimized assay mixture contained 10^{-3} M test compound, 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.

7-Ethoxyresorufin O-deethylase enzyme activity

7-Ethoxyresorufin O-deethylase (EROD) activity was measured by the spectrofluorometric method of Burke *et al.* (34). A typical optimized assay mixture contained 1.0 mM ethoxyresorufin, 10^{-3} M test compound, 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.

2,2-Diphenyl-1-picrylhydrazyl free radical scavenging activity

The radical scavenging assay was determined by the modified method described by Blois (35). BHT and stock solutions of the compounds were prepared at 10^{-2} M in DMSO. A series of solutions in DMSO were diluted to varying concentrations in 96-well microplates. Then, methanolic DPPH solution (100 μM) was added to each well. The plate was shaken and placed in the dark. After 30 min, the optical density (OD) of the solution was read at the 517 nm. The methanolic solution of DPPH served as a control. Percentage inhibition was calculated using the following formula:

$$\%\ \text{Inhibition} = (OD_{control} - OD_{sample})/OD_{control} \times 100$$

where $OD_{control}$ is the absorbance of the control with DMSO and OD_{sample} is the absorbance of the sample in the presence of the

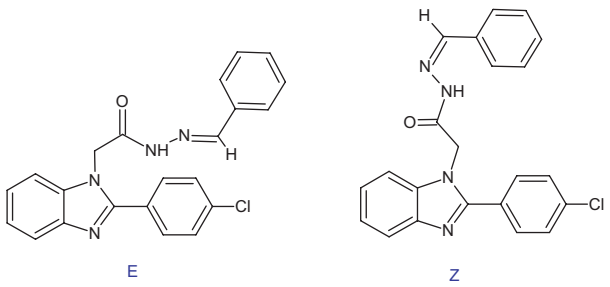


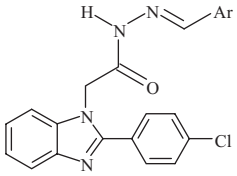
Figure 1: Representation of E and Z configuration of compound **4.**

Table 1: ^{1}H-NMR data for diastereomeric imines in the (E)/(Z) diastereoisomers

Compound	δ ppm–N=CH- (E)	δ ppm–N=CH- (Z)	Diastereoselection Ratio (E)/(Z)[a]
2	8.40	8.21	3/7
3	8.39	8.23	3/7
4	8.26	8.06	2/8
5	8.25	8.04	3/7
6	8.21	8.01	4/6
7	8.22	8.02	3/7
8	8.18	7.99	3/7
9			
10	8.18	7.99	3/7
11	8.18	7.99	3/7
14	8.57	8.38	2/8
15	8.16	7.97	3/7
16	8.15	7.94	3/7
17	8.16	7.97	3/7
18	8.12	7.90	3/7

[a]Data obtained by relative integration of the corresponding imine-attached hydrogens of the (E) and (Z) diastereoisomers.

Table 2: Some physico-chemical properties and spectral findings of compounds **1–18**



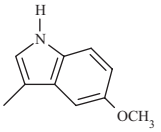
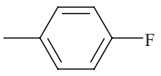
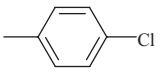
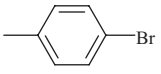
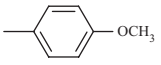
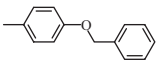
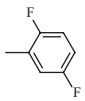
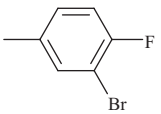
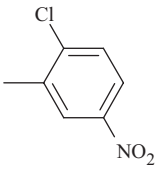
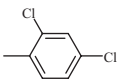
Compound	Ar	Formulas	M.P (°C)	^{1}H-NMR	MS M + H (%)
1		$C_{26}H_{19}ClN_4O$	279–282	5.10, 5.59 (2s, 2H, CH_2), 7.25–8.82 (m, 16H, Ar-H, -N=CH), 11.85, 12.06 (2s, 1H, NH)	439 (72) 441 (28)
2		$C_{26}H_{19}ClN_4O$	270–272	5.09, 5.61 (2s, 2H, CH_2), 7.28–8.40 (m, 16H, Ar-H, -N=CH), 11.91, 12.06 (2s, 1H, NH)	439 (71) 441 (29)
3		$C_{25}H_{20}ClN_5O_2$-1.75H_2O	217–221	3.59 (s, 3H, OCH_3), 5.04, 5.57 (2s, 2H, - CH_2), 6.79–8.39 (m, 13H, Ar-H,-N=CH), 11.48 (s, 1H, NH), 11.52, 11.65 (2s, 1H, NH)	458 (73) 460 (27)
4		$C_{22}H_{17}ClN_4O$-0.25H_2O	230–231	5.07, 5.55 (2s, 2H, CH_2), 7.22–8.26 (m, 14H, Ar-H, -N=CH), 11.82, 11.98 (2s, 1H, NH)	389 (75) 391 (25)
5		$C_{22}H_{16}ClFN_4O$-0.75H_2O	222	5.08, 5.56 (2s, 2H, CH_2), 7.26–7.99–8.25 (m, 13H, Ar-H, -N=CH), 11.84, 12.01 (2s, 1H, NH)	407 (74) 409 (26)
6		$C_{22}H_{16}Cl_2N_4O$-1.85H_2O	252	5.05, 5.54 (2s, 2H, CH_2), 7.27–8.21 (m, 13H, Ar-H, -N=CH), 11.85, 12.05 (2s, 1H, NH)	423 (63) 425 (37)
7		$C_{22}H_{1\ 6}$ BrClN$_4$O	280–281	5.06, 5.54 (2s, 2H, CH_2), 7.27–8.22 (m, 13H, Ar-H, -N=CH), 11.87, 12.05 (2s, 1H, NH)	467 (47) 469 (53)
8		$C_{23}H_{19}ClN_4O_2$-0.75H_2O	210–212	3.79 (s, 3H, OCH_3), 5.04, 5.51 (2s, 2H, -CH_2), 6.98–8.18 (m, 13H, Ar-H,-N=CH), 11.68, 11.83 (2s, 1H, NH)	419 (75) 421 (25)
9		$C_{22}H_{16}$ ClN$_5$O$_3$-1.25H_2O	145 (Bubbl.)	5.12, 5.63 (2s, 2H, CH_2), 7.32–8.55 (m, 13H, Ar-H, -N=CH), 12.06, 12.21 (2s, 1H, NH)	434 (76) 436 (24)
10		$C_{29}H_{23}$ ClN$_4$O$_2$-0.25H_2O	225–228	5.04, 5.51 (2s, 2H, CH_2), 5.16 (s, 2H, OCH_2), 7.06–8.18 (m, 18H, Ar-H, -N=CH), 11.69, 11.85 (2s, 1H, NH)	495 (73) 497 (27)
11		$C_{22}H_{15}ClF_2N_4O$-0.75H_2O	208	5.04, 5.50 (2s, 2H, CH_2), 7.99–7.82-8.18 (m, 11H, Ar-H, -N=CH), 11.92, 12.11 (2s, 1H, NH, exchangeable with D_2O)	425 (74) 427 (26)
12		$C_{22}H_{15}BrClFN_4O$-2H_2O	122	5.08, 5.59 (2s, 2H, CH_2), 7.28–8.22 (m, 12H, Ar-H, -N=CH), 11.92, 12.09 (2s, 1H, NH)	485 (37) 487 (50) 489 (13)
13		$C_{22}H_{15}Cl_2N_5O_3$-3.5H_2O	130 (dec.)	5.11, 5.62 (2s, 2H, CH_2), 7.27–8.72 (m, 12H, Ar-H, -N=CH), 12.16, 12.40 (2s, 1H, NH)	468 (57) 470 (43)
14		$C_{22}H_{15}Cl_3N_4O$-0.5H_2O	263–265	5.08, 5.56 (2s, 2H, CH_2), 7.27–8.57 (m, 12H, Ar-H, -N=CH), 12.02, 12.24 (2s, 1H, NH)	457 (43) 459 (42) 461 (15)

Table 2: *Continued*

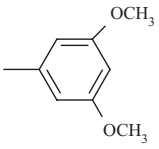
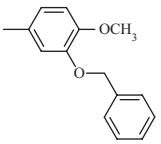
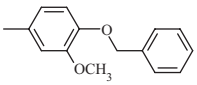
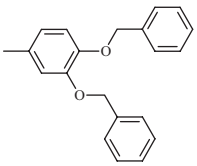
Compound	Ar	Formulas	M.P (°C)	^{1}H-NMR	MS M + H (%)
15		$C_{24}H_{21}ClN_4O_3$	236–238	3.76 (s, 6H, OCH_3), 5.06, 5.54 (2s, 2H, - CH_2), 6.56–8.16 (m, 12H, Ar-H, -N=CH), 11.85, 11.98 (2s, 1H, NH)	449 ((77) 451 (23)
16		$C_{30}H_{25}ClN_4O_3$-0.75H_2O	215	3.80 (s, 3H, OCH_3), 5.06, 5.10 (2s, 2H, - CH_2), 5.48 (s, 2H, OCH_2), 7.00–8.15 (m, 17H, Ar-H, -N=CH), 11.73, 11.83 (2s, 1H, NH)	525 (70) 527 (30)
17		$C_{30}H_{25}ClN_4O_3$-0.33H_2O	186–189	3.79 (s, 3H, OCH_3), 5.07, 5.13 (2s, 2H, - CH_2), 5.67 (s, 2H, OCH_2), 7.08–8.16 (m, 17H, Ar-H, -N=CH), 11.76, 11.86 (2s, 1H, NH)	525 (72) 527 (28)
18		$C_{36}H_{29}ClN_4O_3$	228–229	5.05, 5.44 (2s, 2H, CH_2), 5.13 (s, 2H, OCH_2), 5.17 (s, 2H, OCH_2), 7.06–8.12 (m, 22H, Ar-H, -N=CH), 11.73, 11.84 (2s, 1H, NH)	601 (73) 603 (27)

Table 3: Some physico-chemical properties and spectral findings of compounds **1t–18t**

Compd.	Formulas	M.P (°C)	NMR	MS M + H (%)
1t	$C_{28}H_{21}ClN_4O_2S$-0.9H_2O	285–288	3.84, 3.96 (2H, 2d, j = 17.2 Hz, CH_2-thiazolidine ring C5), 4.88 ((2H, j = 17.6 Hz, AB quartet, CH_2), 6.62 (s, 1H, CH thiazolidine ring C2), 7.09–8.07 (m, 15H, Ar-H), 11.02 (s, 1H, NH)	513 (68) 515 (32)
2t	$C_{28}H_{21}ClN_4O_2S$-0.8$HSCH_2COOH$	262	3.83, 3.99 (2H, 2d, j = 16.0 Hz, CH_2-thiazolidine ring C5), 4.83 ((2H, j = 17.6 Hz, AB quartet, CH_2), 6.01 (s, 1H, CH thiazolidine ring C2), 7.01–8.03 (m, 15H, Ar-H), 10.87 (s, 1H, NH)	513 (69) 515 (31)
3t	$C_{27}H_{22}ClN_5O_3S$-0.8H_2O	305	3.74 (s, 3H, OCH_3), 3.80, 3.90 (2H, 2d, j = 16.0 Hz, CH_2-thiazolidine ring C5), 4.81 (2H, j = 17.1 Hz, AB quartet, CH2), 5.56 (s, 1H, CH thiazolidine ring C2), 6.83–7.85 (m, 12H, Ar-H), 10.72 (s, 1H, NH), 11.22 (s, 1H, NH)	532 (71) 534 (29)
4t	$C_{24}H_{19}ClN_4O_2S$	275–278	3.76, 3.92 (2H, 2d, j = 16.0 Hz CH_2-thiazolidine ring C5), 4.87 (2H, j = 17.6 Hz AB quartet, CH_2), 5.80 (s, 1H, CH thiazolidine ring C2), 7.21–7.70 (m, 13H, Ar-H), 10.83 (s, 1H, NH) ^{13}C: 29.8, 46.1, 62.1, 11.4, 119.8, 123.0, 123.4, 128.3, 129.2, 129.4, 129.8, 131.6, 135.4, 136.9, 138.9, 142.9, 153.0, 166.9, 169.6	463 (77) 465 (23)
5t	$C_{24}H_{18}ClFN_4O_2S$-1.1H_2O-0.1C_2H_5OH	147	3.73, 3.90 (2H, 2d, j = 16.0 Hz, CH_2-thiazolidine ring C5), 4.85 (2H, j = 16.8 Hz, AB quartet, CH_2), 5.80 (s, 1H, CH thiazolidine ring C2), 7.18–7.69 (m, 12H, Ar-H), 10.78 (s, 1H, NH)	482 (70) 484 (30)
6t	$C_{24}H_{18}Cl_2N_4O_2S$-2H_2O	169	3.77, 3.93 (2H, 2d, j = 16.0 Hz , CH_2-thiazolidine ring C5), 4.80 (2H, s, CH_2), 5.83 (s, 1H, CH thiazolidine ring C2), 7.20–7.76 (m, 12H, Ar-H), 10.83 (s, 1H, NH)	497 (60) 499 (40)
7t	$C_{24}H_{18}$ $BrClN_4O_2S$-0.2C_2H_5OH-0.75H_2O	145	3.76, 3.82 (2H, 2d, j = 16.0 Hz CH_2-thiazolidine ring C5), 4.99 (2H, j = 18.0 Hz, AB quartet, CH_2), 5.92 (s, 1H, CH thiazolidine ring C2), 7.26–7.78 (m, 12H, Ar-H), 9.88 (s, 1H, NH)	541 (36) 543 (46) 545 (18)

Table 3: *Continued*

Compd.	Formulas	M.P (°C)	NMR	MS M + H (%)
8t	$C_{25}H_{21}ClN_4O_3S$-0.5$HSCH_2COOH$-0.9H_2O	159	3.76, 3.88 (2H, 2d, j = 14.40 Hz CH_2-thiazolidine ring C5), 3.81 (s, 3H, OCH_3), 4.85 (2H, j = 17.20 Hz, AB quartet, CH_2), 5.76 (s, 1H, CH thiazolidine ring C2), 6.98–7.82 (m, 12H, Ar-H), 10.77 (s, 1H, NH)	493 (78) 495 (22)
9t	$C_{24}H_{18}ClN_5O_4S$-1H_2O	178	3.80, 4.00 (2H, 2d, j = 16.0 Hz, CH_2-thiazolidine ring C5), 4.88 (2H, AB quartet, j = 18.8 Hz CH_2), 6.02 (s, 1H, CH thiazolidine ring C2), 7.17–8.34 (m, 12H, Ar-H), 10.90 (s, 1H, NH)	508 (73) 510 (27)
10t	$C_{31}H_{25}ClN_4O_3S$-0.6C_2H_5OH-1.5H_2O	220 decomp.	3.73, 3.88 (2H, 2d, j = 16.0 Hz, CH_2-thiazolidine ring C5), 4.91 (2H, AB quartet, j = 20.4 Hz, CH_2), 5.24 (s, 2H, OCH_2-), 5.76 (s, 1H, CH thiazolidine ring C2), 6.98–7.70 (m, 19H, Ar-H)	569 (64) 571 (36)
11t	$C_{24}H_{17}ClF_2N_4O_2S$-0.1H_2O	307–310	3.77, 3.95 (2H, 2d, j = 15.6 Hz, CH_2-thiazolidine ring C5), 4.93 (2H, s, CH_2), 5.96 (s, 1H, CH thiazolidine ring C2), 7.20–7.74 (m, 11H, Ar-H), 10.87 (s, 1H, NH)	499 (71) 501 (39)
12t	$C_{24}H_{17}BrClFN_4O_2S$-0.5 $HSCH_2COOH$-0.9C_2H_5OH	128	3.75, 3.98 (2H, 2d, j = 16.0 Hz CH_2-thiazolidine ring C5), 4.89 (2H, s, CH_2), 5.84 (s, 1H, CH thiazolidine ring C2), 7.22–7.86 (m, 11H, Ar-H), 10.84 (s, 1H, NH)	559 (37) 561 (48) 563 (15)
13t	$C_{24}H_{17}Cl_2N_5O_4S$-0.85$HSCH_2COOH$	169	3.81, 3.97 (2H, 2d, j = 16.4 Hz CH_2-thiazolidine ring C5), 4.93 (2H, AB quartet, j = 17.5 Hz , CH_2), 6.19 (s, 1H, CH thiazolidine ring C2), 7.18–8.25 (m, 11H, Ar-H), 11.04 (s, 1H, NH)	542 (63) 544 (37)
14t	$C_{24}H_{17}Cl_3N_4O_2S$-0.85H_2O	297–299	3.77, 3.92 (2H, 2d, j = 15.6 Hz, CH_2-thiazolidine ring C5), 4.94 (2H, s, CH_2), 6.06 (s, 1H, CH thiazolidine ring C2), 7.19–7.74 (m, 11H, Ar-H), 10.96 (s, 1H, NH)	531 (41) 533 (45) 535 (14)
15t	$C_{26}H_{23}ClN_4O_4S$-0.9$HSCH_2COOH$-1.3H_2O	287–289	3.72–3.93 (m, 8H, OCH_3, CH_2-thiazolidine ring C5), 4.89 ((2H, AB quartet, j = 16.0 Hz , CH_2), 5.79 (s, 1H, CH thiazolidine ring C2), 6.55–7.70 (m, 11H, Ar-H), 10.90 (s, 1H, NH) ^{13}C: 29.7, 46.2, 55.9, 62.0, 101.3, 105.9, 11.4, 119.8, 123.0, 123.4, 129.1, 129.5, 131.5, 135.4, 136.9, 141.4, 142.9, 152.9, 161.3, 166.9, 169.7	523 (70) 525 (30)
16t	$C_{32}H_{27}ClN_4O_4S$-0.3$HSCH_2COOH$-0.45H_2O	119	3.70–3.90 (m, 5H, OCH_3,CH_2-thiazolidine ring C5), 4.88 ((2H, AB quartet, j = 17.2 Hz , CH_2), 5.05 (s, 2H, OCH_2-), 5.81 (s, 1H, CH thiazolidine ring C2), 6.96–7.69 (m, 16H, Ar-H), 10.78 (s, 1H, NH)	599 (68) 601 (32)
17t	$C_{32}H_{27}ClN_4O_4S$-0.95$HSCH_2COOH$	106–107	3.71–3.88 (m, 5H, OCH_3, CH_2-thiazolidine ring C5), 4.84 ((2H, AB quartet, j = 17.0 Hz, CH_2), 5.11 (s, 2H, OCH_2-), 5.76 (s, 1H, CH thiazolidine ring C2), 6.87–7.67 (m, 16H, Ar-H), 10.75 (s, 1H, NH)	599 (69) 601 (31)
18t	$C_{38}H_{31}ClN_4O_4S$-0.25 $HSCH_2COOH$-1.4H_2O	127	3.74, 3.86 (2H, 2d, j = 15.6 Hz CH_2-thiazolidine ring C5), 4.86 (2H, AB quartet, j = 17.6 Hz, CH_2), 5.06 (s, 2H, OCH_2-), 5.18 (s, 2H, OCH_2-), 5.78 (s, 1H, CH thiazolidine ring C2), 6.94–7.70 (m, 21H, Ar-H), 10.87 (s, 1H, NH)	675 (66) 677 (34)

compounds. A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged. The standard used in this assay was BHT.

Results and Discussion

Chemistry

The desired benzimidazole derivatives were synthesized according to Scheme 1. Compound **A** having benzimidazole ring was prepared via oxidative condensation of *o*-phenylenediamine, *p*-chlorobenzaldehyde, and sodium metabisulfite. Treatment of compound **A** with ethyl chloroacetate in KOH/DMSO gave the N-alkylated product **B**. Hydrazine hydrate and the ester **B** in ethanol were refluxed for 4 h to give the desired hydrazide compound, (2-(*p*-chlorophenyl)-benzimidazole-1-yl)-acetic acid hydrazide **C**. Compounds **1–18** were obtained by condensing acyl hydrazide **C** with the corresponding aldehyde derivatives in the presence of catalytic amounts of ceric ammonium nitrate (CAN) in ethanol (36) Condensation of **1–18** with thioglycolic acid in toluene yielded 2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(4-oxo-2-aryl-thiazolidine-3-yl) acetamides (**1t–18t**).

In the NMR spectral data, all protons were seen according to the expected chemical shift and integral values. The aromatic protons appeared as multiplet peaks within the range 6.56–8.82 for compounds **1–18** and 6.55–8.34 for compounds **1t–18t**. For the compounds **1–18**, the signals belonging to the benzylidene group were observed in the aromatic region.

The investigation of ^{1}H-NMR spectra of compounds **1–18** indicated the presence of two singlet signals referring to (E) and (Z) geometric isomers about imino hydrogens (CH=N) and cis/trans amide conformers. Karabatsos *et al.* (37) describes that imine-attached hydrogen signal of (E) diastereoisomer is downfielded by 0.2–0.3 ppm from the corresponding hydrogen atom signal of (Z) diastereoisomer. This result showed that the construction of an imino bond of the compounds **1–18** is not a diastereoselective process. Karabatsos *et al.* also suggest that if the hydrazones have an aromatic substituent, it is conceivable that the aromatic ring interacts with the protonated group favoring the (Z) diastereoisomer (Figure 1). In our findings, E and Z diastereoisomers were observed at 8.12–8.57 and 7.90–8.38 ppm, respectively, and the ratio between (E)/(Z) diastereoisomers could be defined from the relative integration of imine-attached hydrogen in the corresponding ^{1}H-NMR spectra (Table 1).

The methylene protons of thiazolidinone (**1t–18t**) displayed two doublets at 3.70–3.84 and 3.57–4.00 ppm because of the nonequivalent geminal methylene protons. Thiazolidinone protons (-CH) (**1t–18t**) resonated as singlets at 5.11–6.62 ppm. The methylene protons (-CH_2CO-) of compounds **1t–18t** were observed at 4.81–4.99 ppm as an AB quartet.

It has been recognized for some time that the two protons of a methylene group adjacent to an asymmetrically substituted carbon or any dissymmetric moiety are magnetically non-equivalent and consequently split each other in the NMR spectra (38). In accord to this expectation, methylene protons of acetamide chain (**1t–5t,7t–11t,13t, and 15t–18t**) appear as an AB quartet at 4.81–4.99 ppm and with a coupling constant 15.93–20.40 Hz. In the other compounds (**6t, 12t,** and **14t**), magnetically equivalent CH_2 protons are observed as a sharp singlet at 4.80–4.94 ppm.

The mass spectra of synthesized compounds showed a M + H ion peak, which is conforming with the molecular formula of the compounds. The structures of the synthesized compounds were consistent with the ^{1}H-, ^{13}C-NMR, and mass spectra. Spectral data are summarized in Tables 1, 2, and 3.

Antioxidant activity in vitro

The *in vitro* effects of the compounds and caffeine in liver microsomal EROD activity are shown in Table 4. All the tested compounds except compound **6t** (24%) showed significant inhibition (50–95%) of EROD activity. Compounds **1, 3, 5, 11, 16, 17, and 18** decreased liver EROD activity in the range of 87–95%, better than that of the specific inhibitor caffeine (85%) at 10^{-3} M concentration. In terms of inhibition on EROD activity, it can be said that 2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(2-arylmethylene amino) acetamide (**1–18**) derivatives displayed better activity than 2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(4-oxo-2-aryl-thiazolidine-3-yl) acetamide (**1t–18t**) counterparts. Among the compounds **1–18**, different aryl groups such as phenyl, 1- or 2-naphthyl or indole-3-yl have exhibited no considerable difference. The most active compound **16** inhibited EROD activity with a **0.360 mM** IC_{50} value, which has 4-methoxy-3-benzyloxy phenyl ring as aryl group, while IC_{50} value of caffeine was **0.520 mM.** Significant inhibitory activities were also observed for compounds **2** (81%), **9** (81%), **4** (84%), **6** (84%), and **14** (83%).

Inhibition of NADPH-dependent lipid peroxidation (Table 4) produced by the compounds in the rat liver microsomes was examined by measuring the formation of the TBARS for their antioxidant capac-

Table 4: Effects of the compounds on the liver LP levels, EROD enzyme, and DPPH free radical scavenging activities *in vitro*[a]

Compound[b]	EROD (pmol/mg/dk)	% of control	LP (nmol/mg/dk)	% of control	DPPH % of control
1	5.20 ± 0.27	13	8.12 ± 0.38	50	24 ± 3.8
2	7.75 ± 0.75	19	7.29 ± 2.13	45	84 ± 0.8
3	4.93 ± 0.63	12	64.99 ± 5.27	399	70 ± 2.2
4	6.69 ± 0.08	16	2.63 ± 0.97	16	88 ± 1.7
5	4.49 ± 0.11	11	11.04 ± 0.95	68	nt
6	6.81 ± 0.11	16	8.12 ± 0.56	50	90 ± 0.6
7	9.95 ± 0.32	24	17.69 ± 2.02	109	70 ± 3.8
8	12.36 ± 1.31	28	6.33 ± 2.53	39	92 ± 1.0
9	8.03 ± 3.16	19	18.52 ± 3.21	114	nt
10	14.95 ± 0.44	36	4.06 ± 0.68	25	72 ± 3.9
11	4.60 ± 6.08	11	7.17 ± 1.35	44	nt
12	17.14 ± 1.86	41	7.17 ± 5.07	44	nt
13	8.49 ± 0.41	20	13.62 ± 1.56	84	nt
14	7.25 ± 1.80	17	15.77 ± 3.04	97	86 ± 3.0
15	10.36 ± 6.74	25	4.54 ± 0.23	28	91 ± 0.6
16	2.13 ± 0.11	5	19.00 ± 6.25	116	85 ± 2.0
17	5.06 ± 0.12	12	10.75 ± 1.02	66	72 ± 0.6
18	5.47 ± 0.20	13	1.80 ± 0.16	11	88 ± 2.2
1t	22.51 ± 1.17	54	4.00 ± 1.36	25	81 ± 1.2
2t	17.93 ± 1.11	43	19.17 ± 1.49	119	59 ± 0.7
3t	11.25 ± 0.42	27	nd	–	66 ± 1.7
4t	19.11 ± 0.25	46	1.67 ± 0.03	10	85 ± 1.5
5t	14.60 ± 1.22	35	2.70 ± 0.48	17	85 ± 0.1
6t	31.89 ± 0.50	76	3.54 ± 0.35	22	96 ± 1.2
7t	20.66 ± 0.70	50	2.60 ± 0.08	16	69 ± 1.4
8t	16.23 ± 1.03	39	3.63 ± 0 19	22	55 ± 1.0
9t	14.35 ± 2.40	35	1.52 ± 0.48	9	73 ± 1.8
10t	11.23 ± 1.61	27	5.64 ± 0.42	34	23 ± 0.6
11t	9.25 ± 1.09	22	3.76 ± 0.91	23	97 ± 0.4
12t	12.76 ± 3.34	31	2.36 ± 0.54	15	54 ± 0.9
13t	14.91 ± 1.81	36	3.29 ± 0.41	20	52 ± 0.7
14t	16.06 ± 0.01	37	9.65 ± 0.30	59	71 ± 2.0
15t	14.22 ± 3.59	34	3.41 ± 0.83	21	62 ± 2.4
16t	13.12 ± 3.25	32	3.53 ± 0.04	22	62 ± 0.6
17t	10.47 ± 0.36	25	4.22 ± 0.35	26	68 ± 0.8
18t	18.85 ± 0.79	45	1.77 ± 0.53	11	76 ± 1.7
BHT			5.68 ± 0.22	35	15 ± 2.9
Caffeine	6.41 ± 0.36	15			
Control[c]	41.53 ± 0.99	100	16.25 ± 1.45	100	100

[a]Each value represents mean ± SD of 2–4 independent experiments.
[b]Concentration in incubation medium (10^{-3} M).
[c]DMSO only, control for compounds.
nt, not tested.

ity. In contrast to EROD activity, in the series of thiazolidinone, all of the compounds except compound **10t** have better LP activities than corresponding methylaminoacetamide derivatives (**1–18**). The most active compound **9t** (2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(4-oxo-2-(3-nitrophenyl-thiazolidine-3-yl) acetamide) caused 91% inhibition (**0.375 mM** IC_{50}) on LP level in rat liver microsomes at 10^{-3} M concentration, while BHT showed 65% inhibition (**0.408 mM** IC_{50}) at the same concentration.

We also tested the DPPH radical scavenger capacities (Table 4) by the synthesized compounds. Almost all of the compounds' DPPH free radical scavenger capacities were not enough, except compounds **1** and **10t**. Compounds **1** and **10t** showed the highest scavenger capacities among the tested compounds on DPPH radical with 76% and 77%, respectively, which are close to BHT (85%).

Conclusion

Compound **10t** appeared to have similar effect for all assay systems. It is plausible to suggest that the inhibition of LP levels and DPPH scavenger capacities could be related to decrease in production of oxygen radicals because of the depression of CYPA1/2. Moreover, the other compounds showed different effect on these parameters. Such contradictory results have also been found in a previous study (39). Each method relates to the generation of a different radical, acting through a variety of mechanism. Therefore, the observation of different effects of the synthesized compounds on EROD activity, LP levels, and DPPH radical scavenger capacity is not surprising, as the mechanism of production of reactive oxygen species using these methods is different.

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