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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of some 3,4-disubstituted-6,7-dihydro-imidazo[2,1-b][1,3]thiazole and 3,4-disubstituted-7,8-dihydro-6H-imidazo[2,1-b][1,3]thiazine derivatives and evaluation of their cytotoxicities against F2408 and 5RP7 cells

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Abstract This article describes the synthesis of 3,4-disubstituted-6,7-dihydro-imidazo[2,1-b][1,3]thiazoles and 3,4-disubstituted-7,8-dihydro-6H-imidazo[2,1-b][1,3]thiazines, having substituted or nonsubstituted phenyl rings at the 5,6 and 2,3 positions, respectively, their cytotoxic effects through noncancer (F2408) and cancer (5RP7) cells, and their detailed ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectral characterization. The title compounds were obtained by the cyclization of 4,5-diaryl-imidazole-2-thione and dihaloalkane (i.e., 1,2-dihaloethane or 1,3-dihalopropane), in the presence of potassium carbonate (K_2CO_3) in *N,N*-dimethyl formamide (DMF). 4,5-Diaryl-imidazole-2-thione was prepared by condensation of α-hydroxyketones (acyloins), which were obtained by treating aldehydes with cyanide, with thioureas in AcOH. The structure of imidazo[2,1-b][1,3]thiazole and imidazo[2,1-b][1,3]thiazine derivatives was confirmed by infrared (IR), ^{1}H-NMR, and ^{13}C-NMR. The cytotoxicities of the synthesized compounds on both of noncancer (F2408) and cancer (5RP7) cells were measured by 3-(4,5-dimethyl-thiazollyl-2)-2,5-diphenyltetrazolium (MTT) assay. In the presence of only lower doses of compounds **9** and **11**, bearing methyl or methoxy substituents on the phenyl ring of imidazo[2,1-b][1,3]thiazole scaffold, the cytotoxic effect was higher on 5RP7 cells than control cells after 24 h.

Keywords Fused azole heterocycles · Bridgehead nitrogen · 6,7-Dihydro-imidazo[2,1-b][1,3]thiazoles · 7,8-Dihydro-6H-imidazo[2,1-b][1,3]thiazines

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Introduction

Among the fused azole heterocycles with bridgehead nitrogen, imidazo[2,1-b][1,3]thiazoles have received considerable attention as a result of their various biological effects. These activities range from analgesic–anti-inflammatory (Palagiano *et al*., 1995); antifungal (Çapan *et al*., 1999), antibacterial (Gadad *et al*., 2000), antimicrobial (Ur *et al*., 2004), antimycobacterial (Cesur *et al*., 1994), antituberculosis (Ulusoy, 2002), antisecretory (Andreani *et al*., 2000), cardiotonic (Andreani *et al*., 1996), cytotoxic (Terasawa *et al*., 2001; Gürsoy and Ulusoy-Güzeldemirci, 2007), antitumor (Andreani *et al*., 2005a) activities to inhibition on 5-lipoxygenase (Bender and Gleason, 1992), mitochondrial nicotinamide adenine dinucleotide dehydrogenase (NADH) (Andreani *et al*., 1999), acetyl cholinesterase (Andreani *et al*., 2005b), and human constitutive androstane receptor (CAR) (Auerbach *et al*., 2005).

The thiazine moiety of imidazo[2,1-b][1,3]thiazines is also an important pharmacophoric group for the benzodiazepin receptor binding activity (Kiec-Kononowicz *et al*., 2001; Broom and Harrington, 2003).

Levamisole (Peterlin-Masic *et al*., 2000) and tetramisole (Feil, 1996) are well-known antihelmintic and immunomodulatory agents with their saturated structure.

Since the precursor imidazole-2-thione itself or its analogs (i.e., 2-mercaptoimidazole and thioeters) are also biologically active as antitumor (Weitzel *et al*., 1966), cytostatic and thyrostatic (Weitzel, 1967), neoplasm inhibitory (Guglielmi, 1968; Athen and Guglielmi, 1969; Kolmar Laboratories Inc, 1973), radioprotective (Skvortsova *et al*., 1978), immunoregulatory (Bender, 1978; Bender *et al*., 1985; Or *et al*., 1996) agents, it may expect that their fused derivatives would have similar or much greater active.

In the light of these findings, we previously described the synthesis and X-ray crystallography study of 3,4-di-p-tolyl-6,7-dihydro-imidazo[2,1-b][1,3]thiazole (Özbey and Meriç, 2006). In continuation of our work on the synthesis of biologically active heterocycles, herein we report the synthesis and cytotoxic properties of 3,4-disubstituted-6,7-dihydro-imidazo[2,1-b][1,3]thiazole and 3,4-disubstituted-7,8-dihydro-6H-imidazo[2,1-b][1,3]thiazine derivatives on noncancer and cancer cell lines.

Methods and materials

Chemistry

Melting points of the compounds were determined using an Electrothermal model 9100 melting-point apparatus and are reported uncorrected. Thin-layer chromatography was performed on silica gel 60 F_{254} aluminum sheets (Merck) using $CHCl_3$/MeOH (98:2) as the development solvent system and exposure to an ultraviolet (UV) lamp (CAMAG CE 12VDC/VAC 50/60 Hz) at 254/366 nm as the visualization method. The structures of the synthesized compounds were elucidated by spectroscopic methods (infrared (IR), ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR), electron ionization-mass spectroscopy (EI-MS). IR spectra were recorded on a JASCO FT-IR-430 spectrophotometer (KBr; ν_{max} is expressed in cm^{-1}), ^{1}H-NMR spectra on a Bruker 400 MHz (Madison, WI) and GEMINI 300 MHz (Varian,

Palo Alto, CA) NMR spectrometers by using tetramethylsilane (TMS) as an internal standard (δ in ppm) in $CDCl_3$; the chemical shift (referenced to solvent signal) is expressed in δ (ppm). Coupling constants (J) values are given in Hertz. Multiplicities are reported as singlet (s), triplet (t), or multiplet (m), but doublets for the aromatic protons of compounds **9–12** are reported in the AA'BB' system. ^{1}H-NMR chemical shifts are relative to TMS ($\delta = 0.00$ ppm) and $CDCl_3$ ($\delta = 7.26$ ppm). ^{13}C-NMR chemical shifts are relative to $CDCl_3$ ($\delta = 77.23$ ppm). All the chemicals and solvents used in this study were of analytical grade (Merck, Aldrich, Sigma, and Fluka). All chemical drawings and calculations were performed by using the ChemDraw Ultra 7.0 and MDL ISIS/Draw 2.5 computer programs.

General procedure for the synthesis of compounds **2a–c** and **4a–c**

For the synthesis of compounds **2a**–**c** (Ide and Buck, 1948) and **4a**–**c** (Gregoire *et al*., 1951; Yoshida *et al*., 1951), known synthesis pathways were followed. The required benzoins (**2a**–**c**) were prepared by the classical benzoin condensation (Ide and Buck, 1948).

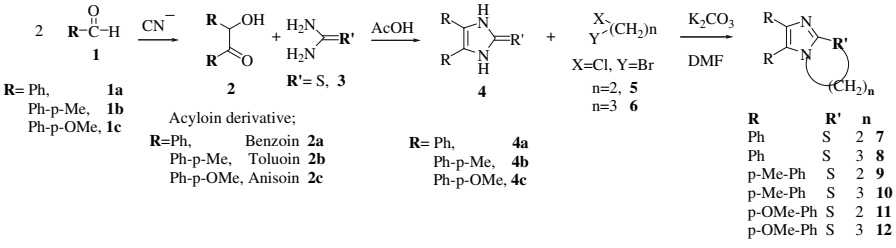
General procedure for the synthesis of compounds **7–12**

The title compounds were obtained by the cyclization (Bender PE, 1978; Bender *et al*., 1985) of equimolar amounts of 4,5-diaryl-imidazole-2-thione (2.5 mmol) and dihaloalkane (2.5 mmol) in the presence of K_2CO_3 (3.75 mmol) in DMF at 130–135°C (see Scheme 1). 4,5-Diaryl-imidazole-2-thione was prepared by condensation (Gregoire *et al*., 1951; Yoshida *et al*., 1951) of α-hydroxyketones (acyloins), which were obtained by treating aldehydes with cyanide, with thioureas in AcOH.

The synthesis of 3,4-disubstituted-6,7-dihydro-imidazo[2,1-b][1,3]thiazole and 3,4-disubstituted-7,8-dihydro-6H-imidazo[2,1-b][1,3]thiazine derivatives

*3,4-Diphenyl-6,7-dihydro-imidazo[2,1-b][1,3]thiazole (**7**) (Mazur et al., 1969; Mohan et al., 1973; Dou et al., 1980; Bender et al., 1985; Pooru et al., 1987)*

A mixture of 4,5-diphenyl-1,3-dihydro-imidazole-2-thione (2.5 mmol; 0.63 g) and 1-bromo-2-chloroethane (2.5 mmol; 0.358 g; 0.225 mL) in 35 mL of DMF was



Scheme 1 General synthesis scheme of the investigated compounds

heated at 130–135°C for 5 hours in the presence of K_2CO_3 (3.75 mmol; 0.518 g). At the end of the reaction, the mixture was allowed to stand until it reached room temperature, then the content of reaction vessel was poured onto iced water and the pH was adjusted to ~11 by 10% NaOH aq. solution. The crude product thus obtained was collected by filtration, dried, and purified by column chromatography ($CHCl_3$:MeOH) (97:3) (Rf:0.5). In order to remove unreacted 1-bromo-2-chloroethane, the product gained from column purification was washed with diethyl ether, dried, with a yield of 52%, (0.362 g). m.p. = 165–166°C. IR (KBr, cm^{-1}): 1683–1436 (C=N and C=C), 773 and 723 (monosubstituted-Ph ring deformation band). ^{1}H NMR: ($CDCl_3$): 3.81 (t, J = 7.2 Hz, 2H), 4.1 (t, J = 7.2 Hz, 2H), 7.15–7.25 (m, 3H), 7.32–7.43 (m, 5H), 7.48–7.52 (m, 2H). ^{13}C NMR: ($CDCl_3$) 34.69 (CH_2), 45.72 (CH_2), 126.57, 126.85, 127.41, 128.14, 128.16, 128.94, 129.13, 130.83, 134.53, 137.15.

*3,4-Diphenyl-7,8-dihydro-6H-imidazo[2,1-b][1,3]thiazine (**8**) (Kovpak and Bagrii, 1970; Schoeberl and Magosch, 1970; Mohan* et al.*, 1973; Bagrii* et al.*, 1975; Dou* et al.*, 1980)*

A mixture of 4,5-diphenyl-1,3-dihydro-imidazole-2-thione (2.5 mmol; 0.6 3g) and 1-bromo-3-chloropropane (2.5 mmol; 0.394 g; 0.247 mL) in 50 mL of DMF was heated at 130–135°C for 4 hours in the presence of K_2CO_3 (3.75 mmol; 0.518 g). At the end of this period, the mixture was allowed to stand until it reached room temperature, then the content of reaction vessel was poured onto iced water and the pH was adjusted to ~11 by 10% NaOH aq. solution. The crude product thus obtained was collected by filtration, dried, and extracted with diethyl ether. The organic layer was evaporated under reduced pressure. Pure compound on TLC monitoring was obtained (TLC solvent system: $CHCl_3$:MeOH, 97:3) with a yield of 43%, (0.312 g). m.p. = 191°C. IR (KBr, cm^{-1}): 1683–1425 (C=N and C=C), 771 and 696 (monosubstituted-Ph ring deformation band). ^{1}H NMR: ($CDCl_3$) 2.21–2.30 (m, 2H), 3.12–3.16 (m, 2H), 3.71–3.77 (m, 2H), 7.08–7.20 (m, 3H), 7.29–7.34 (m, 2H), 7.39–7.47 (m, 5H). ^{13}C NMR: ($CDCl_3$) 23.87, 25.62, 43.78, 126.22, 126.63, 127,94, 128.47, 128.92, 129.14, 130.22, 130.71, 134.08, 137.20, 137.54.

*3,4-Di-p-tolyl-6,7-dihydro-imidazo[2,1-b][1,3]thiazole (**9**)*
(Bender et al.*, 1985; Pooru* et al.*, 1987; Özbey and Meriç, 2006)*

A mixture of 4,5-di-p-tolyl-1,3-dihydro-imidazole-2-thione (2.5 mmol; 0.7g) and 1-bromo-2-chloroethane (2.5 mmol; 0.358 g; 0.225 mL) in 35 mL of DMF was heated at 130–135°C for 5 hours in the presence of K_2CO_3 (3.75 mmol; 0.518 g). At the end of the reaction, the mixture was allowed to stand until it reached room temperature, then the content of reaction vessel was poured onto iced water and the pH was adjusted to ~11 by 10% NaOH aq. solution. The crude product thus obtained was collected by filtration, dried, and purified by column chromatography ($CHCl_3$:MeOH) (97:3) (Rf:0.6). In order to remove unreacted 1-bromo-2-

chloroethane, product was washed with diethyl ether, dried, with a yield of 49% (0.375 g). m.p. = 173°C. IR (KBr, cm^{-1}): 1683–1456 (C=N and C=C), 727 (1,4-disubstituted-Ph ring deformation band). ^{1}H NMR: ($CDCl_3$): 2.29 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.77 (t, J = 6.9 Hz, CH_2), 4.05 (t, J = 6.9 Hz, CH_2), 7.03 (AA'BB', J_{AB} = 8.1 Hz, 2H), 7.18 (AA'BB', J_{AB} = 8.4 Hz, 2H), 7.22 (AA'BB', J_{AB} = 8.4 Hz, 2H), 7.40 (AA'BB', J_{AB} = 8.1 Hz, 2H). ^{13}C NMR: ($CDCl_3$): 21.13 (CH_3), 21.28 (CH_3), 34.62 (CH_2), 45.62 (CH_2), 126.64, 127.03, 127.93, 128.79, 128.95, 129.58, 131.81, 136.03, 137.93, 142.41, 148.47.

3,4-Di-p-tolyl-7,8-dihydro-6H-imidazo[2,1-b][1,3]thiazine (10)

A mixture of 4,5-di-p-tolyl-1,3-dihydro-imidazole-2-thione (2.5 mmol; 0.7 g) and 1-bromo-3-chloropropane (2.5 mmol; 0.394 g; 0.247 mL) in 35 mL of DMF was heated at 130–135°C for 5 hours in the presence of K_2CO_3 (3.75 mmol; 0.518 g). At the end of this period, the mixture was allowed to stand until it reached room temperature, then the content of reaction vessel was poured onto iced water and the pH was adjusted by ~11 by 10% NaOH aq. solution. The crude product thus obtained was collected by filtration, dried, and purified by column chromatography ($CHCl_3$:MeOH, 97:3, Rf: 0.65), with a yield of 65% (0.518 g). m.p. = 206–207°C. IR (KBr, cm^{-1}): 1683–1472 (C=N and C=C), 821 (1,4-disubstituted-Ph ring deformation band). ^{1}H NMR: ($CDCl_3$): 2.20–2.30 (m, 2H), 2.27 (s, CH_3), 2.41 (s, CH_3), 3.12–3.16 (m, 2H), 3.71–3.76 (m, 2H), 6.99 (AA'BB', J_{AB} = 8.1 Hz, 2H), 7.19 (AA'BB', J_{AB} = 8.4 Hz, 2H), 7.23 (AA'BB', J_{AB} = 8.4 Hz, 2H), 7.35 (AA'BB', J_{AB} = 8.1 Hz, 2H). ^{13}C NMR: ($CDCl_3$): 21.10 (CH_3), 21.33 (CH_3), 23.98 (CH_2), 25.70 (CH_2), 43.73 (CH_2), 126.50, 127.38, 128.68, 128.77, 129.64, 130.63, 131.42, 135.71, 137.09, 137.2, 138.31.

*3,4-Bis-(4-methoxy-phenyl)-6,7-dihydro-imidazo[2,1-b][1,3]thiazole (**11**) (Cherkofsky and Sharpe, 1977; Metabio-Fr, 1977; Bender, 1978; Rihiaruto and Pieru, 1979; Goeschke and Ferrini, 1980; Bender, 1981; Bender* et al.*, 1985; Pooru* et al.*, 1987; Cheng* et al.*, 1989; Kolbe* et al.*, 2004)*

A mixture of 4,5-bis-(4-methoxy-phenyl)-1,3-dihydro-imidazole-2-thione (2.5 mmol; 0.78 g) and 1-bromo-2-chloroethane (2.5 mmol; 0.358 g; 0.225 mL) in 30 mL of DMF was heated at 135–137°C for 5.5 hours in the presence of K_2CO_3 (3.75 mmol; 0.518 g). At the end of the reaction, mixture was allowed to stand until it reached room temperature, then the content of reaction vessel was poured onto iced water and the pH was adjusted to ~11 by 10% NaOH aq. solution. The crude product thus obtained was collected by filtration, dried, and purified by column chromatography ($CHCl_3$:MeOH, 97:3, Rf: 0.6). In order to remove unreacted 1-bromo-2-chloroethane, product was washed with diethyl ether, dried, with a yield of 91% (0.767 g). m.p. = 209–210°C. IR (KBr, cm^{-1}): 1600–1458 (C=N and C=C), 833 (1,4-disubstituted-Ph ring deformation band). ^{1}H NMR: ($CDCl_3$): 3.78 (s, OCH_3), 3.80 (t, J = 7.5 Hz, 2H), 3.85 (s, OCH_3), 4.06 (t, J = 7.5 Hz, 2H), 6.77

(AA'BB', J_{AB} = 9.0 Hz, 2H), 6.93 (AA'BB', J_{AB} = 8.7 Hz, 2H), 7.26 (AA'BB', J_{AB} = 8.7 Hz, 2H), 7.43 (AA'BB', J_{AB} = 8.7 Hz, 2H). ^{13}C NMR: ($CDCl_3$): 34.64 (CH_2), 45.58 (CH_2), 55.14 (OCH_3), 55.28 (OCH_3), 113.53, 114.36, 122.35, 126.13, 127.45, 127.91, 130.47, 136.26 (2C), 158.23, 159.36.

*3,4-Bis-(4-methoxy-phenyl)-7,8-dihydro-6H-imidazo[2,1-b][1,3]thiazine (**12**) (Gupta and Pujari, 1978; Rihiaruto and Pieru, 1979; Goeschke and Ferrini, 1980; Bender et al, 1985; Pooru et al., 1987)*

A mixture of 4,5-bis-(4-methoxy-phenyl)-1,3-dihydro-imidazole-2-thione (2.5 mmol; 0.78g) and 1-bromo-3-chloropropane (2.5 mmol; 0.394 g; 0.247 mL) in 35 mL of DMF was heated at 135–137°C for 5 hours in the presence of K_2CO_3 (3.75 mmol; 0.518 g). At the end of this period, mixture was allowed to stand until it reached room temperature, then the content of reaction vessel was poured onto iced water and the pH was adjusted to ~11 by 10% NaOH aq. solution. The crude product thus obtained was collected by filtration, dried, and purified by column chromatography ($CHCl_3$:MeOH, 97:3, Rf: 0.5), with a yield of 72% (0.634 g). m.p. = 191–192°C. IR (KBr, cm^{-1}): 1608–1456 (C = N and C=C), 831 (1,4-disubstituted-Ph ring deformation band). ^{1}H NMR: ($CDCl_3$): 2.21–2.31 (m, 2H), 3.12–3.16 (m, 2H), 3.70–3.75 (m, 2H), 3.74 (s, OCH_3), 3.85 (s, OCH_3), 6.73 (AA'BB', J_{AB} = 9.0 Hz, 2H), 6.95 (AA'BB', J_{AB} = 8.7 Hz, 2H), 7.22 (AA'BB', J_{AB} = 8.7 Hz, 2H), 7.39 (AA'BB', J_{AB} = 9.0 Hz, 2H). ^{13}C NMR: ($CDCl_3$): 23.99 (CH_2), 25.70 (CH_2), 43.62 (CH_2), 55.08 (OCH_3), 55.25 (OCH_3), 113.39, 114.38, 122.50, 127.10, 127.71, 127.96, 132.08, 136.95, 158.02, 159.61.

Biochemistry

Cell cultures

5RP7 cells were derived by transfection of the parenteral F2408 rat embryo fibroblast cells with a pEI plasmid containing c-Ha-*ras* cloned from T24 carcinoma cells (Garbisa *et al*., 1987). Cells were maintained in Dulbecco Modified Eagle Medium (DMEM) (Sigma) supplemented with 10% (v/v) foetal calf serum (FCS) (Gibco), penicillin/streptomycin at 100 Units/mL as adherent monolayers. Both cell lines were incubated at 37°C in humid atmosphere saturated with 5% CO_2.

Stock solutions of 3,4-disubstituted-6,7-dihydro-imidazo[2,1-b][1,3]thiazoles (**7, 9,** and **11**) and 3,4-disubstituted-7,8-dihydro-6H-imidazo[2,1-b][1,3]thiazines (**8, 10,** and **12**) were prepared in dimethyl sulfoxide (DMSO) and further dilutions were made with fresh culture medium. (The concentration of DMSO in the final culture medium was <1%, which had no effect on the cell viability) (Jiang *et al*., 2003).

MTT assay

The mitochondrial activity of 5RP7 and F2508 cells after exposure to title compounds (**7–12**) were determined by colorimetric assay, which detects the

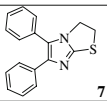
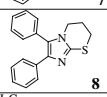
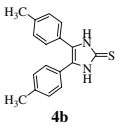
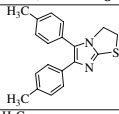
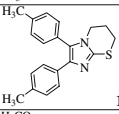
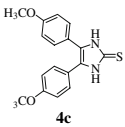
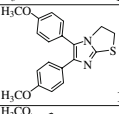
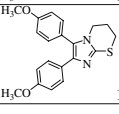
conversion of 3-(4,5-dimethyl-thiazollyl-2)-2,5-diphenyltetrazolium bromide (MTT; Sigma) to formazan (Mosmann, 1983). Briefly, cells were cultured (2×10^4 cell/mL) in 96-well microtiter tissue culture plates and 0.0128, 0.0256, 0.064, 0.128, 0.32, 0.8, 1.6, 3.2, 8,16-40 μg/mL of the title compounds (**7–12**) were added and incubated for 24 and 48 h, after which MTT (5 mg/mL) was added to each well. Absorbance was read at 540 nm on a microplate reader (Elx808-IU Bio-Tek plate reader). Each concentration was repeated in three wells and control cell viability was accepted as 100%.

Results and Discussion

Chemistry

The starting material of the synthesis of compounds **7–12** was thione (Gregoire *et al*., 1951; Yoshida *et al*., 1951) (**4**), which was prepared by condensation of thiourea (**3**) and α-hydroxy ketone (acyloin) (Ide and Buck, 1948) (**2**), obtained in turn from the corresponding aldehyde (**1**). The synthesis of all compounds was achieved through the strategy (Bender, 1978; Bender *et al*., 1985) outlined in Scheme 1.

Table 1 Some characterizations of the compounds[a]

Comp.	Starting Material	Reactant	Target compound[a]	Molecular formula	Molecular weight (g/mol)	m.p.(°C)
7	 **4a**	 **5**	 **7**	$C_{17}H_{14}N_2S$	278.38	165-166
8		 **6**	 **8**	$C_{18}H_{16}N_2S$	292.41	191
9	 **4b**	 **5**	 **9**	$C_{19}H_{18}N_2S$	306.43	173
10		 **6**	 **10**	$C_{20}H_{20}N_2S$	320.46	206-207
11	 **4c**	 **5**	 **11**	$C_{19}H_{18}N_2O_2S$	338.43	209-210
12		 **6**	 **12**	$C_{20}H_{20}N_2O_2S$	352.46	191-192

[a] All compounds exhibited IR, ^{1}H- and ^{13}C-NMR spectra consistent with structures. (See Experimental section for detail)

The detailed synthesis approach for the 3,4-disubstituted-6,7-dihydro-imidazo[2,1-b][1,3]thiazoles **(7, 9,** and **11)** (Table 1) and 3,4-disubstituted-7,8-dihydro-6H-imidazo[2,1-b][1,3]thiazines **(8, 10,** and **12)** (Table 1) are described in the Experimental section.

Conclusion

The title compounds (**7–12**) were prepared from the appropriate acyloin **2**, which were condensed with thiourea (**3**) and treated with dihaloalkane (1-bromo-2(or 3)-chloro-ethane (or propane) (**5, 6**).

Reaction of aryl aldehyde (**1a–c**) in the presence of NaCN in a simple one-pot reaction gave acyloins (**2a-c**), which were subjected to condensation with urea (herein thiourea, **3**) to give 4,5-diaryl-imidazole-2-thione (**4a–c**). Cyclization of these compounds by *vicinal* or *isolated* dihaloalkanes (**5** or **6**) afforded the title imidazole[2,1-b][1,3]thiazoles (**7, 9,** and **11**) and thiazines (**8, 10,** and **12**). It should be noted that the use of t-BuOK in 1,4-dioxane instead of K_2CO_3 in DMF did not provide the target compounds.

The physical data of compounds (**7–12**), in agreement with the assigned structures, are reported under the experimental protocols.

Since some peaks belong to precursor thione (**4a–c**) disappeared on the IR spectra, i.e., at 3300–3100 cm^{-1} (NH) and 1250–1020 cm^{-1} (C=S) stretching absorption, the structures of **7–12** were confirmed. The disappearance of the NH and C=S bands at 3300–3100 cm^{-1} and 1250–1020 cm^{-1}, respectively, in the spectra of the target compounds (**7–12**) provided evidence for the imidazo[2,1-b][1,3]thiazole and imidazo[2,1-b][1,3]thiazine structure.

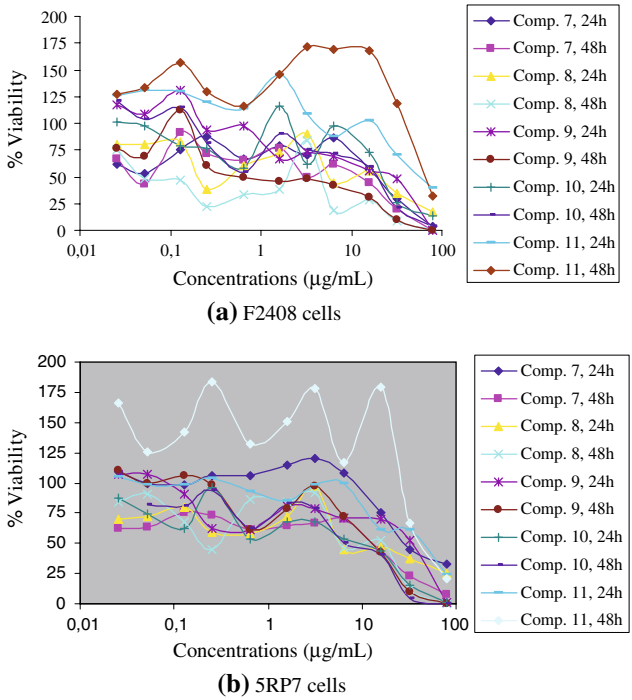
^{1}H- and ^{13}C-NMR data for compounds **7–12** are presented in the experimental section for each compound. For all the synthesized compounds **7–12**; the aromatic protons that belonged to the phenyl attached at the fourth and fifth position of the imidazole ring gave a signal at 6.73–7.52 ppm. The aromatic methyl protons of compounds **9** and **10** were observed at 2.27–2.41 ppm as a singlet. The aromatic methoxy protons of compounds **11** and **12** gave signal at 3.74–3.85 ppm as a singlet. $–CH_2–$ protons of thiazole were signaled as triplets at 3.81 and 4.01 ppm ($J = 7.2$ Hz) (**7**), at 3.77 and 4.05 ppm ($J = 6.9$ Hz) (**9**), and at 3.80 and 4.06 ppm ($J = 7.5$ Hz) (**11**), respectively. On the other side, $–CH_2–$ protons of thiazine were observed as multiplets (**8**, **10,** and **12**).

Aliphatic and aromatic carbons have a signal in the expected region of the ^{13}C-NMR spectra of compounds **7–12**.

In order to obtain information about the strereochemistry of the molecules and to confirm the assigned structures, we previously carried out an X-ray analysis of compound **9** (Özbey and Meriç, 2006).

Biochemistry

Methylthiotetrazole produces a dark blue formazan product when incubated with living cells. The MTT ring is cleaved only in active mitochondria (Mosmann, 1983).



(a) F2408 cells

(b) 5RP7 cells

Fig. 1 Cytotoxic effects of compounds **7–11** on (a) F2408 and (b) 5RP7 cells measured by the MTT assay. Cells were incubated with increased concentrations of Compounds **7–11** for 24 and 48 h and the response were measured by the MTT assay. Values are means ± standard error on the mean (SEM) of two independent experiments performed in triplicates. Results were measured as percentage absorbance of MTT (absorbance rate on compounds and noncancer cells). The standard deviation (SD) for the assay was 10% of the mean or less

The cytotoxic effects of imidazo[2,1-b][1,3]thiazoles (**7,9,11**) and imidazo[2,1-b][1,3]thiazines (**8, 10**) against 5RP7 and F2408 cell lines are shown in Fig. 1.

Some compounds showed dose- and time-dependent cytotoxic effects on both cell lines.

7 showed high cytotoxic effects against F2408 control cells after 24 and 48 h. However 5RP7 (H-ras active rat cells) cells treated with the same compound exhibited cytotoxic effects at high concentrations and longer incubation time (48 h) (Fig. 1).

Compound **8** was also found to be toxic to both cell lines. After treatment with lower concentrations (0.0128, 0.0256, and 0.064 µg/mL) of compound **8**, the cytotoxic effect was found to be 30%, 28%, and 21% on 5RP7 cells and 20%, 21%, and 18% on F2408 cells, respectively. Except the results obtained from the treatment with compound **8** at 0.0128 µg/mL, the differences between the cytotoxic effects were not significant (Fig. 1). The effect of compound **10** was quite similar to that of compound **8**. However this compound seemed to be slightly more effective in 5RP7 cells than the control cells at lower concentrations. 5RP7 cells exhibited 87% viability at 0.0128 µg/mL of compound **10** after 24 h; under the same

Table 2 IC_{50} values of the tested compounds (**7–11**) for F2408 and 5RP7 cell lines

Comp	IC_{50}^{a} for F2408 (μg/mL)	IC_{50}^{a} for 5RP7 (μg/mL)
7	9.5	15
8	10	2.9
9	15	18
10	12	4.6
11	29	22

[a] The concentration required to inhibit the cellular growth by 50% (after 24 h of drug exposure); see the Experimental section for more details . Values are expressed as mean ± SD ($n = 3$). The standard deviation (SD) for the assay was 10% of the mean or less

conditions 100% viability was detected in control cells. Even after two- and fivefold increases of the concentrations the viability was detected at the same ratio.

In the presence of only lower doses of compounds **9** and **11**, the cytotoxic effect was higher on 5RP7 cells than on the control cells after 24 h (Fig. 1).

The 50% inhibitory concentration (IC_{50}) value for the 5RP7 cell line was determined to be 15 μg/mL of compound **7**, 2.9 μg/mL of compound **8**, 18 μg/mL of compound **9**, 4.6 μg/mL for compound **10**, and 22 μg/mL for compound **11** (Table 2).

The IC_{50} value for the F2408 cell line was determined to be 9.5 μg/mL for compound **7**, 10 μg/mL for compound **8**, 15 μg/mL for compound **9**, 12 μg/mL for compound **10**, and 29 μg/mL for compound **11** (Table 2).

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