Synthesis and Pharmacological Evaluation of 2-Mercapto-4-Substituted-Naphtho[2,1-*b*]furo[3,2-*d*]pyrimidines

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2-Hydroxy-1-naphthonitrile (1) on treatment with different α-haloketones affords corresponding 2-acyl-3-aminonaphtho[2,1-*b*]furans (2a-2d). The compounds 2a-2d on reacting with ammonium thiocyanate and benzoyl chloride produce N-acyl-N1-(2-acylnaphtho[2,1-*b*] furan)thiourea (3a-3d), which on further refluxing with sodium hydroxide yield 2-mercapto-4-acylnaphtho[2,1-*b*]furo[3,2-d]pyrimidines (4a-4d). These on stirring with chloroacetic acid give S-(4-acylnaphtho[2,1-*b*]furo[3,2-d]pyrimidine)-mercaptoacetic acid (5a-5d). The structures of newly synthesized compounds have been established by elemental analysis and spectral studies. In addition, they have been screened for antimicrobial, diuretic, and antiinflammatory activities. The compounds 4c, d, 5c and 5d were found to be active against bacteria and fungi. The compounds 4c, 5a, 5c, and 5d showed significant diuretic activity having T/S values of 0.89, 0.68, 0.62, and 0.68, respectively. The compounds 5c and 5d showed considerable antiinflammatory activity having percentage protection value of 55.64 and 63.7.

Nitrogen, oxygen, and sulphur containing heterocyclic compounds have received considerable attention due to their wide range of pharmacological activities. Pyrimidine-based heterocyclic compounds are of interest as potential bioactive molecules and exhibit analgesic,[1] antihypertensive,[2] antipyretic,[3] antiviral[4], and antiinflammatory activites.[5] These are also associated with nucleic acid, antibiotic, antimalarial, anticancer drugs.[6] Many of the pyrimidine derivatives are reported to possess potential CNS depressant properties.[7] There are few reports concerning pyrimidine condensed with oxygen heterocycles.[8-9] The naphthofuran derivatives have been shown to exhibit cytotoxic activity[10] and various derivatives of naphtho[2,1-*b*]furan fused with pyrimidine ring were synthesized and evaluated for antibacterial, antifungal, diuretic, and anthelmintic activities in our laboratory.[11-13] Hence, it was thought of interest to synthesize new derivatives of naphtho[2,1-*b*]furopyrimidines by simple method and investigate them for biological and pharmacological activities.

The key starting materials 2-acyl-3-aminonaphtho[2,1-*b*]furans (2a-2d) were synthesized from 2-hydroxy-1-naphthonitrile with phenacyl bromide/chloroacetone/4-chloro-phenacyl bromide/ 4-hydroxy-phenacyl bromide in presence of K_2CO_3 in dry acetone.[14] N-Benzoyl-N[1]-(2-acylnaphtho[2,1-*b*]furan)-thiourea (3a-3d) were prepared by the reaction of ammonium thiocyanate and benzoyl chloride with 2a-2d in dry acetone. N-Benzoyl-N[1]-(2-phenylnaphtho[2,1-*b*]furan)thiourea in the presence of sodium hydroxide underwent intramolecular cyclization to produce 2-mercapto-4-phenylnaphtho[2,1-*b*]furo[3,2-*d*]pyrimidine (4a) in aqueous medium. S-(4-phenylnaphtho[2,1-*b*]furo[3,2-*d*]pyrimidine)mercaptoacetic acid (5a) was prepared by the reaction of (4a) with chloroacetic acid in presence of aqueous Na_2CO_3.

MATERIALS AND METHODS

Melting points were recorded in open capillaries and are uncorrected. The IR spectra (KBr) are recorded on a Shimadzu FT-IR Spectrophotometer. The NMR spectra are recorded on Bruker-400 MHz Spectrometer using TMS as internal reference. Mass spectra were recorded using Shimadzu GC-MS instrument. Purity of the compounds was checked by TLC. For diuretic and antiinflammatory activity studies, adult, healthy albino rats (Wistar strain) of either sex weighing 100-200 g were used. All the animals were maintained under standard conditions

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and had access to pelleted animal feed and water. The study protocols were approved by the Institutional Animal Ethics Committee (CPCSEA Regd. No. 144).

Synthesis of 2-hydroxy-1-naphthonitrile (1):
A mixture of 2-hydroxy-1-naphthaldoxime (0.1 mol) and acetic anhydride (20 ml) was refluxed for 30 min and excess of acetic anhydride was removed by distillation under reduced pressure. The dark colored dense liquid leftover gave naphtho[1,2-d]isoxazole, to this ethanol (100 ml) and freshly prepared sodium ethoxide (0.2 mol) [Prepared by adding freshly cut dry sodium (0.2 mol) into absolute ethanol (50 ml) at 0º] were added. The mixture was stirred for 30 min at room temperature and poured into ice water (500 ml), on acidification with dil hydrochloric acid 2-hydroxy-1-naphthonitrile was obtained as yellow crystalline solid. It was collected by filtration and recrystallized from ethanol.

Synthesis of 2-acyl-3-aminonaphtho[2,1-*b*]furan (2a-2d):
To a solution of 2-hydroxy-1-naphthonitrile (1) (0.02 mol) in dry acetone (100 ml), phenacyl bromide (0.02 mol) and anhydrous potassium carbonate (0.2 mol) were added and the reaction mixture was refluxed on water bath for 8 h. The potassium salt was filtered off and washed thoroughly with acetone. Removal of solvent from the filtrate and subsequent tritution with ethanol gave 2-benzoyl-3-aminonaphtho[2,1-b]furan[14] (2a). The compounds 2b-2d were synthesised using chloroacetone, 4-chloro-phenacyl bromide, 4-hydroxy-phenacyl bromide in place of phenacylbromide by the same method described above.

Synthesis of N-benzoyl-N^1-(2-acylnaphtho[2,1-*b*]furan)thiourea (3a-3d):
To a solution of ammonium thiocyanate (0.011 mol) in dry acetone (25 ml), benzoyl chloride (0.01 mol) was added slowly while stirring. The reaction mixture was heated at reflux for 15 min. A solution of 2-benzoyl-3-aminonaphtho[2,1-*b*]furan (2a) (0.01 mol) in dry acetone (50 ml) was added slowly to the above solution so as to maintain reflux condition. After the addition was complete, the mixture was stirred for 90 min at room temperature, compound 3a, which separated as solid precipitate on pouring in ice cold water was collected by filtration, washed with water, cold ethanol and recrystallised from ethanol.

The compounds N-benzoyl-N^1-(2-acetylnaphtho[2,1-*b*]furan)-thiourea (3b), N-benzoyl-N^1-(2-(4-chloro-benzoyl)-naphtho[2,1-*b*]furan)-thiourea (3c) and N-benzoyl-N^1-(2-(4-hydroxy-benzoyl)-naphtho[2,1-*b*]furan)-thiourea (3d) were synthesised from 2-acetyl-3-aminonaphtho[2,1-*b*]furan (2b), 2-(4-chloro-benzoyl)-3-aminonaphtho[2,1-*b*]furan (2c), 2-(4-hydroxy-benzoyl)-3-aminonaphtho[2,1-*b*]furan (2d), respectively, following the similar procedure.

Synthesis of 2-mercapto-4-phenylnaphtho[2,1-*b*]furo[3,2-*d*]pyrimidine (4a):
A mixture of 3a (0.06 mol), sodium hydroxide (0.08 mol), and water (50 ml) was heated under reflux for 15 min, cooled, diluted with water and acidified with dilute hydrochloric acid. The solid that separated was filtered, washed with water, dried and recrystallised from aqueous DMF to get 2-mercapto-4-phenylnaphtho[2,1-*b*]furo[3,2-*d*]pyrimidine (4a). The compounds 4b-4d were synthesized from 3b-3d in a similar manner.

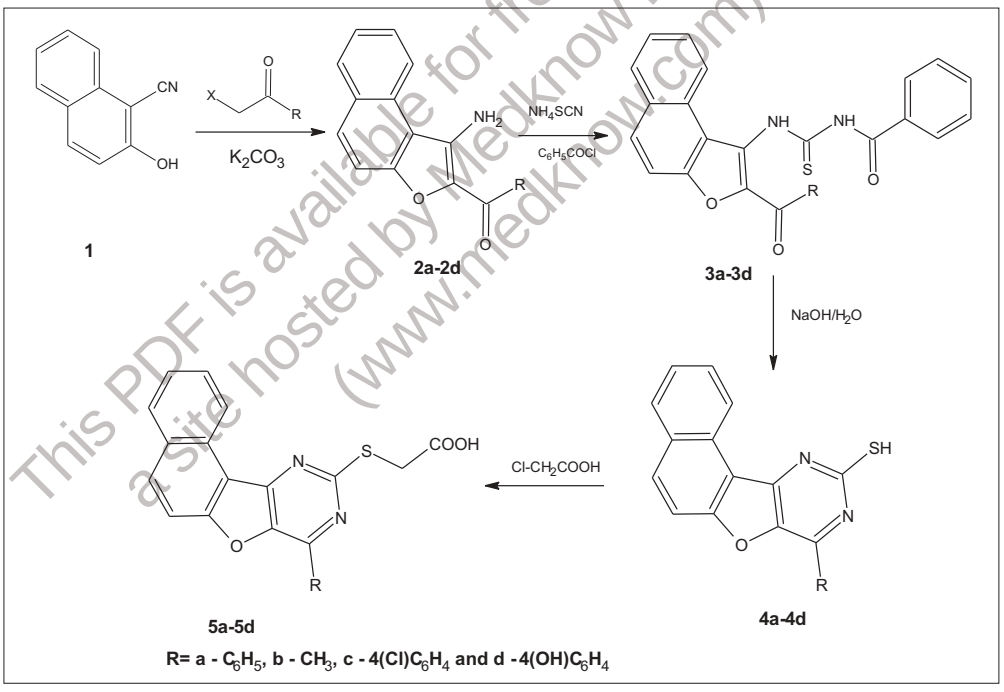
Synthesis of S-(4-phenylnaphtho[2,1-*b*]furo[3,2-*d*]pyrimidine)mercaptoacetic acid (5a):
To a solution of chloroacetic acid (0.005 mol) and sodium carbonate (0.00275 mol) in water (10 ml) was added slowly, while stirring for 30 min, a solution of 4a (0.0055 mol) in sodium hydroxide (0.015 mol) in water (10 ml) and the reaction mixture was stirred overnight at room temperature, acidified with dilute hydrochloric acid and extracted with chloroform. The organic layer was dried over sodium sulphate, the solvent was removed by distillation under reduced pressure to obtain solid, which was recrystallised from ethanol. Physical data of newly synthesized compounds reported in Table 1. The compounds 5b-5d were synthesised from 4b-4d, respectively, following the similar procedure described above. The synthetic route is shown in Scheme 1.

Antimicrobial activity:
The *in vitro* antimicrobial activity was carried out against 24 h old cultures of two bacteria and two fungi by cup-plate method.[15-16] Compounds 4a-4d and 5a-5d were tested for their antibacterial activity against *Pseudomonas aerugenosa* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger* and *Curvularia lunata*. Chloramphenicol and fluconazole were used as standards for antibacterial and antifungal activity, respectively. The compounds were tested at a concentration of 0.001 mol/ml in DMF against all organisms. The zone of inhibition was compared with the standard drug after 24 h of incubation at 25° for antibacterial activity and 48 h at 30° for antifungal activity. Results are reported in Table 2.

Diuretic activity:
The diuretic activity was evaluated on Wistar rats by

TABLE 1: CHARACTERISATION DATA OF THE SYNTHESIZED COMPOUNDS 3a-d, 4a-d AND 5a-d

Compound	R	M.P.°	Yield (%)	Mol. formula	Found (calculated) %		
					C	H	N
3a	C_6H_5	156	73	$C_{27}H_{18}N_2O_3S$	71.90 (71.98	3.96 4.03	6.15 6.22)
3b	CH_3	144	75	$C_{22}H_{16}N_2O_3S$	67.92 (68.02	4.01 4.15	7.15 7.21)
3c	4-Cl-C_6H_5	167	68	$C_{27}H_{17}N_2O_3SCl$	66.79 (66.87	3.45 3.53	5.70 5.78)
3d	4-OH-C_6H_5	186	65	$C_{27}H_{18}N_2O_4S$	69.45 (69.51	3.80 3.89	5.91 6.00)
4a	C_6H_5	174	70	$C_{20}H_{12}N_2OS$	72.99 (73.15	3.59 3.68	8.46 8.53)
4b	CH_3	162	67	$C_{15}H_{10}N_2OS$	67.57 (67.65	3.68 3.78	10.45 10.52)
4c	4-Cl-C_6H_5	203	62	$C_{20}H_{11}N_2OSCl$	66.16 (66.21	2.97 3.06	7.65 7.72)
4d	4-OH-C_6H_5	220	60	$C_{20}H_{12}N_2O_2S$	69.68 (69.75	3.44 3.51	8.00 8.13)
5a	C_6H_5	210	83	$C_{22}H_{14}N_2O_3S$	68.29 (68.38	3.58 3.65	7.16 7.25)
5b	CH_3	193	78	$C_{17}H_{12}N_2O_3S$	62.86 (62.95	3.66 3.73	8.57 8.64)
5c	4-Cl-C_6H_5	232	73	$C_{22}H_{13}N_2O_3SCl$	62.70 (62.78	3.02 3.11	6.59 6.66)
5d	4-OH-C_6H_5	248	70	$C_{22}H_{14}N_2O_4S$	65.59 (65.66	3.45 3.51	6.89 6.96)



Scheme 1: Synthetic route for the synthesis of 4a-4d and 5a-5d

reported method by Lipschitz.[17] Rats of either sex, weighing between 100-200 g were divided into 10 groups, each containing six animals. Group 1 served as control and received aqueous solution of tween-80 (0.1%, 5 ml). Group 2 received 40 mg/kg body weight of frusemide in tween-80 (0.1%, 5 ml) in distilled water orally and served as standard. The groups 3-10 received orally the test compounds at the dose of 30 mg/kg body weight in tween-80 (0.1%, 5 ml). Each group of animals was kept in different metabolic cages provided with a wire mesh at the bottom and a funnel to collect urine. Sieves made up of stainless steel were placed on the funnel to retain feces. The rats were fed with standard diet and water *ad libitum*. Food and water were withdrawn 24 h prior to the experiment. Urine excreted was collected after 5 h and the values are tabulated in Table 3.

TABLE 2: RESULTS OF ANTIMICROBIAL ACTIVITY OF THE COMPOUNDS 4a-d AND 5a-d

Compound	Zone of inhibition in mm			
	P. aerugenosa	*S. aureus*	*Aspergillus niger*	*Curvularia lunata*
4a	12	14	15	10
4b	10	08	12	08
4c	18	16	16	18
4d	16	15	18	18
5a	14	14	15	15
5b	12	12	12	10
5c	16	18	15	18
5d	17	18	16	18
Chloramphenicol	24	26	-	-
Fluconazole	-	-	22	24
DMF	+ve	+ve	+ve	+ve

DMF is used as control, +ve indicates growth of microbes. *Diameter of disc is 5 mm

TABLE 3: RESULTS OF DIURETIC ACTIVITY OF THE COMPOUNDS 4a-d AND 5a-d

Compound	Group	Volume of urine collected in ml after 5 h	T/S (Lipschitz value)
Control	1	8	0.27
Frusemide (standard)	2	29	1.00
4a	3	16	0.55
4b	4	12	0.41
4c	5	26	0.89
4d	6	14	0.48
5a	7	20	0.68
5b	8	16	0.55
5c	9	18	0.62
5d	10	20	0.68

Number of animal used in each group is 6. Tween-80 (0.1%) is used as control. 'T' stands for urine collected for test compounds and 'S' stands for urine collected for standard drug

Antiinflammatory activity:

The antiinflammatory activity was evaluated by rat paw edema method. Edema represents the early phase of inflammation and carragenin-induced paw edema is the simplest and most widely used model for studying the antiinflammatory activity of chemical compounds. This method is based on plethysmographic measurement of carragenin-induced acute rat paw edema[18-22] produced by sub plantar injection of carragenin in hind paw of the rat. The method described by Wilhlmi and Domenjoz[23] later modified by Sirodia and Rao[24] was used for measuring the paw volume.[25-27]

For this study, Wistar rats of either sex, weighing between 100 and 200 g, were used and divided into 10 groups, of four animals each. The Group 1 served as control and received tween-80 (0.1%, 1 ml) solution orally. The Group 2 received ibuprofen in tween-80 (0.1%, 1 ml) at a dose of 40 mg/kg body weight and served as standard. The groups 3-10 received orally the test compounds mentioned at the dose of 30 mg/kg body weight in Tween-80 (0.1%, 1 ml) solution. These drugs were administered 1 h before the injection of an irritant, carragenin. After 1 h all the animals were injected subcutaneously with a suspension of carragenin in tween-80 (0.1%, 0.05 ml) solution to the left hind paw in the subplantar region and the paw volume was measured immediately. After 3 h the paw volume was measured in control, in standard and in test groups. Percent inhibition of paw volume was calculated by using the formula, % inhibition = (1-Vt/Vc) ×100, where Vt = mean increase in the paw volume in test animals group. Vc = mean increase in the paw volume in control group. Statistical analysis was carried to determine % protection and the results are presented in Table 4.

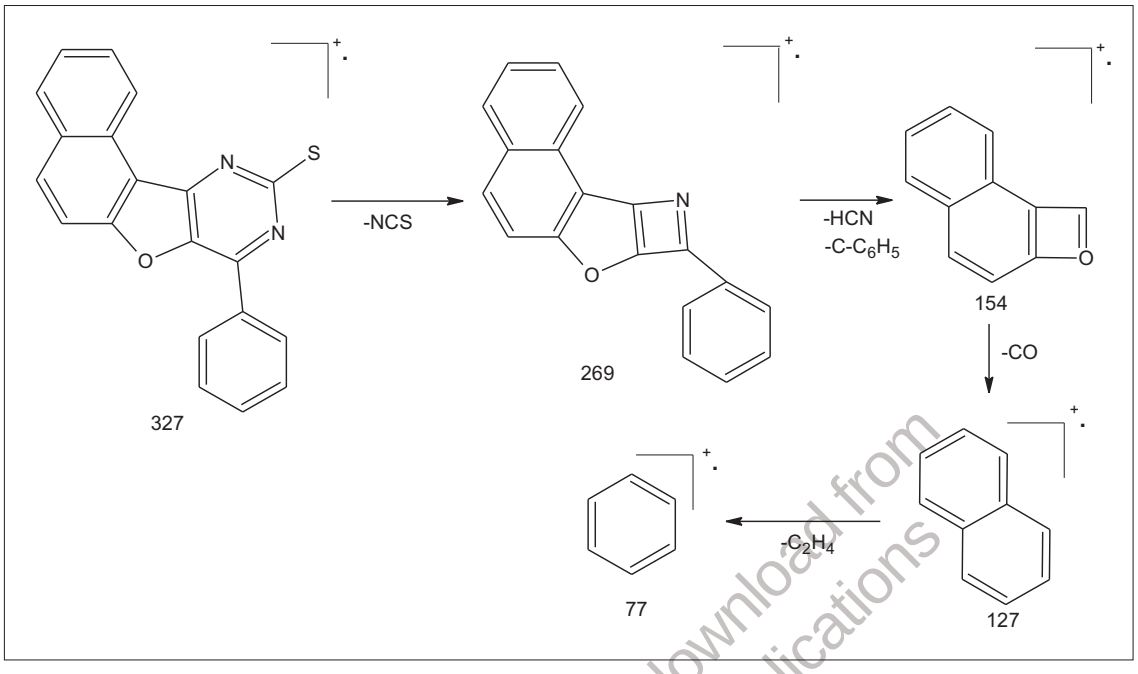
RESULTS AND DISCUSSION

The structures of newly synthesised compounds were elucidated by IR, NMR, mass spectral studies and elemental analysis. The IR spectrum of 3a exhibited the absorption bands at 1672 cm^{-1} (C=O ketone) and 1660 cm^{-1} (C=O amide). In ^1H NMR spectrum ($CDCl_3$) multiplet at δ 7.2-8.4 (m, 16H, Ar-H), singlets at δ 9.4 (s, 1H, NHCS) and δ 12.7 (s, 1H, NHCO) were observed. The ^{13}C NMR spectrum showed peaks at δ 166.73 due to C=S, and peaks at δ 180.7 (COPh) and 183.8 were due to C=O (NHCOPh) carbon atoms. The mass spectrum did not contain molecular ion peak, which may be due to instability of molecular ion. However, other peaks observed at m/z 286, 180, 154, 127, 77 are consistent with the fragmentation pattern of 3a. The IR and ^1H NMR spectra of 3b-3d were in agreement with their structures.

TABLE 4: RESULTS OF ANTIINFLAMMATORY ACTIVITY OF THE COMPOUNDS 4a-d AND 5a-d

Compound	Group	Paw volume ± S.E.M. after 3 h	Inhibition (%) of edema after 3 h
Control	1	1.24 ± 0.152	100
Ibuprofen (standard)	2	0.30 ± 0.037	75.80
4a	3	0.65 ± 0.028	47.58
4b	4	0.67 ± 0.025	45.56
4c	5	0.62 ± 0.047	49.59
4d	6	0.62 ± 0.025	49.59
5a	7	0.57 ± 0.047	53.62
5b	8	0.60 ± 0.070	51.61
5c	9	0.55 ± 0.028	55.64
5d	10	0.45 ± 0.028	63.70

Number of animals used for each group is 4. S.E.M stands for standard error mean



Scheme 2: Mass fragmentation pattern of 4a

The IR spectrum of 4a exhibited the absorption band at 1596 cm^{-1} (C=N). In its ^1H NMR spectrum ($CDCl_3$), a broad singlet at δ 5.0 (b, 1H, SH) and multiplets at δ 7.3-9.2 (m, 11H, Ar-H) were observed. ^{13}C NMR spectrum showed peaks at δ 163.8 due to C=N (C, NCS) and δ 157.4 due to C=N carbon atoms of pyrimidine ring. The mass spectral data showed molecular ion peak at m/z 328 (M^+) corresponding to its molecular weight and peaks at m/z 327, 269, 154, 127, 77 (Scheme 2). The IR spectrum of 5a exhibited the absorption band at 1625 cm^{-1} (C=N) and 1680 cm^{-1} (C=O) stretching. In ^1H NMR spectrum ($CDCl_3$) singlet at δ 4.0 (s, 2H, S-CH_2), multiplet at δ 7.3-9.2 (m, 11H, Ar-H) and singlet at δ 12.8 (s, 1H, COOH) were observed. ^{13}C NMR spectrum showed peaks at δ 163.73 due to C=O (C, COOH) and peaks at δ 157.50 and 153.49 due to C=N carbon atoms of pyrimidine ring. Peak at δ 34.7 was due to CH_2 (-S-CH_2) carbon atom. In the mass spectrum molecular ion peak was not observed, which may be due to instability of molecular ion and peaks at m/z 342, 327, 164, 127, 77 were in consistent with the fragmentation pattern. Analytical data of the compounds supports for the proposed structures.

All the synthesized compounds showed weak antimicrobial activity. The results of antimicrobial activity revealed that compounds 4c, 4d, 5c, and 5d were found to be active against bacteria and fungi. The compounds 4c, 5a, 5c, and 5d showed significant diuretic activity having T/S value of 0.89, 0.68, 0.62 and 0.68, respectively, as compared with that of frusemide. The compounds 5c and 5d showed considerable antiinflammatory activity having % protection value at 55.64 and 63.7 as compared with that of Ibuprofen with % protection value of 75.80.

From the above results it can be concluded that the compounds having electron-withdrawing group (C_6H_5), exhibit more activity than the compounds having electron-releasing group (CH_3). Further introduction of chlorine atom in benzene ring increases diuretic activity and introduction of hydroxyl group increases antiinflammatory activity.

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