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Synthesis, biological and pharmacological activities of 2-methyl-4*H*-pyrimido[2,1-b][1,3]benzothiazoles

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ABSTRACT

*Anilines **1a-h** on reaction with potassium thiocyanate and bromine in acetic acid yielded substituted 2-amino[2,1-b][1,3]benzothiazoles **3a-h**. The compounds **3a-h** on reaction with ethyl acetoacetate and pheylidine acetoacetate**,** yielded, 2-methyl-4-H-pyrimido[2,1-b][1,3]benzothiazole-4-ones **4a-h** and ethyl 4-phenyl-2-methyl-4H–pyrimido[2,1-b][1,3]benzothiazole-3-carboxylates **5a-h** respectively. Pheylidine acetoacetate was conveniently prepared by employing Knoevnagel method using diethyl amine as catalyst. The newly synthesized compounds have been characterized by analytical data IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The compounds were then evaluated for antibacterial, antifungal, and anti-inflammatory activities.*

Key words: 2-Aminobenzothiazoles, pyrimidobenzothiazoles, biological activities, pharmacological activities.

INTRODUCTION

Benzothiazoles make a broader class of nitrogen and sulfur containing compounds with wide range of therapeutic activities viz., antifungal[1], antibacterial[2], anthelmintic[3], antimalarial[4], analgesic[5] anti-inflammatory[6], anticancer[7] etc. Benzothiazoles when

combined with biologically active heterocycles such as pyrimidine have exhibited potent pharmacological activities with improved pharmacokinetic properties[8, 9]. Receiving impetus from the above observations, synthesis and biological activity of benzothiazoles containing fused pyrimidine ring was undertaken.

MATERIALS AND METHODS

Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on Perkin Elmer and Nicolet FT-IR spectrophotometers. NMR spectra were recorded on AMX and Brucker 400MHz.

Synthesis of substituted 2-Aminobenzothiazoles (3a-h)

A mixture of 3-chloro- 4-flouroaniline (0.01 mol, 1.45 g) and potassium thiocyanate (8 g) were added to glacial acetic acid (20 mL), pre-cooled to 0^o C. A solution of bromine (1.6 mL) in acetic acid (6.0 mL) was added slowly with constant stirring. The temperature was maintained at 0^o C through out the addition. After all the bromine has been added, the solution was stirred for an additional 2 hr at 0^o C and at room temperature for 10 hr. It was allowed to stand over night, during which an orange residue settled at the bottom, water (6 mL) was added quickly and the slurry was heated to 85^o C on a steam bath and filtered hot. The orange residue was placed in a reaction flask and treated with glacial acetic acid (10 mL) heated again to 85^o C and filtered hot. The filtrates were combined, cooled and neutralized with conc. ammonia solution to pH 6. The dark yellow precipitate was collected re-crystallized (twice) from benzene. After treating with activated charcoal, it gave colorless plaques of 2-amino-5-chloro-6-flurobenzothiozole. The dry material ($59^0/_0$ yield) melted at 207 to 209^o C.

Similarly compounds **3b-h** were synthesized using appropriately substituted aromatic amines.

Synthesis of 6-chloro-2-methyl-7-fluoro-4- pyrimido[2,1-*b*] [1,3]benzothiazole - 4-ones (4a-h)

The compound **3a (**2.02 g, 0.01 mol) was refluxed with an excess of ethyl acetoacetate (6.5g, 0.05 mol) for 2 hr, when a white solid settled at the bottom. It was filtered and fused at 180^o C in an oil bath. The compound was then recrystallised from absolute ethanol and dried. The dried material (yield 85%), melted at 248^o C.

Similarly the compounds **4b-h** were synthesized from the compounds **3b-h**.

Synthesis of phenylidene acetoacetates

A mixture of ethyl acetoacetate (0.1 mol, 13.01g) of and benzaldehyde (10.61g) was stirred mechanically in the presence of diethyl amine (1 ml) and pyridine (1 ml). The reaction temperature was maintained below 5^o C when a yellow crystalline solid separated at the bottom. The product, phenylidine aceoacetate, that separated as solid was filtered, washed with 95% ethanol and dried. After recrystallization with absolute ethanol the dried material (yield 95%) melted at 158^o C.

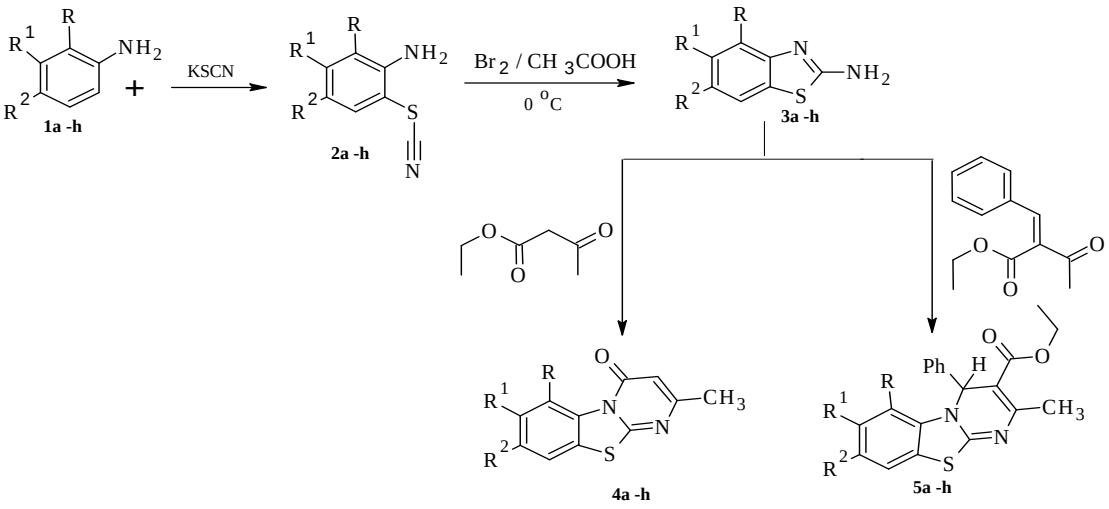
Synthesis of 6-chloro-2-methyl-7-fluoro-4H-pyrimido[2,1-*b*][1,3]benzothiazole -4-ones (5a-h)

The **3a** (2.02 g) and phenylidine acetoacetate (2.18g)**,** were treated with sodium ethoxide (0.46 g of sodium dissolved in 10 cm^3 of absolute ethanol) and refluxed for 3 hr. The reaction mixture was cooled, the solvent was evaporated and the residue thus obtained was purified by recrystaliization using absolute ethanol and dried. The dried material (yield 72%), melted at 252^o C.

Similarly the compounds **5b-h** were synthesized from **3b-h.**

The sequence of reactions is depicted in the scheme

Scheme



Compd.	R	R^1	R^2	Compd.	R	R^1	R^2
a	H	Cl	F	e	H	H	Cl
b	CH_3	H	H	f	H	H	NO_2
c	H	CH_3	H	g	H	H	OH
d	H	H	CH_3	h	H	H	COOH

Analytical data of the all the synthesized compounds has been summarized in Table 1.

Antimicrobial activity:

In vitro antibacterial activity was determined by agar well diffusion method[10] against 24 hr old cultures of *Staphylococcus aureus, Micrococcus luteus, Eshcerichia coli,* and *Pseudomonas aerugenosa* using 0.001 mol/mL of ofloxacin and ampicillin as standards. The compounds were tested at the concentration of 0.001 mol/ml in N, N-dimethylformamide for all the organisms. The zone of inhibition was compared with the standard drugs after 24 hr incubation at 37^o C.

Similarly antifungal activity was carried out agar well diffusion method[11] against *Aspergillus niger* and *Aspergillus flavus* using 0.001mol/mL of fluconazole as standard. The test samples were prepared in N,N-dimethylformamide at the concentration of 0.001/ml . The zone of

inhibition was compared with the standard drug after 72 hr incubation at 25° C. The results of antimicrobial activity are presented in Table 2.

Table 1- Physical characterization data of synthesized compounds

Compound	R	R^1	R^2	Molecular Formula	M.P.^{0}C	% Yield	*m/z*
4a	H	Cl	F	$C_{11}H_6N_2OSClF$	248	72	268
4b	CH_3	H	H	$C_{12}H_{20}N_2OS$	240	76	230
4c	H	CH_3	H	$C_{12}H_{20}N_2OS$	240	78	230
4d	H	H	CH_3	$C_{12}H_{20}N_2OS$	241	78	230
4e	H	H	Cl	$C_{11}H_7N_2OClS$	247	75	250
4f	H	H	NO_2	$C_{11}H_7N_3O_3S$	210	76	261
4g	H	H	OH	$C_{11}H_8N_2O_2S$	296	76	232
4h	H	H	COOH	$C_{12}H_8N_2O_3S$	222	72	260
5a	H	Cl	F	$C_{20}H_{16}N_2O_2SClF$	252	73	402
5b	CH_3	H	H	$C_{21}H_{20}N_2O_2S$	237	76	364
5c	H	CH_3	H	$C_{21}H_{20}N_2O_2S$	242	78	364
5d	H	H	CH_3	$C_{21}H_{20}N_2O_2S$	243	80	364
5e	H	H	Cl	$C_{21}H_{17}N_2O_2SCl$	238	74	384
5f	H	H	NO_2	$C_{20}H_{17}N_3O_4S$	258	80	395
5g	H	H	OH	$C_{20}H_{18}N_2O_3S$	271	79	366
5h	H	H	COOH	$C_{20}H_{18}N_2O_4S$	269	76	394

Table 2-Antimicrobial activity of the synthesized compounds

	Zone of inhibition in mm					
	Antibacterial activity			Antifungal activity		
Compd	*S. aureus*	*M. Luteus*	*E. coli*	*P. aerugenosa*	*A. niger*	*A. flavus*
5a	13	15	11	15	19	23
5b	16	18	19	20	18	17
5c	15	18	17	16	15	16
5d	10	11	13	11	23	19
5e	15	10	09	12	20	18
5f	13	15	14	11	19	17
5g	16	18	11	15	23	18
5h	14	16	17	15	15	13
7a	16	11	13	15	19	16
7b	15	14	18	15	23	16
7c	16	18	20	15	16	11
7d	09	13	10	11	23	18
7e	14	13	12	15	18	15
7f	16	13	15	14	20	20
7g	13	18	15	11	16	14
7h	09	10	13	11	23	19
Standard 1	33	36	28	36	-	-
Standard 2	28	33	24	30	-	-
Standard 3	-	-	-	-	18	18

Standard-1: ofloxacin, Standard-2:ampicilin trihydrate, Standard-3 :fluconazole, Sample concentration: 0.001mol/mL, Sample volume: 1mL in each well

Dose fixation

LD_{50} of the title compounds was determined according to OECD guidelines[11] 423. The compounds were tolerated to a maximum dose of 50mg / kg body weight when tested against albino rats. Therefore, 5mg / kg was taken as effective dose for the purpose evaluation of anti-inflammatory.

Table 3- Anti-inflammatory activity of the synthesized compounds

Compound	Group	Mean rat paw volume ± SEM after 3 hr	% Protection
Control	1	1.81 ± 0.010	
Standard	2	0.63 ± 0.020	65.20
4a	3	1.02 ± 0.081	43.65
4b	4	0.91 ± 0.020	49.23
4c	5	0.90 ± 0.010	50.28
4d	6	0.98 ± 0.030	45.86
4e	7	0.99 ± 0.010	45.31
4f	8	0.98 ± 0.010	45.86
4g	9	0.85 ± 0.020	53.04
4h	10	0.89 ± 0.020	50.83
Control	1	2.58 ± 0.780	
Standard	2	0.45 ± 0.260	82.56
5a	3	1.03 ± 0.330	60.08
5b	4	0.93 ± 0.120	63.96
5c	5	0.92 ± 0.210	64.34
5d	6	0.86 ± 0.300	66.70
5e	7	0.85 ± 0.140	67.07
5f	8	0.82 ± 0.210	68.22
5g	9	0.85 ± 0.220	67.06
5h	10	0.11 ± 0.630	95.73

± SEM = Standard Error Mean; Number of animals n = 6

Anti-inflammatory activity

The anti-inflammatory activity was evaluated by rat paw edema method. Edema represents the early phase of inflammation and carragenin-induced paw edema is the simplest and widely

accepted model for studying the anti-inflammatory activity of chemical compounds. This method is based on plethysmographic measurement of carragenin-induced rat paw edema produced by sub plantar injection of carragenin in hid paw of the rat[12-15]. The method described by Willhemi and Domenjoz[16], later modified by Sirodia and Rao[17] was used for measuring the paw volume[18-20].

For this study, Wistar rats of either sex, weighing between 100 and 200 g, were used and divided into 10 groups of six animals each. The group 1 served as control and received tween-80 (0.1%, 1 cm^3) solution orally. The group 2 received diclofenac sodium in tween-80 (0.1%, 1 cm^3) 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 cm^3) solution. These compounds were administered 1 hr before injection of an irritant, carragenin. After 1 hr all the animals were injected subcutaneously with a suspension of carragenin in tween-80 (0.1%, 0.5 cm^3) solution to the left hind paw in the sub plantar region and the paw volume was measured immediately. After 3 hr the paw volume was measured in control, in standard and in test groups. Percent inhibition of paw volume was calculated by using formula, % inhibition = (1-Vt/Vc) X 100, where Vt = mean increase in the paw in test animals, Vc = mean increase in the paw volume in control group. Statistical analysis was carried out to determine % protection and the results are presented in Table 3.

RESULTS AND DISCUSSION

2-Aminothiocyanates were thought to be appropriate intermediates for the synthesis of title compounds. Thus various 2-aminothiocyanates **2a-h** were synthesized by reacting substituted anilines **1a-h** with potassium thiocyanate. These substituted anilines were selected to assess the impact of inductive effect and mesomeric effects on biological and pharmacological activities. In all the cases, as expected, thiocyanato group entered ortho position with respect to amino group. The compounds **2a-h** underwent smooth oxidative cyclization when treated with bromine in presence of acetic acid[21] and yielded corresponding 2-amiobenzothiazoles **3a-h** in good yield. The structures of the compounds **3a-h** were confirmed by spectral data. The IR spectrum of **3a** exhibited absorption band at 3475 cm^{-1} due to N-H stretching vibration and two absorption bands at 812 $cm^{-1}$and 715 cm^{-1} were assigned to aromatic C-H out of plane deformation. Two peaks at δ 7.45 and δ 7.85 due to two aromatic protons and D_2O exchangeable peak at δ 7.66 due to two –NH_2 protons were observed in ^{1}H NMR spectrum of **3a.** ^{13}C NMR spectrum of **3a** showed peaks at δ 168, 153, 151,150, 131, 118 and 109 assignable to carbon atoms of **3a**.The structure assigned to **3a** was further supported by its mass spectrum which exhibited M^+ and M^{+2} peaks at m/z 202 and 204 as anticipated. Fragmentation pattern also was in agreement with the assigned structure.

2-Aminothiazoles **3a-h** served as excellent intermediates for the synthesis of pyrimidobenzothiazoles. Thus, the compounds **3a-h** on reaction with ethyl acetoacetate produced correspondingly substituted 2-methyl-4H-pyrimido[2.1-b][1,3]benzothiazole-4-ones **4a-h** in good yield. The spectral data of **4a** was in agreement with structure assigned to it. Its IR spectrum was conspicuous by the absence of absorption band at 3475 $cm^{-1}$and appearance of absorption band at 1685 cm^{-1} due to amide carbonyl group. The ^{1}H NMR spectrum of **4a** showed singlet at δ 3.9 due three protons of methyl group, singlet at δ 3.9 due to CH proton of

pyrimidine ring and two singlets at δ 8.2 and δ 8.2 assignable to remaining two aromatic protons. The structure of **4a** was well supported by its ^{13}C NMR spectrum which exhibited peaks due to C=O carbon at δ 165 and C=N carbon at δ 160. Its mass spectrum showed molecular ion peak at m/z 270 (M^{+2}) and 268 (M^{+}). The other peaks appearing at 240,201, 151 and 93 were in accordance with expected fragmentation pattern.

Another route for constructing pyrimidine ring over thiazole moiety, involved condensation of compounds **4a-h** with phenylidine acetoacetate, The required phenylidine acetoacetate was prepared by employing Knoevnagel reaction between ethyl acetoacetate and benzaldehyde in presence of diethyl amine and pyridine as catalysts. Synthesis of substituted ethyl 4-aryl-2-methyl-4H-pyrimido[2,1b]p1,3]benzothiazole-3-carboxylates **5a-h** was accomplished by reacting 2-aminothiazoles **3a-h** with phenylidine acetoacetate using sodium ethoxide in ethanol. The formation of compounds **5a-h** was confirmed by spectral analysis. A strong absorption band at 1726 cm^{-1} due to ester carbonyl group, and band at 1263 cm^{-1}due to C=S appeared in IR spectrum of compound **5a**. ^{1}H NMR spectrum of **5a** exhibited a triplet at δ 1.4 and a quartet at δ 4.4 due to the protons of $–CH_2$-CH_3, a singlet at δ 2.1 due protons of $–CH_3$, another singlet at δ 3.9 due to CH proton of pyrimidine ring, where as the aromatic protons of phenyl ring appeared δ 7.0 to 7.5 and the protons of benzothiazole moiety appeared at δ 8.0 to 8.4. In support of the structure assigned to **5a** its ^{13}C NMR spectrum was recorded, which showed peaks assignable to various carbon atoms of **5a.**

In vitro antibacterial activity of the compounds was carried out by agar well diffusion method against 24 hr culture of S*typhalococcus aureus, Micrococcus luteus, Eshcerichia coli and Pseudomonas aerugenosa*. The compounds **4a** and **5c** exhibited excellent activity against *E. coli*. Antifungal activity was performed on *Aspegillus niger* and *aspergillus flavus.* The compounds **4a-h** exhibited excellent activity against *A.nige* , and **5a,5b, 5d-5f** and **5h** showed good *A. flavus.*

Anti-inflammatory activity was carried out by rat paw edema method on albino rats (Wistar strain) and result indicated that the compound **5h** inhibited significantly carageenin induced rat paw edema to the extent of 95.73 % in comparison with the standard drug (82.56%). The compounds having electronegative hydrophilic functionality exhibited moderate to good activity.

For carrying out experiments with animals, approval from Institutional Animal Ethics Committee in accordance with “Principles of Laboratory Animal Care” was obtained as per certificate No. 3, 2003-04, issued to Sree Siddaganga College of Pharmacy, Tumkur, Karnataka.

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