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

Mohammad Amir,* Harish Kumar and Sadique A. Javed

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110 062, India

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Abstract—Some 6-substituted-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole derivatives (**4a**–**f** and **5a**–**d**) have been synthesized by cyclisation of 4-amino-5-[1-(6-methoxy-2-naphthyl)ethyl]-3-mercapto-(4*H*)-1,2,4-triazole (**3**) with various substituted aromatic acids and aryl/alkyl isothiocyanates, through a single step reaction. The target compounds were pharmacologically evaluated for their anti-inflammatory and analgesic potentials by known experimental models. Several of these showed significant activity. Very low ulcerogenic index was observed for potent compounds.

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain and inflammation. Most currently used NSAIDs have limitations for therapeutic uses, since they cause gastrointestinal and renal side effects, which are inseparable from their pharmacological activities. These compounds act via inhibition of the enzyme cyclooxygenase, thus preventing prostaglandin synthesis. In the early 1990s, it was discovered that the enzyme exists as two isomers, one constitutive (COX-1) and the other inducible (COX-2).[1] COX-1 is an enzyme that is constitutively expressed and provides cytoprotection in the gastrointestinal (GI) tract; whereas inducible COX-2 mediates inflammation.[2–4] The traditional NSAIDs in current use non-selectively inhibit COX-1 and COX-2. In fact, most of them show greater selectivity for COX-1 than COX-2.[5] Consequently long-term therapy with nonselective NSAIDs may cause gastrointestinal complications ranging from stomach irritation to life-threatening GI ulceration and bleeding.[6] Therefore, selective COX-2 inhibitors with better safety profile have been marketed as a new generation of NSAIDs.[7,8] But careful prospective examination of coxibs has revealed unexpected cardiovascular adverse effects.[9] Thus there remains a compelling need for effective NSAIDs with an improved safety profile.

It has been reported in the literature that modification of the carboxyl function of representative NSAIDs results in retained anti-inflammatory activity and reduced ulcerogenic potential.[10–13] In addition 1,2,4-triazoles,[14–16] 1,3,4-thiadiazoles[17,18] and their condensed heterocyclic derivatives, particularly 1,2,4-triazolo[3,4-*b*]thiadiazoles,[19,20] have been reported to possess anti-inflammatory activity. In continuation of our research programme on the synthesis of 5-membered heterocyclic derivatives of arylalkanoic acids,[21–23] we report herein the synthesis and pharmacological profile of 1,2,4-triazolo[3,4-*b*]thiadiazole derivatives of naproxen. These compounds exhibited analgesic and anti-inflammatory potencies similar to those of naproxen, but with significantly lesser gastric damage.

3-[1-(6-Methoxy-2-naphthyl)ethyl]-6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**4a**–**f** and **5a**–**d**) were prepared according to the procedure outlined in Scheme 1. The required dithiocarbazinate[24] was synthesized by reacting acid hydrazide with carbondisulfide and potassium hydroxide in ethanol. This salt underwent ring closure with an excess of 99% hydrazine hydrate to give the 4-amino-5-[1-(6-methoxy-2-naphthyl)ethyl]-3-mercapto-(4*H*)-1,2,4- triazole (**3**).[25] Thus resulted triazole (**3**) was then further converted to the title compounds[26] (**4a**,**b**,**e**,**f**[27] and **4c**,**d**) in a one-pot reaction, by condensation

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* Corresponding author. Tel.: +91 11 26059878; fax: +91 11 26059688x5307; e-mail: mamir_s2003@yahoo.co.in

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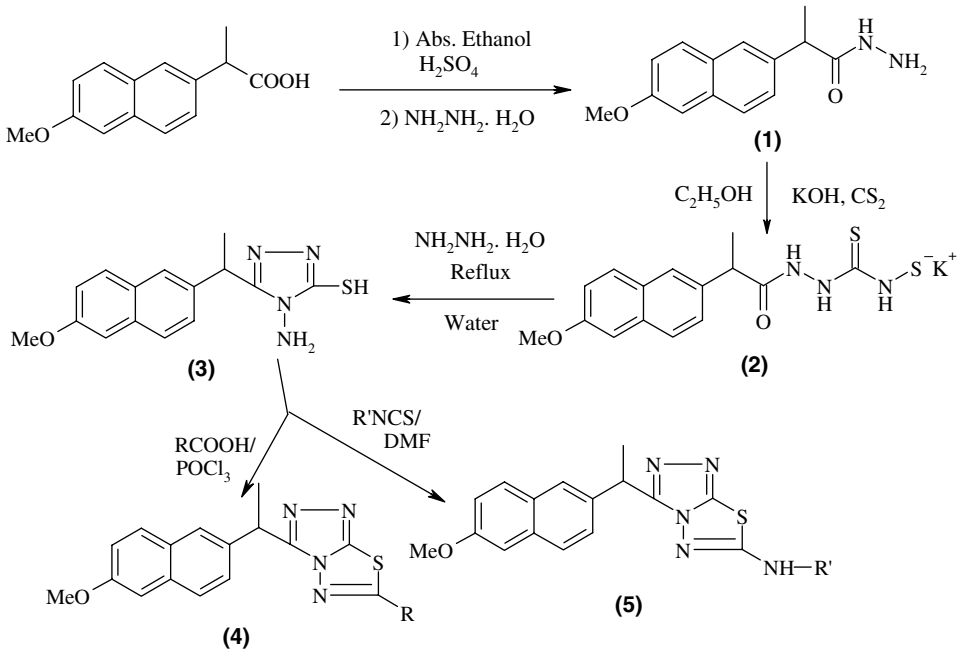

R = a: C_6H_5; b: 4-Cl-C_6H_4; c: 2,4-$(Cl)_2$-C_6H_3; d: 2,4-$(Cl)_2$-$C_6H_3OCH_2$; e: 2-NH_2-C_6H_4; f: 4-NH_2-C_6H_4
R' = a: *n*-C_4H_9; b: C_6H_5; c: 4-F-C_6H_4; d: 2,4-$(CH_3)_2$-C_6H_3

Scheme 1.

with aromatic acids in the presence of $POCl_3$. The synthesis of compounds **5a**–**d**[28] was accomplished in a single step by reacting the triazole (**3**) with aryl/alkyl isothiocyanates in the presence of DMF. The structure of synthesized compounds was confirmed by elemental analysis and spectral data (IR, ^{1}H NMR, Mass).[29]

All the synthesized compounds **4a**–**f** and **5a**–**d** were tested for their anti-inflammatory activity[30] at an equimolar oral dose relative to 30 mg/kg naproxen. The compounds showed anti-inflammatory activity ranging from 41% to 82.50% (Table 1), whereas standard drug naproxen showed 81.81% inhibition after 4 h. The anti-inflammatory activity data showed that the compound having phenyl group (**4a**) at C-6 possesses highest activity (81.05%, $p > 0.05$). Replacement of phenyl group by 4-chlorophenyl (**4b**) resulted in a slight decrease of anti-inflammatory activity (80.30%, $p > 0.05$). Further it was observed that the presence of 2,4-dichlorophenoxy methyl group (**4d**) and 4-aminophenyl group (**4f**) at C-6 showed similar activity (78.02%, $p > 0.05$). Exchange of these by 2,4-dichlorophenyl (**4c**) and 2-aminophenyl (**4e**) groups reduced the activity (68.93%, $p < 0.05$; 54.54%, $p < 0.01$, respectively). It was observed that the replacement of phenyl/substituted phenyl group by *n*-butyl amino, phenyl amino and 4-fluorophenyl

Table 1. Anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of the synthesized compounds (**4a**–**f** and **5a**–**d**)

Compound	Anti-inflammatory activity (% inhibition ± SEM)[#]	Analgesic activity[##]			Ulcerogenic activity (severity index ± SEM)[#]	nmol MDA content ± SEM/ 100 mg tissue[#]
	After 4 h	Pre-treatment (0 h)	Post-treatment (4 h)	% inhibition		
4a	81.05 ± 2.17	1.57 ± 0.210	2.98 ± 0.225[c]	89.50	0.500 ± 0.00[b]	4.86 ± 0.10[b]
4b	80.30 ± 0.96	1.28 ± 0.084	1.62 ± 0.148	26.70	0.583 ± 0.00[b]	5.27 ± 0.26[b]
4c	68.93 ± 1.40[a]	1.80 ± 0.076	2.47 ± 0.174[d]	37.20	0.750 ± 0.11[b]	5.63 ± 0.35[b]
4d	78.02 ± 1.82	1.43 ± 0.146	2.58 ± 0.156[d]	80.30	0.667 ± 0.16[b]	5.38 ± 0.25[b]
4e	54.54 ± 3.32[b]	XX	XX	XX	XX	XX
4f	78.02 ± 1.82	1.36 ± 0.104	2.15 ± 0.142[d]	58.10	0.833 ± 0.21[b]	5.85 ± 0.22[b]
5a	40.90 ± 3.52[b]	XX	XX	XX	XX	XX
5b	43.93 ± 3.03[b]	XX	XX	XX	XX	XX
5c	40.14 ± 4.90[b]	XX	XX	XX	XX	XX
5d	76.51 ± 2.17	1.44 ± 0.156	1.92 ± 0.222	33.30	0.833 ± 0.21[b]	6.11 ±0.40[b]
Naproxen	81.81 ± 2.65	1.17 ± 0.086	2.03 ± 0.039[c]	73.50	2.250 ± 0.11	9.04 ± 0.24
Control	—	—	—	—	0.00	3.25 ± 0.05

[XX]Not tested.
[#]Relative to 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 (pre-treatment) and data were analyzed by paired Student's t test for $n = 6$; [c]$p < 0.0001$, [d]$p < 0.005$.

Table 2. Effect of compound **4a** on serum enzymes, total proteins and total albumin

Compound	SGOT U/mL[#]	SGPT U/mL[#]	Alkaline phosphatase[#]	Total protein g/dl[#]	Total albumin g/dl[#]
Control	148.67 ± 1.50	27.67 ± 0.84	13.06 ± 0.25	1.80 ± 0.01	1.67 ± 0.01
4a	150.00 ± 1.90	30.17 ± 1.22	20.75 ± 1.07[b]	2.10 ± 0.02[a]	1.98 ± 0.03[a]

[#]Relative to control and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for $n = 6$; [a]$p < 0.05$, [b]$p < 0.01$.

amino groups (**5a–c**) resulted in a sharp decrease ($p < 0.01$) of activity, except in compound **5d**, where the presence of a 2,4-dimethylphenyl amino group showed good activity (76.51%, $p > 0.05$).

The compounds that showed anti-inflammatory activity higher than 65% were further tested for their analgesic activity[31] at an equimolar oral dose relative to 30 mg/kg naproxen. Compounds **4a–d**, **4f** and **5d** showed analgesic activity ranging from 26.7% to 89.5%, whereas the standard drug naproxen showed 73.5% inhibition. It was noted that the compound **4a** showing highest anti-inflammatory activity also exhibited highest analgesic activity (89.5%), whereas compound **4b** showed sharp decrease in analgesic activity (26.7%), although it showed high anti-inflammatory activity (80.30%). Compound **4d** having 2,4-dichlorophenoxymethyl group also showed high analgesic activity (80.30%). The remaining compounds showed reduced analgesic activity. These compounds were further screened for their acute ulcerogenic activity.[32] The compounds were tested at an equimolar oral dose relative to 90 mg/kg naproxen. The maximum reduction in ulcerogenic activity (0.500 ± 0.00) was found in compound **4a** having phenyl group at position 6 of thiadiazole ring. Rest of the tested compounds also showed better GI safety profile as compared to naproxen, as illustrated in Table 1.

All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation.[33] Lipid peroxidation is measured as nanomole of malondialdehyde (MDA)/100 mg of gastric mucosa tissue. Naproxen showed maximum lipid peroxidation (9.04 ± 0.24), whereas control group showed 3.25 ± 0.05. It was found that all cyclised 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 lesion, and results further suggested that their protective effect can be related to the inhibition of lipid peroxidation in the gastric mucosa. It has been speculated that naproxen acts non-selectively probably because of its relatively small size, tolerated by both COX-1 and COX-2. It appears that bulkier structures are more likely to confer selectivity to COX-2 due to its wider active site. Thus it seems that the conversion of carboxylic group of naproxen to triazolo thiadiazolo moiety might have caused COX-2 inhibitory activity, resulting in significant anti-inflammatory activity with less ulcerogenicity.

The compound **4a** showing potent anti-inflammatory activity with reduced ulcerogenicity and lipid peroxidation was further studied for its hepatotoxic effect. The compound was studied for its effect on biochemical parameters[34–36] (serum enzymes, total protein and total albumin) and histopathology of liver.[37] As shown in Table 2, activities of liver enzymes (SGOT, SGPT, alkaline phosphatase), total protein and total albumin remained almost the same with respect to control values. Histopathological studies of liver samples of test compound **4a** (Fig. 2) do not show any significant pathological changes in comparison to control group (Fig. 1). No hepatocyte necrosis or degeneration was seen in the sample.

In summary various triazolo thiadiazole derivatives of naproxen were prepared with the objective of developing

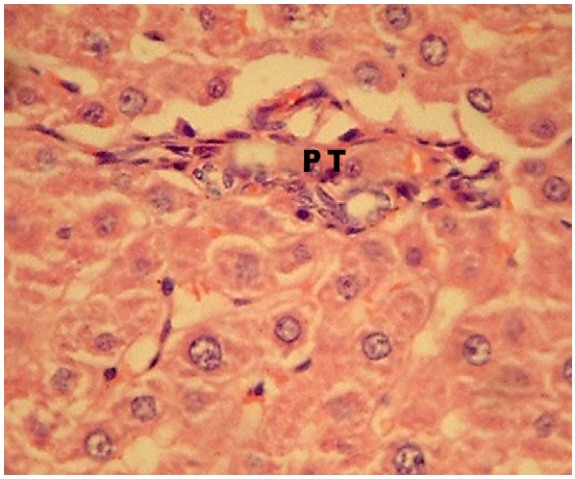

Figure 1. Control: Section of liver, showing normal hepatic parenchyma with portal triad structures (400×).

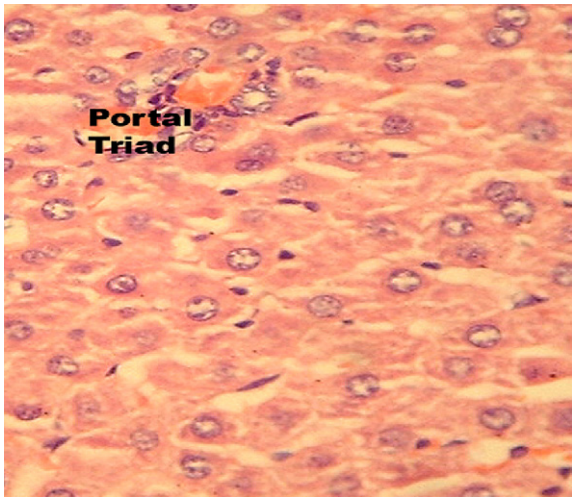

Figure 2. 4a: Section of liver, showing normal portal triad structures (400×).

better anti-inflammatory molecules with minimal ulcerogenic activity. Among these, the compound 6-phenyl-3-[1-(6-methoxy-2-naphthyl)ethyl][1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazole (**4a**) showed maximum anti-inflammatory and analgesic activity. It also showed maximum reduction of severity index along with minimum lipid peroxidation, with no hepatocyte necrosis or degeneration. Therefore it was concluded that triazolo thiadiazole derivatives of naproxen might afford a safer alternative to naproxen for the treatment of inflammatory disease and pain.

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References and notes

1. Hla, T.; Neilson, K. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7384.
2. Marnett, L.; Kalgutkar, A. S. *Trend. Pharmacol. Sci.* **1999**, *20*, 465.
3. Dannhardt, G.; Kiefer, W. *Eur. J. Med. Chem.* **2001**, *36*, 109.
4. Almansa, C.; Alfon, J.; De Arriba, A.; Cavalcanti, F.; Escamilla, I.; Gomez, L.; Miralles, A.; Soliva, R.; Bartolli, J.; Carceller, E.; Merlos, M.; Garcia-Rafanell, J. *J. Med. Chem.* **2003**, *46*, 3463.
5. Jackson, L.; Hawkey, C. *Exp. Opin. Invest. Drugs* **1999**, *8*, 963.
6. Allison, M.; Howatson, A.; Torrance, C.; Lee, F.; Russell, R. *N. Engl. J. Med.* **1992**, *327*, 749.
7. Kalgutkar, A. S. *Exp. Opin. Invest. Drugs* **1999**, *9*, 831.
8. Tally, J. J.; Bertenshaw, R. S.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Kellogg, M. S.; Kobolt, C. M.; Yuan, J.; Zhang, Y. Y.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 1661.
9. Dange, J. M.; Supuran, C. T.; Pravnito, D. *J. Med. Chem.* **2005**, *48*, 2251.
10. Galanakis, D.; Kourounakis, A. P.; Tsiakitzis, K. C.; Doulgkeris, C.; Rekka, E. A.; Gavalas, A.; Kravaritou, C.; Charitos, C.; Kourounakis, P. N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3639.
11. Kalgutkar, A. S.; Marnett, A. B.; Crews, B. C.; Remmel, R. P. *J. Med. Chem.* **2000**, *43*, 2860.
12. Duflos, M.; Nourrisson, M. R.; Brelet, J.; Courant, J.; Le Baut, G.; Grimaud, N.; Petit, J. Y. *Eur. J. Med. Chem.* **2001**, *36*, 545.
13. Kalgutkar, A. S.; Crews, B.; Rowlinson, S.; Garner, C.; Seibert, K.; Marnett, L. *Science* **1998**, *280*, 1268.
14. Labanauskas, L.; Udrenaite, E.; Gaidelis, P.; Brukstus, A. *Farmaco* **2004**, *59*, 255.
15. Mullican, M.; Wilson, M.; Commor, D.; Kostlan, C.; Schrier, R.; Dyer, R. *J. Med. Chem.* **1993**, *36*, 1090.
16. Amir, M.; Khan, M. S. Y.; Zaman, M. S. *Ind. J. Chem.* **2004**, *B*, 2189.
17. Schenone, S.; Brullo, C.; Bruno, O.; Bondavalli, F.; Ranise, A.; Filippelli, W.; Rinaldi, B.; Capuanob, A.; Falconeb, G. *Bioorg. Med. Chem.* **2006**, *14*, 1698.
18. Palaska, E.; Sahin, G.; Kelicen, P.; Durlu, N. T.; Altinok, G. *Farmaco* **2002**, *57*, 101.
19. Mathew, V.; Keshavayya, J.; Vaidya, V. P. *Eur. J. Med. Chem.* **2006**, *41*, 1048.
20. Udupi, H. R.; Setty, R. S.; Srinivasullu, N.; Pasha, Y. T.; Aggarwal, N. K. *Ind. J. Heterocycl. Chem.* **2003**, *12*, 361.
21. Amir, M.; Kumar, S. *Archiv Der Pharmazie* **2005**, *338*, 24.
22. Amir, M.; Kumar, S. *Die Pharmazie* **2005**, *60*, 175.
23. Amir, M.; Kumar, S. *Acta Pharm.* **2007**, *57*, 31.
24. Synthesis of potassium dithiocarbazinate: potassium hydroxide (0.03 M) was dissolved in absolute ethanol (50 mL). The solution was cooled in ice bath and 6-methoxy-α-methyl-2-naphthalene acetic acid hydrazide (0.02 M) was added with stirring. To this carbondisulfide (0.025 M) was added in small portions with constant stirring. The reaction mixture was agitated continuously for 12 h at room temperature. The precipitated potassium dithiocarbazinate was collected by filtration, washed with anhydrous ether (100 mL) and dried in vacuum. The potassium salt thus obtained (65% yield) was used in the next step without further purification.
25. Synthesis of 4-amino-5-[1-(6-methoxy-2-naphthyl)ethyl]-3-mercapto-(4*H*)-1,2,4- triazole: a suspension of potassium dithiocarbazinate (0.02 M) in water (5 mL) and hydrazine hydrate (99%, 0.04 M) was refluxed for 18–20 h with occasional shaking. The colour of the reaction mixture changed to green with the evolution of hydrogen sulfide gas. A homogeneous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with water (20 mL). On acidification with acetic acid the required triazole was precipitated out. Yield 60%, mp 212 °C; IR (KBr, ν cm^{-1}): 3280 (NH), 2930 (CH), 1596 (C=C), 1628 (C=N), 1260 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (d, J = 7 Hz, 3H, CH_3), 3.64 (s, 3H, OCH_3), 4.20 (q, J = 7 Hz, 1H, CH), 4.47 (s, 2H, NH_2), 6.85–7.44 (m, 6H, Ar-H), 11.61 (br s, 1H, SH); MS: *m/z* 300 (M^+). Anal. Calcd for $C_{15}H_{16}N_4OS$: C, 59.98; H, 5.37; N, 18.65; S, 10.61. Found: C, 59.96; H, 5.39; N, 18.62; S, 10.60.
26. General method for the synthesis of 3-[1-(6-methoxy-2-naphthyl)ethyl]-6-substituted[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazoles: an equimolar mixture of 4-amino-5-[1-(6-methoxy-2-naphthyl)ethyl]-3-mercapto-(4*H*)-1,2,4-triazole (0.10 M), aromatic acids (0.10 M) in phosphorus oxychloride (10 mL) was refluxed for 5 h. The reaction mixture was cooled to room temperature and then gradually poured onto 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: 54–71%.
27. Rao, A. S.; Sudheendra; Udupi, R. H. *Ind. J. Heterocycl. Chem.* **2006**, *15*, 291.
28. General method for the synthesis of 3-[1-(6-methoxy-2-naphthyl)ethyl]-6-alkyl/aryl amino[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles: an equimolar mixture of 4-amino-5-[1-(6-methoxy-2-naphthyl)ethyl]-3-mercapto-(4*H*)-1,2,4-triazole (0.10 M), aryl/alkyl isothiocyanate (0.10 M) in dimethylformamide (20 mL) was refluxed for 20–22 h. The reaction mixture was cooled to room temperature and then gradually poured onto crushed ice with stirring. The mixture was allowed to stand overnight, yield: 49–57%.
29. Physical and analytical data of the selected compounds: Compound **4a**: yield 71%, mp 152 °C; IR (KBr ν cm^{-1}): 2938 (C–H), 1605 (C=N), 1263 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.98 (d, J = 7 Hz, 3H, CH_3), 3.89 (s, 3H, OCH_3), 4.81 (q, J = 7 Hz, 1H, CH), 7.09–7.81 (m,

11H, Ar-H); MS: *m/z* 386 (M^+). Anal. Calcd for $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50; S, 8.30. Found: C, 68.39; H, 4.70; N, 14.53; S, 8.32.

Compound **4c**: yield 59%, mp 172 °C; IR (KBr ν cm^{-1}): 2927 (C–H), 1631 (C=N), 1269 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.95 (d, J = 7 Hz, 3H, CH_3), 3.89 (s, 3H, OCH_3), 4.74 (q, J = 7 Hz, 1H, CH), 6.86–7.72 (m, 9H, Ar-H); MS: *m/z* 455 (M^+); Anal. Calcd for $C_{22}H_{16}Cl_2N_4OS$: C, 58.03; H, 3.54; N, 12.30; S, 7.04. Found: C, 58.00; H, 3.55; N, 12.33; S, 7.00.

Compound **4d**: yield 62%, mp 162 °C; IR (KBr ν cm^{-1}): 2919 (C–H), 1606 (C=N), 1264 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.94 (d, J = 7 Hz, 3H, CH_3), 3.90 (s, 3H, OCH_3), 4.72 (q, J = 7 Hz, 1H, CH), 5.23 (s, 2H, OCH_2), 6.78–7.70 (m, 9H, Ar-H); MS: *m/z* 485 (M^+). Anal. Calcd for $C_{23}H_{18}Cl_2N_4O_2S$: C, 56.91; H, 3.74; N, 11.54; S, 6.61. Found: C, 56.90; H, 3.77; N, 11.55; S, 6.60.

Compound **4e**: yield 60%, mp 272 °C; IR (KBr ν cm^{-1}): 2930 (C–H), 1626 (C=N), 1258 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.90 (d, J = 7 Hz, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.65 (q, J = 7 Hz, 1H, CH), 5.06 (s, 2H, NH_2), 6.85–7.48 (m, 10H, Ar-H); MS: *m/z* 401 (M^+). Anal. Calcd for $C_{22}H_{19}N_5OS$: C, 65.82; H, 4.77; N, 17.44; S, 7.99. Found: C, 65.80; H, 4.75; N, 17.42; S, 7.97.

Compound **4f**: yield 63%, mp 242 °C; IR (KBr ν cm^{-1}): 2916 (C–H), 1654 (C=N), 1260 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.57 (d, J = 7 Hz, 3H, CH_3), 3.92 (s, 3H, OCH_3), 4.42 (q, J = 7 Hz, 1H, CH), 4.78 (s, 2H, NH_2), 6.67–7.88 (m, 10H, Ar-H); MS: *m/z* 401 (M^+). Anal. Calcd for $C_{22}H_{19}N_5OS$: C, 65.82; H, 4.77; N, 17.44; S, 7.99. Found: C, 65.86; H, 4.79; N, 17.46; S, 8.00.

Compound **5b**: yield 57%, mp 134 °C; IR (KBr ν cm^{-1}): 3372 (NH), 2930 (C–H), 1634 (C=N), 1265 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.59 (d, J = 7 Hz, 3H, CH_3), 3.90 (s, 3H, OCH_3), 4.25 (q, J = 7 Hz, 1H, CH), 7.33–7.96 (m, 11H, Ar-H), 12.67 (br s, 1H, NH); MS: *m/z* 401 (M^+). Anal. Calcd for $C_{22}H_{19}N_5OS$: C, 65.82; H, 4.77; N, 17.44; S, 7.99. Found: C, 65.84; H, 4.81; N, 17.47; S, 7.96.

Compound **5c**: yield 49%, mp 128 °C; IR (KBr ν cm^{-1}): 3290 (NH), 2919 (C–H), 1617 (C=N), 1248 (C–O–C); ^1H NMR (300 MHz, DMSO-d_6): δ 1.53 (d, J = 7 Hz, 3H, CH_3), 3.99 (s, 3H, OCH_3), 4.40 (q, J = 7 Hz, 1H, CH), 6.72–7.32 (m, 10H, Ar-H), 12.38 (br s, 1H, NH); MS: *m/z* 419 (M^+); Anal. Calcd for $C_{22}H_{18}FN_5OS$: C, 62.99; H, 4.32; N, 16.70; S, 7.64. Found: C, 62.96; H, 4.30; N, 16.74; S, 7.67.

Compound **5d**: yield 52%, mp 192 °C; IR (KBr ν cm^{-1}): 3318 (NH), 2938 (C–H), 1604 (C=N), 1267 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.70 (d, J = 7 Hz, 3H, CH_3), 2.59 (s, 3H, CH_3), 3.05 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.46 (q, J = 7 Hz, 1H, CH), 7.11–7.76 (m, 9H, Ar-H), 13.38 (br s, 1H, NH); MS: *m/z* 429 (M^+). Anal. Calcd for $C_{24}H_{23}N_5OS$: C, 67.11; H, 5.40; N, 16.30; S, 7.46. Found: C, 67.10; H, 5.38; N, 16.33; S, 7.42.

30. Amir, M.; Kumar, S.. *Eur. J. Med. Chem.* **2004**, *39*, 535.
31. Adeyemi, O. O.; Okpo, O. S.; Okpaka, O. *J. Ethnopharmacol.* **2004**, *90*, 45.
32. Cioli, V.; Putzolu, S.; Rossi, V.; Barcellona, S. P.; Corradino, C. *Toxicol. Appl. Pharmacol.* **1979**, *50*, 283.
33. Ohkawa, H.; Ohishi, N.; Yagi, K. *Anal. Biochem.* **1979**, *95*, 351.
34. Reitman, S.; Frankel, S. *Am. J. Clin. Pathol.* **1957**, *28*, 56.
35. King, E. J.; Armstrong, A. R. A. *Can. Med. Assoc. J.* **1934**, *31*, 376.
36. Varley, H. *Practical Clinical Biochemistry*, 1st ed.; CBS Publishers and Distributors: New Delhi, 1988, pp 236.
37. Luna, L. G. *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed.; McGraw-Hill: New York, 1968, pp 567.