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Preliminary communication

Synthesis of some bioactive 2-bromo-5-methoxy-*N*′-[4-(aryl)-1,3-thiazol-2-yl]benzohydrazide derivatives

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Abstract

Eight novel 2-bromo-5-methoxy-*N*′-[4-(aryl)-1,3-thiazol-2-yl]benzohydrazide derivatives were prepared and characterized by analytical and spectral analyses. All the compounds were screened for their analgesic, antifungal and antibacterial activities and three of the compounds were screened for antiproliferative activity. Two of the newly synthesized compounds exhibited promising analgesic activity and one compound exhibited in vitro antiproliferative activity.

Keywords: Synthesis; Bioactive; 2-Bromo-5-methoxy; Thiazolylbenzohydrazide

1. Introduction

Antimicrobial activity of thiazole derivatives has been extensively studied by many researchers [1–3]. Organic compounds bearing thiazoles of different pharmacodynamic nuclei were found to possess antiinflammatory activity [4,5]. Compounds containing thiazole ring system are used as antiviral agents and some are used as pesticides [6,7]. Antitumor and cytotoxic activities of thiazole derivatives are well known in the literature [8–10]. As a continuation of our research work to explore potent bioactive thiazole containing molecules [11–13], eight new 2-bromo-5-methoxy-*N*′-[4-(aryl)-1,3-thiazol-2-yl]benzohydrazide derivatives were prepared and characterized by analytical and spectral methods. All the eight compounds were screened for their analgesic, antifungal and antibacterial activities. Three of the compounds have also been screened for their antiproliferative activity.

2. Results and discussion

2.1. Chemistry

Methyl-2-bromo-5-methoxybenzoate was converted to 2-bromo-5-methoxybenzoic acid hydrazide [19,20], which was then converted to 1-(2-bromo-5-methoxybenzoyl) thiosemicarbazide **1** by treating with KSCN and conc. HCl. 1-(2-Bromo-5-methoxybenzoyl) thiosemicarbazide **1** was then refluxed with different aromatic acyl bromides **2a–h** in ethanol to obtain 2-bromo-5-methoxy-*N*′-[4-(aryl)-1,3-thiazol-2-yl]-benzohydrazide derivatives **3a–h**, Scheme 1. All the compounds were isolated in good yield after recrystallisation from methanol–DMF mixture. Few compounds were characterized by IR, ^{1}H NMR and mass spectral analyses. Characterization data of the compounds are given in Table 1.

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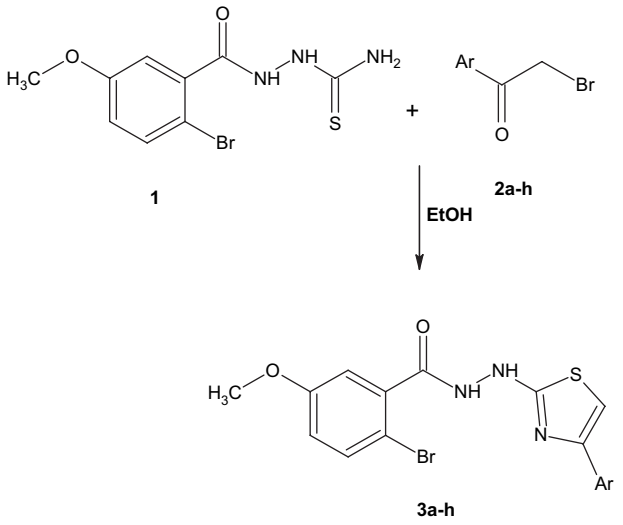


Scheme 1.

The ^{1}H NMR spectrum of compound **3a** gave a singlet at δ 3.88 due to three protons of the $-OCH_3$ group. Two doublets at δ 6.93 ($J = 9.0$ Hz) and δ 7.63 ($J = 9.0$ Hz), two singlets at δ 7.05 and δ 7.14 and a doublet of doublet at δ 7.9 ($J = 9.0$ Hz) are due to aromatic protons. A singlet at δ 8.5 is due to $-$NH protons and another singlet at δ 10.7 is due to $-$OH proton. FABMS displayed a peak at *m/z* 463 (70%) that corresponds to the molecular ion peak. Another peak at *m/z* 465 (75%) corresponds to M + 2 ion peak. Elemental analysis also gave satisfactory results for all the compounds. Spectral data of other compounds are given in Section 4.

2.2. Biological activities

2.2.1. Analgesic activity (acetic acid induced writhing test)

All the newly synthesized compounds were evaluated for their analgesic activity by acetic acid induced writhing test. Swiss albino mice (25–30 g) of either sex were used for the experiment. The mice were divided into 10 groups of two each and was given the drugs in 2% gum acacia orally by gavage feeding as shown in Table 2. Group 1 was given 2% gum acacia. Group 2 was given diclofenac sodium at a dose of 2.5 mg/kg. Groups 3–10 were administered the test compounds **3a**–**h**, respectively, at a dose of 50 mg/kg suspended in 10 ml/kg of 2% gum acacia orally by gavage feeding. Writhing was induced 1 h later by intraperitoneal injection of 10 ml/kg of 0.6% acetic acid in distilled water [17]. The number of writhes was counted for 15 min immediately after the acetic acid injection. The percentage of protection was calculated. The analgesic activity was compared with diclofenac sodium (2.5 mg/kg) and the results are given in Table 2. The experiment was conducted after obtaining approval from Institutional Animal Ethics Committee.

The results show that the analgesic activity of compound **3a** (50 mg/kg) is comparable to that of standard drug diclofenac sodium (2.5 mg/kg). Compounds **3e** and **3g** have also exhibited promising analgesic activity. The exact structure–activity relationship is not clear from our studies. The higher activity of **3a** may be due to the presence of bioactive salicylamide moiety, which is a moiety present in antihypertensive drug Labetalol. More studies have to be conducted to get conclusive results.

2.2.2. Antiproliferative activity

Three of the newly synthesized compounds such as **3a**, **3b** and **3d** were screened for their anticancer activity at NIH, Bethesda, Maryland, USA under the Drug Discovery Programme of NCI as per the procedure suggested by Boyd and Paull [18] in a primary three cell line-one dose antitumor assay against NCI-H (lung), MCF-7 (breast) and SF-268 (CNS). In the current protocol each cell line is inoculated on a preincubated microtiter plate. Test agents are added at a single concentration and the culture is incubated for 48 h. End point of determinations is made with sulphorhodamine B, a protein binding dye. Compounds which reduce the growth of any one of the cell line to 32% or less (negative numbers indicate cell killing) are passed on for evaluation in a panel of 60 cell lines over a 5-long dose range. In the present screening programme (Table 3) only **3a**, 2-bromo-5-methoxy-*N*′-[4-(4-hydroxy-3-benzamido)-1,3-thiazol-2-yl]benzohydrazide was selected for 60 cell line screening and it showed some antiproliferative activity on the whole panel of 60 cells derived from seven cancer cells namely lung, colon, melanoma, renal, ovarian, CNS and leukemia. Their GI_{50}, TGI and LC_{50} values were determined.

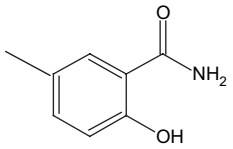
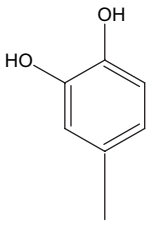
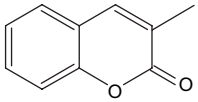
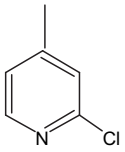
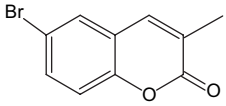
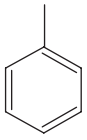
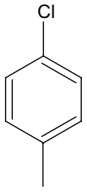
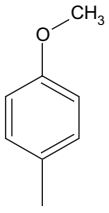
The 60 cell line screening results show that compound **3a** showed highest antiproliferative activity against *leukemia* MOLT-4 cell line with $GI_{50} = 22.6$, $TGI = 57.0$ and $LC_{50} \geq 100$. The compound was also active against *leukemia* RPMI-8226 [$GI_{50} = 27.9$, $TGI = 92.5$ and $LC_{50} \geq 100$] and SR [$GI_{50} = 29.8$, $TGI \geq 100$ and $LC_{50} \geq 100$] at concentrations below 30 μM. For all other cell lines, the compound was ineffective. As observed in analgesic activity, presence of bioactive salicylamide moiety enhances the antiproliferative activity also.

2.2.3. Antibacterial and antifungal activities

All the newly synthesized compounds **3a**–**h** were evaluated for their antibacterial and antifungal activities by disc diffusion method and serial plate dilution method [14–16]. Furacin was used as standard antibacterial drug and Itraconozole was used as standard antifungal drug. The results are given in Table 4.

Compound **3b** bearing 3,4-dihydroxyphenyl moiety exhibited maximum antibacterial activity. Compounds **3a** and **3d** bearing salicylamide and 2-chloropyridinyl moieties exhibited maximum antifungal activity among the tested compounds. In our earlier studies [11–13], we have reported the promising antimicrobial activity of thiazoles bearing salicylamide, 4-dihydroxyphenyl and 2-chloropyridinyl moieties. Presence of these potent moieties may be the reason for higher activity of **3a** and **3d**.

Table 1
Physical and analytical data of the compounds **3a–h**

Compound no.	Ar-	Yield[a] (%)	Mp (°C)	Molecular formula	Nature of product
3a		90	228–232	$C_{18}H_{15}BrN_4O_4S$	Off white crystals
3b		90	192–196	$C_{17}H_{14}BrN_3O_4S$	Yellow crystals
3c		90	200–204	$C_{20}H_{14}BrN_3O_4S$	Yellow microcrystals
3d		64	232–236	$C_{16}H_{12}ClBrN_4O_2S$	Dark yellow crystals
3e		80	250–254	$C_{220}H_{13}Br_2N_3O_4S$	Yellow crystals
3f		65	162–164	$C_{17}H_{14}BrN_3O_2S$	Off white crystals
3g		89	228–230	$C_{17}H_{13}BrClN_3O_2S$	Light yellow crystals
3h		85	228 (dec.)	$C_{18}H_{26}BrN_3O_3S$	Cream microcrystals

[a] All the yields are on isolated basis. All the compounds gave satisfactory results for elemental analysis. Recrystallisation solvent, methanol–DMF mixture (1:9).

Table 2
Analgesic activity (acetic acid induced writhing test) of compounds **3a–h**

Group	Drug	Dose (mg/kg)	Average time taken for onset writhing (s)	No. of writhes for 15 min	Protection (%)
1	2% gum acacia	10 ml/kg	246	16	–
2	Diclofenac sodium	2.5	692	6	62.5
3	**3a**	50	84	7	56.25
4	**3b**	50	432	14	12.5
5	**3c**	50	480	10	37.5
6	**3d**	50	506	10	37.5
7	**3e**	50	454	8	50.0
8	**3f**	50	524	14	12.5
9	**3g**	50	496	9	43.75
10	**3h**	50	336	12	25

$N = 2$ in each group.

3. Experimental

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Merck silica gel 60 F_{254} coated aluminium plates. IR spectra were recorded on Shimadzu-FTIR spectrometer in KBr (ν_{max} in cm^{-1}). ^1H NMR spectra were recorded in $CDCl_3$ and in DMSO-d_6 on a Varian (300 MHz) spectrometer using TMS as an internal standard and ^{13}C NMR spectra were recorded in $CDCl_3$ and in DMSO-d_6 on a Varian (75 MHz) spectrometer. FABMS spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas.

Compound 2-bromo-5-methoxybenzohydrazide was prepared from methyl-2-bromo-5-methoxybenzoate [19] by treating with hydrazine hydrate in methanol; mp 172–174 °C. ^1H NMR (300 MHz): δ 3.79 (s, 3H, $-OCH_3$), δ 6.84 (dd ($J = 8.8$ Hz), 1H, Ar–H), δ 6.95 (d ($J = 2.96$ Hz), 1H, Ar–H), δ 7.45 (d ($J = 8.8$ Hz), 1H, Ar–H), δ 9.26 (br s, 1H, –NH).

3.1. Synthesis of 1-(2-bromo-5-methoxybenzoyl) thiosemicarbazide (*1*) [20]

2-Bromo-5-methoxybenzohydrazide (50 g, 0.204 mol), potassium thiocyanate (25 g, 0.267 mol) and 40 ml of conc. HCl in 400 ml of water were refluxed for 4 h. A white solid appeared on cooling and then the solid was filtered and dried. Yield 51.2 g (74.4%); mp 190–192 °C. IR (KBr, cm^{-1}): 3280 (NH), 1670 (CONH), 1360 (C=S).

Table 3
Antiproliferative activity screening data of the compounds **3a**, **3b** and **3d**

Compound	NCI code	Growth percentage[a]		
		NCI-H	MCF-7	SF-268
3a	NSC 736958	17	19	55
3b	NSC 736960	101	86	111
3d	NSC 736959	95	87	111

Fixed concentration (100 μM; standard NCI protocol).
[a] Active when growth percentage is <32% for any one of the three line cells.

3.2. Synthesis of 2-bromo-5-methoxy-N′-[4-(aryl)-1,3-thiazol-2-yl]benzohydrazide (*3a–h*)

1-(2-Bromo-5-methoxybenzoyl) thiosemicarbazide (**1**) (1 g, 0.0032 mol) was refluxed with appropriate acyl bromide (0.0032 mol) in 10 ml ethanol for 10 h and kept overnight. The solid separated on cooling was filtered and the crude products were recrystallised from 10% methanol in DMF. All the compounds were isolated in 64–90% yields. The characterization data are given in Table 1.

4. Spectral and analytical data

4.1. 2-Bromo-5-methoxy-N′-[4-(4-hydroxy-3-benzamido)-1,3-thiazol-2-yl]benzohydrazide (*3a*)

^1H NMR: ($CDCl_3$, 300 MHz) δ 3.88 (s, 3H, $-OCH_3$), δ 6.93 (d ($J = 2.9$, 9.0 Hz), 1H, Ar–H), δ 7.05 (s, 1H, Ar–H), δ 7.14 (s, 1H, Ar–H), δ 7.63 (d ($J = 9.0$ Hz), 1H, Ar–H), δ 7.9 (dd ($J = 9.0$ Hz), 2H, Ar–H), δ 8.5 (s, 2H, –NH–NH–, exchangeable with D_2O), δ 10.7 (s, 1H, –OH); FABMS: *m/z* 463 (M^+, 70%), 465 (M + 2, 75%). Anal. Calcd for $C_{18}H_{15}BrN_4O_4S$: %C, 46.66; %H, 3.26; %N, 12.09. Found: %C, 46.29; %H, 3.17; %N, 11.89.

4.2. 2-Bromo-5-methoxy-N′-[4-(3,4-dihydroxyphenyl)-1,3-thiazol-2-yl]benzohydrazide (*3b*)

^1H NMR: ($CDCl_3$, 300 MHz) δ 3.84 (s, 3H, $-OCH_3$), δ 6.82 (d ($J = 8.2$ Hz), 1H, Ar–H), δ 6.87 (d ($J = 8.2$ Hz), 1H, Ar–H), δ 6.97 (m, 2H, Ar–H), δ 7.07 (d ($J = 8.29$ Hz), 1H, Ar–H), δ 7.19 (t, 2H, Ar–H), δ 7.62 (d ($J = 8.79$ Hz), 1H, Ar–H), δ 11.33 (s, 2H, –NH–NH–, exchangeable with D_2O); FABMS: *m/z* 440 (M + 1, 100%), 436 (M^+, 95%) 438 (M + 2, 100%). Anal. Calcd for $C_{17}H_{14}BrN_3O_4S$: %C, 46.80; %H, 3.23; %N, 9.63. Found: %C, 46.59; %H, 3.17; %N, 9.36.

4.3. 2-Bromo-5-methoxy-N′-[4-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]benzohydrazide (*3c*)

^1H NMR: ($CDCl_3$, 300 MHz) δ 3.85 (s, 3H, $-OCH_3$), δ 6.92 (dd ($J = 3.15$, 8.81 Hz), 1H, Ar–H), δ 7.12 (d ($J = 2.95$ Hz), 1H, Ar–H), δ 7.33 (s, 1H, Ar–H), δ 7.35 (s, 1H, Ar–H), δ 7.51 (t, 1H, Ar–H), δ 7.54 (s, 1H, Ar–H), δ 7.23 (d ($J = 7.94$ Hz), 1H, Ar–H), δ 7.78 (s, 1H, Ar–H), δ 8.61 (s, 1H, Ar–H), δ 10.49 (s, 2H, –NH–NH–, exchangeable with D_2O); FABMS: *m/z* 474 (M + 2, 100%), 472 (M^+, 98%). Anal. Calcd for $C_{20}H_{14}BrN_3O_4S$: %C, 50.86; %H, 2.99; %N, 8.90. Found: %C, 50.47; %H, 3.24; %N, 8.78.

4.4. 2-Bromo-5-methoxy-N′-[4-(4-chlorophenyl)-1,3-thiazol-2-yl]benzohydrazide (*3g*)

^1H NMR: ($CDCl_3$, 300 MHz) δ 3.86 (s, 3H, $-OCH_3$), δ 7.0 (dd ($J = 2.66$, 8.88 Hz), 1H, Ar–H), δ 7.25 (t, 1H, Ar–H), δ 7.55 (s, 1H, Ar–H), δ 7.58 (s, 1H, Ar–H), δ 7.23 (dd

Table 4
Antibacterial and antifungal activities of the compounds **3a–h** (MIC values in mg/ml)

Compound	Antibacterial activity				Antifungal activity			
	Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Penicillium marneffei*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Trichophyton mentagrophytes*
3a	–	12.5	–	12.5	12.5	60	12.5	12.5
3b	50	12.5	6.25	50	–	12.5	50	100
3c	50	–	–	50	12.5	–	100	100
3d	–	50	–	–	12.5	50	12.5	12.5
3e	–	–	–	50	12.5	–	10	12.5
3f	–	–	–	–	100	–	–	–
3g	–	–	50	–	–	50	–	50
3h	–	–	50	–	100	–	50	–
Furacin	12.5	12.5	6.25	12.5	–	–	–	–
Itraconozole	–	–	–	–	<16	<16	<16	<16

($J = 9.0$ Hz), 4H, Ar–H), δ 8.31 (s, 2H, –NH–NH–, exchangeable with D_2O); FABMS: *m/z* 440 (M + 1, 100%), 439 (M^+, 60%), 438 (M − 1, 75%). Anal. Calcd for $C_{17}H_{13}BrClN_3O_2S$: %C, 46.54; %H, 2.99; %N, 9.58. Found: %C, 46.32; %H, 2.86; %N, 9.36.

5. Conclusion

Eight novel 2-bromo-5-methoxy-*N*′-[4-(aryl)-1,3-thiazol-2-yl]benzohydrazide derivatives were prepared and screened for analgesic, antifungal, antibacterial and anticancer activities. Compound **3a** exhibited promising analgesic activity. Compound **3b** exhibited maximum antibacterial activity and compounds **3a** and **3d** exhibited maximum antifungal activity among the tested compounds. Compound **3a** further displayed some antiproliferative activity and the presence of salicylamide group may be contributing to its enhanced biological activity.

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