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Synthesis and pharmacological evaluation of condensed heterocyclic 6-substituted 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives of isoniazid

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ABSTRACT

The significance of this study was to prepare various isoniazid derivatives by introducing the isoniazid core into several molecules to explore the possibilities of some altered biological activities. Series of 6-substituted-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole (**3a–g**) and 1,3,4-oxadiazole (**4a–g** and **5**) derivatives of isoniazid were synthesized in satisfactory yield and pharmacologically evaluated for their anti-inflammatory, analgesic, ulcerogenic, and lipid peroxidation activities by known experimental models.

Isoniazid, an organic compound, is a prodrug and must be activated by bacterial catalase. It is activated by catalase-peroxidase hemoprotein, KatG which couples the isonicotinic acyl with nicotinamide adenine dinucleotide (NADH) to form isonicotinic acyl-NADH complex. This complex indirectly inhibits the synthesis of mycolic acid required for the mycobacterial cell wall.[1] It is bactericidal to rapidly-dividing mycobacteria but is bacteriostatic if the mycobacterium is slow-growing.[2]

Isoniazid is metabolized by the liver mainly by acetylation and dehydrazination. The *N*-acetylhydrazine metabolite is believed to be responsible for the hepatotoxic effects seen in patients treated with isoniazid. CYP2E1 is reportedly involved in INH-induced hepatotoxicity in humans.[3] For these reasons, in our study, we tried to eliminate in vivo acetylation by arylamine *N*-acetyltransferase (NAT) to form inactive acetylated drug by replacing hydrazide moiety of INH with triazolo-thiadiazole and 1,3,4-oxadiazole heterocycles.

A survey of literature revealed that substituted-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole rings, have received much attention during recent years on account of their prominent potential as antifungal,[4] anti-inflammatory,[5] anti-tumor,[6] analgesic,[7] and antibacterial agents.[8] A triazolo-thiadiazole system may be viewed as a cyclic analog of two very important components-thiosemicarbazide[9,10] and biguanide,[11] which often display diverse biological activities. Similarly, 1,3,4-oxadiazoles is a class of heterocycle which have attracted significant interest in medicinal chemistry and they have a wide range of pharmaceutical and biological activities.[12,13] Therefore, it was planned to synthesize hybrid compounds that comprise both the isoniazid and the aforementioned heterocyclic ring systems. Such hybridization was designed in order to investigate the effect of structural variation on the anticipated anti-inflammatory-analgesic, ulcerogenic and lipid peroxidation activities.

6-Substituted-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole (**3a–g**) and 1,3,4-oxadiazole (**4a–g** and **5**) were prepared according to the procedure outlined in Scheme 1. The required dithiocarbazinate[14] was synthesized by reacting acid hydrazide with carbon disulfide and potassium hydroxide in ethanol. This salt underwent ring closure with an excess of 99% hydrazine hydrate to give the 4-amino-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol (**2**).[15]Hence resulted triazole (**2**) was then converted to the title compounds[16] (**3a–g**) in a one-pot reaction, by condensation with aromatic acids in the presence of $POCl_3$. The synthesis of compounds[17] (**4a–g**) was accomplished in a single step by reacting the acid hydrazide of starting drug, isoniazid with aromatic acids in the presence of $POCl_3$ and compound[18] **5** by reacting with carbon disulfide and potassium hydroxide in ethanol, respectively. The structure of synthesized compounds was confirmed by elemental analysis and spectral data (IR, ^{1}H NMR, and LRMS).[19]

The anti-inflammatory activities[20] of the synthesized compounds **3a–g, 4a–g**, and **5** were evaluated. The compounds were

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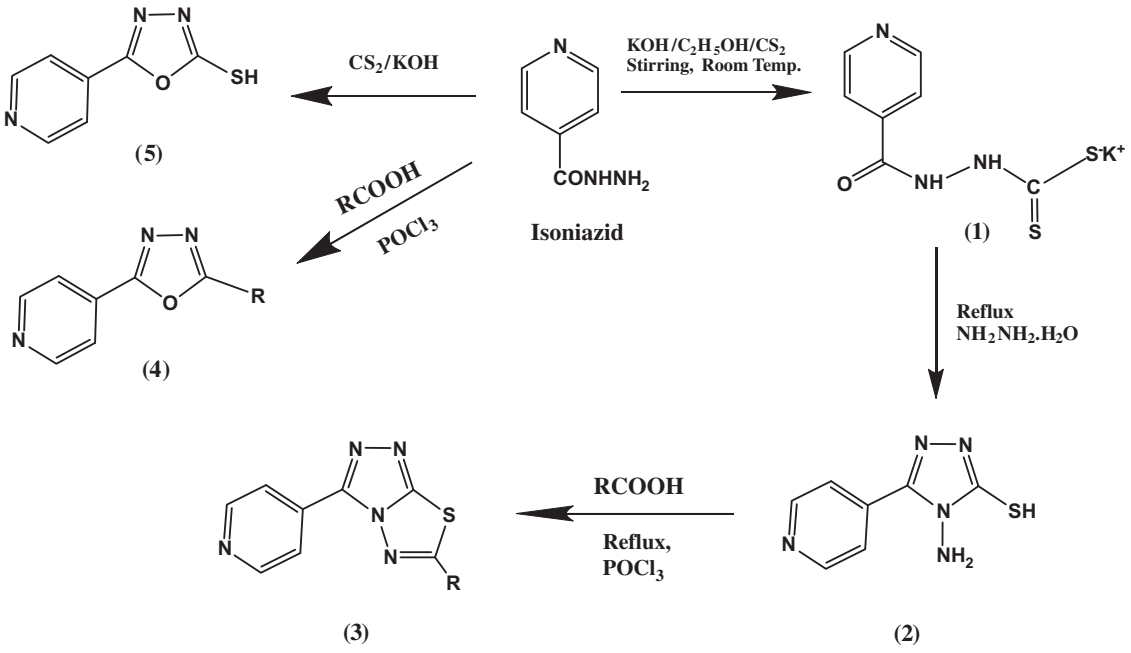

Scheme 1. Reagents: **3**, **4** [(a) R = C_6H_5; (b) R = 2-C_6H_4Cl; (c) R = 2,4-$C_6H_3Cl_2$; (d) R = 2-$C_6H_4CH_3$; (e) R = 2-$C_6H_4OCOCH_3$; (f) R = OC_6H_5(phenoxy); (g) R = 4-$C_6H_4NO_2$].

Table 1
Biological data of 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives of isonicotinic acid hydrazide (**3a–g**, **4a–g**, and **5**)

Compound	Anti-inflammatory activity (% inhibition ± SEM after 4 h)≠	Analgesic activity (% inhibition ± SEM after 4 h)#	Ulcerogenic activity (severity index ± SEM)≠	nmol MDA content ± SEM/100 mg tissue≠
3a	66.36 ± 2.56[a]	56.06 ± 3.25[d]	0.666 ± 0.10[b]	5.78 ± 0.10[b]
3b	45.41 ± 3.10[b]	37.27 ± 3.73[d]	1.417 ± 0.08[b]	8.80 ± 0.06[b]
3c	69.32 ± 2.17[a]	61.81 ± 2.45[d]	0.750 ± 0.17[b]	6.71 ± 0.16[b]
3d	79.79 ± 1.75[a]	62.54 ± 2.27[d]	0.471 ± 0.11[a]	4.16 ± 0.17[b]
3e	39.80 ± 3.73[b]	25.00 ± 3.94[d]	2.250 ± 0.24	8.82 ± 0.14[b]
3f	63.55 ± 2.23[a]	52.27 ± 2.81[d]	1.500 ± 0.00[b]	7.32 ± 0.19[b]
3g	86.32 ± 1.09	74.32 ± 1.20[c]	0.324 ± 0.08[a]	3.47 ± 0.19[a]
4a	82.63 ± 2.59	68.21 ± 1.65[c]	1.667 ± 0.13[b]	4.87 ± 0.26[b]
4b	37.74 ± 3.20[b]	29.54 ± 3.27[d]	2.000 ± 0.10[b]	8.11 ± 0.13[b]
4c	84.00 ± 1.66	70.37 ± 1.67[c]	0.386 ± 0.10[a]	3.29 ± 0.15
4d	43.38 ± 3.94[b]	31.81 ± 3.71[d]	2.266 ± 0.20	7.33 ± 0.16[b]
4e	63.36 ± 2.00[a]	57.57 ± 2.45[d]	0.831 ± 0.16[b]	5.19 ± 0.22[b]
4f	71.23 ± 2.83[a]	62.14 ± 3.74[d]	0.731 ± 0.24[b]	3.33 ± 0.19
4g	54.01 ± 3.22[b]	44.05 ± 3.76[d]	1.762 ± 0.26[b]	6.85 ± 0.14[b]
5	62.88 ± 2.45[a]	54.28 ± 3.96[d]	1.327 ± 0.21[b]	5.78 ± 0.10[b]
Isoniazid	51.62 ± 3.20[b]	45.90 ± 2.05[d]	3.75 ± 0.11[b]	9.10 ± 0.14[b]
Ibuprofen	82.69 ± 1.65	73.52 ± 1.00	2.315 ± 0.18	7.51 ± 0.16
Control	—	—	0.00 ± 0.00	3.26 ± 0.01

≠ Relative to their respective standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for $n = 6$; [a]$P < 0.05$; [b]$P < 0.01$.
Relative to normal and data were analyzed by paired Student's *t*-test for $n = 6$; [c]$P < 0.0001$; [d]$P < 0.005$.

tested at an equimolar oral dose relative to 70 mg/kg ibuprofen. The tested compounds showed anti-inflammatory activity ranging from 37.74 ± 3.20% to 86.32 ± 1.09%, whereas the standard and parent drug ibuprofen and isoniazid showed 82.69 ± 1.65% and 51.62 ± 3.20% inhibition, respectively, after 4 h (Table 1). It was observed that the triazolo-thiadiazole derivatives having 4-nitrophenyl (**3g**) at sixth position possess highest activity (86.32 ± 1.09%) comparable to that of ibuprofen (82.69 ± 1.65%). Further it was observed that the presence of 2-chlorophenyl moiety (**3b**) at C-6 showed decrease of activity (45.41 ± 3.10%) and replacement of this group by acetyl phenyl group (**3e**) resulted in sharp decrease of anti-inflammatory activity (39.80 ± 3.73%). In oxadiazole derivatives highest activity (84.00 ± 1.66%) was found in **4c** having 2,4-dichlorophenyl group at second position. When the phenyl group was replaced by mercapto group **5** the activity was found to be moderate (62.88 ± 2.45%).

All compounds were further tested for their analgesic activity[21] at the same oral dose and showed good to moderate analgesic activity in comparison to their respective standard and parent drugs. The compounds showed analgesic activity ranging from 25.00 ± 3.94% to 74.32 ± 1.20% inhibition, whereas parent isoniazid and standard drug ibuprofen showed 45.90 ± 2.05% and 73.52 ± 1.00% inhibition, respectively (Table 1).

The compound **3g** having 4-nitrophenyl group at C-6 position of triazolo-thiadiazole ring (74.32 ± 1.20%), and 2,4-dichlorophenyl group, present at the second position on oxadiazole ring **4c**, showed the maximum activity (70.37 ± 1.67%), equivalent to that of the standard drug ibuprofen (73.52 ± 1.00%). The 2-mercapto group **5** at second position of oxadiazole ring showed moderate analgesic activity (54.28 ± 3.96%).

The results showed that an electron-donating group (**3g**) increases the analgesic activity of the triazolo-thiadiazole derivatives

Table 2
Effect of compounds **3g** and **4c** on serum enzymes, total proteins, and total albumin

Compound	SGOT[a] (U/mL)	SGPT[a] (U/mL)	Alkaline phosphatase[a]	Total protein[a] (g/dL)	Total, albumin[a] (g/dL)
Control	143.71 ± 1.10	32.46 ± 0.76	12.17 ± 0.16	1.72 ± 0.02	1.63 ± 0.02
Isoniazid	151.25 ± 1.56**	40.21 ± 0.64	8.31 ± 0.21	1.55 ± 0.05	1.48 ± 0.07
Ibuprofen	140.14 ± 1.12**	34.26 ± 0.72**	11.67 ± 0.18	1.74 ± 0.06	1.71 ± 0.01
3g	138.32 ± 1.22**	29.34 ± 0.64**	12.76 ± 0.13**	1.78 ± 0.08	1.77 ± 0.15
4c	136.24 ± 1.20**	28.67 ± 0.67**	13.79 ± 0.19**	1.76 ± 0.02	1.72 ± 0.05

[a] Relative to control and data were analyzed by ANOVA followed by Dunnett's multiple comparison test, for $n = 6$; $^{**}P < 0.01$.

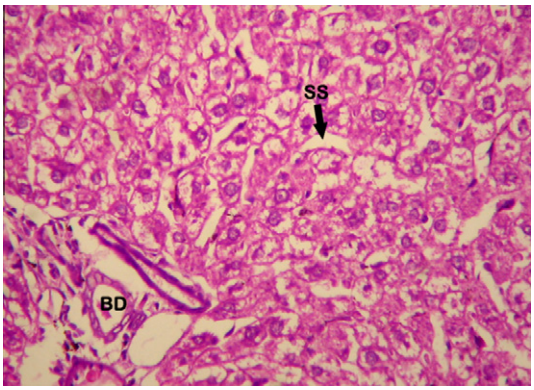

Figure 1. Compound **3g**: section of liver showing hepatocytes with mild swelling and clearing of cytoplasm. SS, sinusoidal space; BD, bile duct (100×).

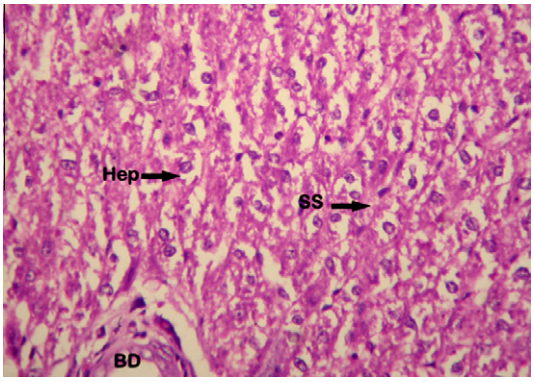

Figure 2. Compound **4c**: section of liver, showing hepatocytes with mild clearing of cytoplasm. Hep, hepatocyte; SS, sinusoidal space; BD, bile duct (100×).

whereas electron-withdrawing group (**4c**) increases the analgesic activity of the 1,3,4-oxadiazole derivatives of isoniazid. It was noted that the compound **3g** and **4c** showing highest anti-inflammatory activity also exhibited highest analgesic activity (74.32 ± 1.20% and 70.37 ± 1.67%) as well as those exhibiting lowest anti-inflammatory activity **3e** and **4b**, also exhibited lowest analgesic activity (25.00 ± 3.94% and 29.54 ± 3.27%). It was further interestingly noted that parent isoniazid does not show any fruitful results for both anti-inflammatory (51.62 ± 3.20%) and analgesic activity (45.90 ± 2.05%) as compared to their triazolo-thiadiazole and 1,3,4-oxadiazole derivatives which showed interesting results for both the activities.

The compounds were further tested for their acute ulcerogenic activity[22] at an equimolar oral dose relative to 210 mg/kg ibuprofen and parent drug isoniazid. The tested compounds showed low ulcerogenic activity ranging from 0.324 ± 0.08 to 2.266 ± 0.20, compared to standard ibuprofen and parent drug isoniazid having high severity index of 2.315 ± 0.18 and 3.750 ± 0.11, respectively (Table 1). It has been reported in literature that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation.[23] Therefore, an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of lipid per oxidation.

The lipid peroxidation is measured as nmol of MDA/100 mg of tissue. The isoniazid (parent drug) and ibuprofen (standard drug) showed the maximum lipid per oxidation (9.10 ± 0.14 and 7.51 ± 0.16), whereas the control group showed 3.26 ± 0.01. It was found that all the condensed derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation (Table 1). Thus these studies showed that synthesized compounds have inhibited the induction of gastric mucosal lesions and the results further suggested that their protective effect might be related to the inhibition of lipid per oxidation in the gastric mucosa. Further it has been reported that isoniazid causes hepatotoxicity due to the presence of hydrazine moiety in their molecule. Hence, conversion of hydrazine group of isoniazid to triazolo-thiadiazole and 1,3,4-oxadiazole moiety might have caused COX-2 inhibitory activity, resulting in significant anti-inflammatory activity with less ulcerogenicity.

The compounds **3g** and **4c**, derivatives of triazolo-thiadiazole and 1,3,4-oxadiazole, respectively, showing potent anti-inflammatory and analgesic activities with reduced ulcerogenicity and lipid peroxidation, were further studied for their hepatotoxic effect in comparison with parent isoniazid and standard ibuprofen drugs. Both compounds were studied for their effect on biochemical parameters[24,25] (serum enzymes, total proteins, and total albumin), as shown in Table 2 and liver histopathological[26] testing were also carried out. The studies of the liver samples do not show any significant pathological changes in comparison to control group (Figs. 1 and 2).

In Summary, various triazolo-thiadiazole and 1,3,4-oxadiazole derivatives of isoniazid were prepared. Among these the compound 6-(4-nitrophenyl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**3g**) and 2-(2,4-dichlorophenyl-5-(pyridin-4-yl)-1,3,4-oxadiazole (**4c**) showed maximum anti-inflammatory and analgesic activity. Therefore, it was concluded that triazolo-thiadiazole and 1,3,4-oxadiazole derivatives of isoniazid might afford a safer alternative to isoniazid for the treatment of inflammatory disease, pain and hepatotoxicity caused by acetyl hydrazine, a metabolite of isoniazid.

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14. Karabasanagouda, T.; Adhikari, V. A.; Shetty, S. N. *Eur. J. Med. Chem*. **2007**, *42*, 521. Compound **1**: Yield 75%, mp 184 °C; IR (KBr, ν cm^{-1}): 3300 (NH), 1670 (C=O), 1088 (C=S); ^{1}H NMR (DMSO-d_6): δ 10.76 (br s, 1H, CONH), 9.23 (br s, 1H, NH),7.76, 8.64 (m, 4H, Py) MS: m/z 250 (M$^+$). Anal. Calcd for $C_7H_6KN_3OS_2$: C, 33.40; H, 2.36; N, 16.76; S, 25.48. Found: C, 33.45; H, 2.41; N, 16.72; S, 25.51.
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16. General method for the synthesis of 6-substituted-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles: an equimolar mixture of 4-amino-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol (0.10 mol) and aromatic acids (0.10 mol) in phosphorus oxychloride (20 mL) was refluxed for 5 h. The reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixture was allowed to stand overnight and the solid separated out was filtered, treated with dilute sodium hydroxide solution and washed thoroughly with cold water, yield: 60–70%.
17. General method for the synthesis of 2-substituted-5-(pyridin-4-yl)-1,3,4-oxadiazole: an equimolar mixture of isonicotinic acid hydrazide (0.001 mol) and aromatic acids (0.001 mol) was dissolved in phosphorus oxychloride (20 mL) and refluxed for 20 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus separated out was filtered, washed with water, dried and recrystallised from ethanol, yield: 65–75%.
18. General method for the synthesis of 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol: a mixture of isonicotinic acid hydrazide (0.005 mol), KOH (0.005 mol) and carbon disulfide (5 mL) in ethanol (50 mL) was refluxed on a steam bath for 12 h. The solution was then concentrated, cooled and acidified with dilute HCl. The solid mass that separated out was filtered, washed with ethanol, dried and recrystallized from ethanol. Yield: 74%, mp 166 °C; IR (KBr, ν cm^{-1}): 1638 (C=N), 1521 (C=C aromatic), 1430 (C–O–C oxadiazole), 1164 (SH); ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.94, 8.74 (m, 4H, Py), 13.03 (s, 1H, SH); MS: m/z 179 (M$^+$). Anal. Calcd for $C_7H_5N_3OS$: C, 46.95; H, 2.83; N, 23.48; S, 17.91. Found: C, 46.92; H, 2.81; N, 23.45; S, 17.89.
19. Physical and analytical data of the selected compounds: Compound **3g**: yield 70%, mp 204 °C; IR (KBr, ν cm^{-1}): 3164 (C–H), 1238 (N–N=C triazolo-thiadiazole), 1674 (C=N), 1544 (C=C), 696 (C–S–C), 1316 (NO$_2$); ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.92, 8.76 (m, 4H, Py), 7.24–7.28 (m, J = 12 Hz, 4H Ar-H); MS: m/z 324 (M$^+$); Anal. Calcd for $C_{14}H_8N_6O_2S$: C, 51.87; H, 2.52; N, 25.94; S, 9.92. Found: C, 51.85; H, 2.49; N, 25.91; S, 9.89.
Compound **4c**: yield 71%, mp 164 °C; IR (KBr, ν cm^{-1}): 1646 (C=N), 1548 (C=C), 1434 (C–O–C oxadiazole), 836 (C–Cl); ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.94, 8.67 (m, 4H, Py), 7.12–7.14 (m, J = 6 Hz, 3H, Ar-H); MS: m/z 291 (M$^+$). Anal. Calcd for $C_{13}H_7Cl_2N_3O$: C, 69.99; H, 4.10; N, 18.86. Found: C, 53.45; H, 2.42; N, 14.38.
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