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Preliminary communication

Synthesis and antibacterial activity of some 5-guanylhydrazone/thiocyanato-6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide derivatives †

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Abstract – 6-Arylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamides **3** on Vilsmeier-Haak reaction produced 5-formyl-6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamides **4**, while **3** on treatment with potassium thiocyanate in the presence of bromine in acetic acid produced 5-thiocyanato-2-sulfonamides **6**. Interaction of **4** with aminoguanidine hydrochloride in ethanol produced the corresponding 5-guanylhydrazone derivatives **5**. Compounds **5** and **6** showed a high degree of antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus* comparable to that of sulfamethoxazole and Norfloxacin. However, they were found to show moderate activity against *Salmonella typhi*, *Pseudomonas aeruginosa* and *Pneumococci*. © 2000 Éditions scientifiques et médicales Elsevier SAS

5-guanylhydrazone / thiocyanatoimidazo[2,1-b]-1,3,4-thiadiazoles-2-sulfonamides / synthesis / antibacterial activity

1. Introduction

The development of sulfonamides is a fascinating and informative area in medicinal chemistry, highlighting the role of skillful planning and serendipity in drug research. Sulfonamide derivatives are widely used in various conditions including gastrointestinal and urinary tract infections. They are preferred due to the ease of administration, wide spectrum of antimicrobial activity, non-interference with the host defense mechanism and relative freedom from problems of superinfection. Andreani et al. [1] reported that some imidazo[2,1-b]thiazoles are more active than levamisole.

In view of the above and in continuation of our research [2, 3] on fused imidazo[2,1-b]-1,3,4-thiadiazoles, we report here the synthesis and antibacterial activity of some 5-guanylhydrazone/thiocyanato-6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide derivatives **5** and **6** (*figure 1*). Both **5** and **6** showed promising activity during antibacterial screening against *Escherichia coli* and *Staphylococcus aureus*, and moderate activity against *Salmonella typhi*, *Pseudomonas aeruginosa* and *Pneumococci*.

2. Chemistry

The title compounds were prepared using a synthetic method closely related to the general procedure that we normally employed to obtain bicyclic imidazo[2,1-b]-1,3,4-thiadiazole derivatives [4]. In the present case the condensation of **1** and **2** involved attack of an electrophile α-bromoketone on the more basic *endo* nitrogen (3-N) of 2-amino-1,3,4-thiadiazole–5-sulfonamide **1** followed by dehydrocyclisation of the intermediate **3A** in boiling anhydrous ethanol to yield 6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamides **3**. The products **3** were subjected to Vilsmeier-Haack reaction which involved attack of chloroimmonium ion at 5-position to give 5-formyl derivatives **4**. During this reaction, the dimethylformamide also reacted with the NH_2 function of the 2-sulfonamido group converting it to N-(dimethylaminomethino)sulfonamide group. Reaction of **4** with aminoguanidine hydrochloride gave 5-guanylhydrazones **5** which belonged to the E configuration (*figure 1*). A

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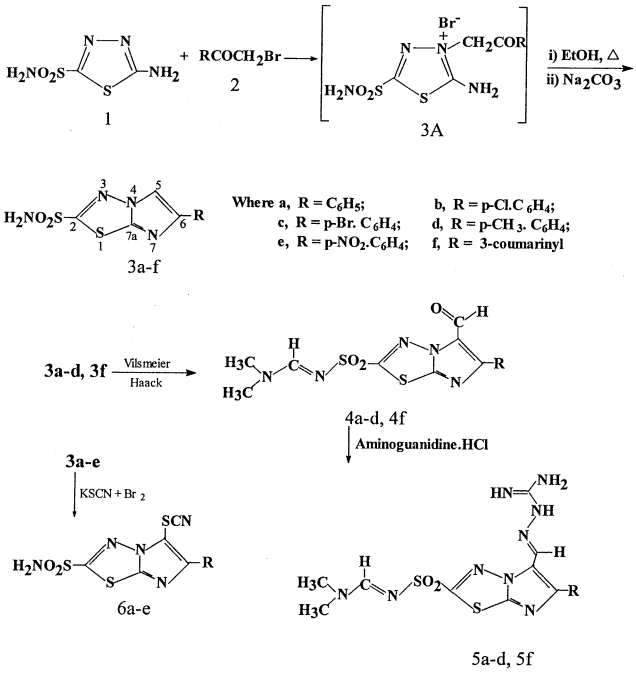

Figure 1.

reaction of **3** with KSCN in the presence of bromine in acetic acid produced 5-thiocyanato derivatives **6**. The correct structural assignments to these products were made by the UV, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. In particular, it must be pointed out that the correct interpretation of the IR and ^{1}H-NMR spectra of the 5-thiocyanato-6-phenyl derivative **6a** gave firm basis of the structural assignments to all other compounds of the series, whereas mass spectral data obtained by using FAB technique and ^{1}H-NMR were the basis for 5-guanylhydrazones **5** (*tables I* and *II*).

5-H of **3** generally appeared as a singlet between δ 8.9–9.2 ppm in ^{1}H-NMR, whereas this singlet disappeared in the case of 5-formyl derivatives **4**. Formyl proton of **4** appeared around δ 10 ppm. Such observation was first reported by O'Daly et al. [5] in the case of 5-formylimidazo[2,1-b]thiazole derivatives.

^{1}H-NMR of **5** showed consistent peaks: particularly the attribution of peaks at δ 12.1 (guanyl NH), 8.55 (5-CH=N), δ 8.40 (–SO_2N=CH) and 7.75–7.50 (aryl protons) and 3.25, 3.00 for –$N(CH_3)_2$ are correct (table I). Compound **5d** showed an additional signal for Ar–CH_3 at δ 2.30 and **5f** showed 6-(3-coumarinyl) protons as a singlet at δ 8.60 and a multiplet at 8.00–7.30 ppm. The singlets at δ 8.55 and δ 8.40 indicated that these compounds are single pure geometrical isomers. On the basis of reported nuclear overhauser enhancement difference spectroscopic experiments [6–8], it was inferred that CH=N– and SO_2–N=CH– of **5** belonged to E-isomer structure (*figure 1*).

We noticed that electron impact mass spectra of **5a–c** did not show the molecular ion peaks at 70 eV. However,

Table I. UV, IR and ^{1}H-NMR spectral data of 5-guanylhydrazone-6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylamino-methino)]sulfonamides **5a–d** and **5f**.

Compound	Ethanol λ_{max} (nm)	IR (KBr): cm^{-1}	^{1}H-NMR (δ ppm) in DMSO-d_6			Aryl	–$N(CH_3)_2$
			=N–NH–	–CH=N-SO_2	5–CH=N		
5a	290, 248, 226	3 412 (b), 1 683, 1 640, 1 319, 1 146, 1 134, 1 078, 911, 850, 670	12.10 s	8.40 s	8.50 s	7.75–7.50 m	3.25 s; 3.00 s
5b	289, 257, 225	3 443 (b), 1 677, 1 634, 1 498, 1 405, 1 313, 1 152, 1 084, 917, 846	12.10 s	8.40 s	8.55 s	7.75–7.50 d	3.25 s; 3.00 s
5c	288, 264, 225	3 412 (b), 1 677, 1 634, 1 492, 1 424, 1 319, 1 152, 985, 924, 850	12.20 s	8.40 s	8.55 s	7.75–7.60 d	3.25 s; 3.00 s
5d	294, 248, 226	3 425 (b), 1 678, 1 641, 1 418, 1 326, 1 147, 1 091, 918, 850	12.10 s	8.40 s	8.55 s	7.70–7.30 d; 2.30 (ArCH_3) s	3.25 s; 3.00 s
5f	349, 292, 225	3 425 (b), 1 727, 1 678, 1 647, 1 486, 1 425, 1 313, 1 153, 1 128, 1 036, 918, 850	12.10 s	8.40 s	8.55 s	8.60 s; 8.00–7.30 m	3.25 s; 3.00 s

Table II. IR and ^{1}H-NMR spectral data of 5-thiocyanato-6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamides **6a–e**.

Compound	IR (KBr) cm^{-1}		^{1}H-NMR (δ ppm) in DMSO-d_6	
	NH_2	SCN	$-SO_2NH_2$	Aryl
6a	3 375, 3 264	2 165	8.80 s	8.0–7.45 m
6b	3 345, 3 172	2 159	8.80 s	8.0–7.60 d
6c	3 320, 3 240	2 159	8.80 s	8.0–7.60 d
6d	3 363, 3 197	2 166	8.80 s	8.0–7.40 d, 2.13 ($ArCH_3$) s
6e	3 329, 3 290	2 159	8.80 s	8.40–8.25 d

when a fast atomic bombardment technique was applied, molecular ion peaks of **5a**, **5b**, **5c** were observed at m/z 419, 453.5 and 498, respectively, confirming their structures. In all these cases, the fragmentation pattern was similar and showed peaks at M^+, 289, 165, 154, 136, 120, 107, 89, 77, 69, 55, 41 and 27.

Thiocyanation of **3a** to **6a** was clearly seen by the appearance of a band around 2 165 cm^{-1} (SCN) in its IR spectrum and absence of a 5-H singlet around 9.0 δ ppm in ^{1}H-NMR spectrum. Structure of **6a** was further supported by MS showing molecular ion peak (m/z) at 337 and ^{13}C-NMR showing signals at δ 102, 110, 148, 152, 166 (C-2, C-5, C-6, C-7a, SCN) and carbons of Ph group at 127–132.5, respectively. Similarly, the structures of remaining compounds **6b–d** were confirmed by their IR and ^{1}H-NMR spectra (*table II*).

3. Results and discussion

Compounds **5** and **6** showed significant antibacterial activity against *E. coli* and *S. aureus*, while they were found to show moderate activity against *S. typhi*, *P. aeruginosa* and *Pneumococci*. The starting compounds **3a–f** were found to possess lesser activity (table III). The most active compounds were **5b**, **5c**, **6b**, **6c**, and **6e** against both *E. coli* and *S. aureus*, which were approximately equipotent in activity and comparable to that of sulfamethoxazole and Norfloxacin at all concentrations. Both the reference drugs exhibit antibacterial activity by inhibiting dihydrofolate reductase and bacterial DNA gyrase, respectively. The antibacterial activity exhibited by **5b–5d** against *S. aureus* was more than that against *E. coli*. Compounds **6b**, **6c** and **6e** showed superior activity against *S. aureus* even at 50 µg concentration. From the results, it is evident that the presence of a 5-guanylhydrozone and 5-thiocyanato group of **3** resulted in producing good antibacterial activity. Particularly 6-p-chlorophenyl, 6-p-bromophenyl and 6-p-nitrophenyl derivatives showed increased activity. All these compounds can be considered as potential candidates for antibacterial activity against both Gram-positive and Gram-negative organisms.

4. Experimental protocols

4.1. Chemistry

Thin layer chromatography by pre-coated silica gel plates (Merck 60 F 254) was used to control the purity of the products; all the compounds were designated as pure when they showed a single spot after elution with chloroform/methanol mixture (95:5). Detection of components was made by UV light and/or treatment with iodine vapours. Melting points were determined by open capillary method and are uncorrected. Elemental analysis, indicated by the symbols of the elements, were within ± 0.4% of the theoretical values. Commercially available pure solvents and chemicals were used throughout the work.

The UV spectra in ethanol were recorded on a Jasco V 530 spectrophotometer, IR spectra (KBr) were recorded on Nicolet Impact-410 FT, ^{13}C- and ^{1}H-NMR spectra were recorded from a solution in DMSO-d_6 on a Varian VXR 500 spectrometer: chemical shift values are reported in δ units (ppm) relative to tetramethylsilane used as internal standard. Mass spectra were recorded on a JEOL MS-DX-303 spectrometer.

The starting 6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamides **3a–f** and 5-formyl-6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamides **4a–d** and **4f** were prepared following this laboratory method [3, 9]. Compounds **3d** and **4d** are new compounds. 6-p-Methylphenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide **3d** was obtained by the condensation of 2-amino-1,3,4-thiadiazole-5-sulfonamide with p-methylphenacylbromide as yellow needles, 51% yield, m.p. 240 °C (d). Anal. $C_{11}H_{10}N_4O_2S_2$ (294); C, H, N. 5-Formyl-6-p-methylphenylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]-sulfonamide **4d** was

Table III. In vitro antibacterial activity data of **3a–f***, **5a–d**, **5f** and **6a–e**[1].

Compound	*Escherichia coli* zone of inhibition in mm			*Staphylococcus aureus* zone of inhibition in mm			*Salmonella typhi* zone of inhibition in mm		*Pseudomonas aeruginosa* zone of inhibition in mm		*Pneumococci* zone of inhibition in mm	
	0.05 mL	0.1 mL	0.15 mL	0.05 mL	0.1 mL	0.15 mL	0.1 mL	0.15 mL	0.1 mL	0.15 mL	0.1 mL	0.15 mL
5a	14	18	22	14	18	20	14	18	14	18	12	14
5b	15	20	27	20	24	28	18	18	14	24	16	20
5c	15	20	24	22	24	27	14	18	14	24	14	18
5d	12	18	22	18	19	22	18	18	14	24	14	18
5f	15	18	22	14	16	18	14	20	14	14	14	18
6a	14	16	23	16	18	22	16	20	12	16	14	18
6b	16	18	24	25	26	27	14	22	12	14	18	20
6c	14	18	23	24	25	26	18	20	14	14	14	18
6d	12	16	20	17	20	24	14	18	12	16	16	20
6e	16	20	24	24	26	28	16	22	12	18	18	20
Sulfamethoxazole	18	22	24	12	18	27	29	32	–	–	–	–
Norfloxacin	30	30	30	30	30	30	36	40	20	27	37	42

[1] A majority of the compounds showed the resistance against *S. typhi*, *P. aeruginosa* and *Pneumococci* at a concentration of 50 μg/mL, hence these values are not included in the table. Control (DMF) did not show any activity. Cup diameter was 7 mm for 0.05 mL and 0.1 mL, and 10 mm for 0.15 mL. * Compounds **3a–f** did not show activity against *S. aureus* and *P. aeruginosa*; however, they were moderately active against *E. coli*, *S. typhi* and *Pneumococci* (16–22 mm).

prepared by treating **3d** with freshly prepared Vilsmeier reagent and recrystallised from ethanol as yellow needles, 83% yield, m.p. 222–225 °C. Anal. $C_{15}H_{15}N_5O_3S_2$ (377); C, H, N.

4.2. 5-Guanylhydrazone-6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamides **5**

General method:

5-Formyl-6-phenylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamide **4a** (3.63 g, 0.01 mol) was dissolved in 150 mL of ethanol and treated with (0.01 mol) of aminoguanidine hydrochloride prepared from treating the suspension of aminoguanidine bicarbonate in ethanol with excess of 37% hydrochloric acid. The reaction mixture was refluxed for 30 min and the resulting precipitate was collected by filtration and dried. The HCl salt thus obtained was suspended in ice cold water and basified with aqueous ammonia solution. The free base **5a** thus formed was filtered, dried and recrystallised from ethanol as yellow flakes in 70% yield, m.p. 222–226 °C. Anal. $C_{15}H_{17}N_9O_2S_2$ (419); C, H, N.

5-Guanylhydrazone-6-p-chlorophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamide **5b** was obtained in 65% yield starting from 3.97 g of 5-formyl-6-p-chlorophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamide **4b** as yellow needles, m.p. 228–232 °C dec. Anal. $C_{15}H_{16}ClN_9O_2S_2$ (453.5); C, H, N.

5-Guanylhydrazone-6-p-bromophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamide **5c** was obtained in 65% yield starting from 4.42 g of 5-formyl-6-p-bromophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamide **4c** as yellow flakes, m.p. 232–234 °C dec. Anal. $C_{15}H_{16}BrN_9O_2S_2$ (498); C, H, N.

5-Guanylhydrazone-6-p-methylphenylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamide **5d** was obtained in 70% yield starting from 3.77 g of 5-formyl-6-p-methylphenylimidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamide **4d** as yellow needles, m.p. 215–220 °C dec. Anal. $C_{16}H_{19}N_9O_2S_2$ (433); C, H, N.

5-Guanylhydrazone-6-(3-coumarinyl)imidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamide **5f** was obtained in 68% yield starting from 4.31 g of 5-formyl-6-(3-coumarinyl)imidazo[2,1-b]-1,3,4-thiadiazole-2-[N-(dimethylaminomethino)]sulfonamide **4f** as brown flakes, m.p. 218–222 °C dec. Anal. $C_{18}H_{17}N_9O_4S_2$ (487); C, H, N.

4.3. 5-Thiocyanato-6-arylimidazo-[2,1-b]-1,3,4-thiadiazole-2-sulfonamides **6**

General method:

To a mixture of 6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide (0.01 mol) and potassium thiocyanate (1.56 g, 0.016 mol) in glacial acetic acid (50 mL) was added (at 0–5 °C) bromine (1.6 g, 0.01 mol) in glacial

acetic acid (20 mL) dropwise with stirring. Then stirring was continued for 30 min at 15–18 °C and then at room temperature for 30 min. The reaction mixture was poured into ice water. The precipitate that separated was filtered, dried and recrystallised from ethanol. Compound **6a** was obtained in 70% yield starting from 2.8 g of **3a** as pale yellow needles, m.p. 232–234 °C. Anal. $C_{11}H_7N_5O_2S_3$ (337); C, H, N.

5-Thiocyanato-6-p-chlorophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide **6b** was obtained in 65% yield starting from 3.14 g of 6-p-chlorophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide **3b** as colourless needles, m.p. 211–213 °C. Anal. $C_{11}H_6ClN_5O_2S_3$ (371.5); C, H, N.

5-Thiocyanato-6-p-bromophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide **6c** was obtained in 60% yield starting from 3.59 g of 6-p-bromophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide **3c** as colourless needles, m.p. 216–218 °C. Anal. $C_{11}H_6BrN_5O_2S_3$ (416); C, H, N.

5-Thiocyanato-6-p-methylphenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide **6d** was obtained in 72% yield starting from 2.94 g of 6-p-methylphenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide **3d** as pale yellow needles, m.p. 220–222 °C. Anal. $C_{12}H_9N_5O_2S_3$ (351); C, H, N.

5-Thiocyanato-6-p-nitrophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide **6e** was obtained in 75% yield starting from 3.25 g of 6-p-nitrophenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide **3e** as yellow needles, m.p. 250–255 °C dec. Anal. $C_{11}H_6N_6O_4S_3$ (382); C, H, N.

4.4. Antibacterial activity

Cup plate method [10] using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of **3a–f**, **5a–d**, **5f** and **6a–e** against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *S. typhi* and *Pneumococci* (local strains). The agar medium was purchased from HI media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound (5 mg) was dissolved in 5 mL of dimethylformamide (1 000 µg/mL). Volumes of 0.05 mL, 0.1 mL and 0.15 mL of each compound were used for testing.

The cups were made by scooping out agar medium with a sterilised cork borer in a Petri dish which were streaked with the organisms. The solution of each test compound (0.05, 0.1 and 0.15 mL) were added separately in the cups and Petri dishes were subsequently incubated at 37 °C for 48 h. Sulfamethoxazole and Norfloxacin were used as standard reference drugs and dimethylformamide as a control which did not reveal any inhibition. Zone of inhibition produced by each compound was measured in mm and the results are presented in *table III*.

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