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SYNTHESIS AND ANTI-BACTERIAL SCREENING OF SOME NOVEL FUSED IMINO PYRIMIDO BENZOTHIAZOLE AND ITS SCHIFF'S BASES

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ABSTRACT

Benzotiazole and pyrimidine have been reported to act as effective pharmacophores. However, much less work as been carried out on these heterocycles fused with each other. In the present study, we report the synthesis of a series of 8-chloro-3-(phenylcarbonoimidoyl)-10a-H-pyrimido [2,1-*b*][1,3]benzothiazole-2-thiol. This series was synthesized by the reaction of 8-chloro-2-sulfanyl-10a-H-pyrimido [2,1-*b*][1,3]benzothiazole-3-carbaldehyde with various aromatic amines in presence of dimethyl formamide and sodium sulphide. 2, 8-dichloro-10a-H-pyrimido[2, 1-*b*][1,3]benzothiazole-3-carbaldehyde was prepared by reaction of N-(6-chloro-1, 3-benzothiazole-2-yl)acetamide with Vilsmeier-Haack reagent. All the synthesized compounds were screened for their anti-inflammatory and anti-bacterial activities. The results of both the studies confirmed the biological potential of these fused heterocycles which can be further used as therapeutic lead for generating new drug candidates.

KEYWORDS: Benzothiazole, Pyrimidine, Vilsmeier Haack reagent, Anti-inflammatory activity and Anti-bacterial activity

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INTRODUCTION

The compounds encompassing benzothiazoles and pyrimidines are of great interest and have been extensively used in medicinal chemistry [1,2]. However, literature survey revealed that very little work has been carried out on synthesis of fused pyrimido benzothiazole heterocycles. These heterocycles have been reported to act as effective pharmacophores. Benzothiazoles are widely found in bioorganic and medicinal chemistry with applications in drug discovery and have very significant antitumor [3-5], antiviral [6], anti-HIV [7], and anti-microbial activities [8,9]. Benzothiazoles can be used for the treatment of autoimmune and inflammatory diseases, [10] in the prevention of solid organ transplant rejection, epilepsy [11-13], amyotrophic lateral sclerosis [14] and analgesia [15]. Further industrial applications as antioxidants [16,17], vulcanization accelerators [18] and a dopant in light emitting organic electroluminescent devices [19] have also been reported. Also, pyrimidine derivatives form an important component for a number of useful drugs and are associated with many biological, pharmaceutical and therapeutic activities [20]. Fused pyrimidine derivatives with other heterocycles have been reported as antiviral, anti-inflammatory [21], anti-HIV [22], anti-tubercular [23], anti-neoplastic [24], anti-malarial [25], diuretic [26], cardiovascular [27] agents. Pyrimidine compounds are also used as hypnotic drugs for the nervous system [28].

It is surmised that fused pyrimido benzothiazoles would also possess interesting pharmacological properties. These observations prompted us to synthesize a variety of pyrimido benzothiazoles and its substituted derivatives. Moreover, we have successfully attempted to prepare some novel benzothiazoles containing imino pyrimidines on which very less research work has been carried out. The newly synthesized compounds were further evaluated for anti-inflammatory and anti-bacterial potential and their activities were compared with conventional marketed drugs. The anti-inflammatory activity was determined by the method of carrageenan-induced hind paw oedema in albino rats using diclofenac sodium as standard. Also, the anti-bacterial activity was evaluated by cup-plate method along with the determination of minimum inhibitory concentration. This activity was tested against gram positive bacteria; *S. aureus* and *B. subtillus* and gram negative bacteria *P. aeruginosa* and *E. coli.* The results of both the studies confirmed the biological potential of these fused heterocycles which can be further used as therapeutic lead for generating new drug candidates.

MATERIALS AND METHODS

(i) Synthesis of 2-amino-6-chloro benzothiazole (I)

Procedure: In a round bottom flask, potassium thiocyanate 8gm (0.08mol) and p-chloro aniline 1.45gm (0.01 mol) were taken and glacial acetic acid (20 ml) was added to it. The mixture was placed in freezing mixture of ice and salt (0-5°C) and mechanically stirred, of bromine (1.6 ml) and glacial acetic acid (6 ml) was added to it from a dropping funnel at such a rate that the temperature never raised above room temperature. After all the bromine was added in about 105 minutes, the solution was stirred for 2 hours below room temperature and at room temperature for 10 hours. It was then allowed to stand overnight and during this period, an orange precipitate settled at the bottom. Water (6 ml) was added quickly and slurry was heated at 85°C on a water bath and filtered while hot. The orange residue was placed in a reaction flask and treated with of glacial acetic acid (10 ml), heated again to 85°C and filtered quickly. The combined filtrate was then cooled and neutralized with concentrated ammonia solution to pH-6. A dark yellow precipitate was collected and recrystallized from a mixture of benzene : ethanol (1:1) which gave yellow flakes of 2-amino-6-chloro-(1,3)-benzothiazole. After drying in oven at 80°C, the dry product (1 gm,

51.02%) was obtained. Melting point: 183-187°C.

(ii) Synthesis of N-(6-chloro-1, 3-benzothiazole-2-yl)acetamide (II)

Procedure: 6-chloro-2-aminobenzothiazole (0.035mol) was dissolved in 25ml of pyridine with efficient stirring. Acetyl chloride (0.02 mol) was added dropwise to it and stirred for 1 hour at room temperature. The reaction mixture was poured on ice cold water to obtain yellow coloured product, which was then filtered, washed with water, dried and recrystallized from ethyl acetate. Melting point: 163-167°C.

(iii) Synthesis of 2, 8-dichloro-10aH-pyrimido [2, 1-b][1,3]benzothiazole-3-carbaldehyde (III)

Procedure: Phosphorus oxychloride (98.28 mmol) was added dropwise to DMF (34.65 mmol) taken in a round bottom flask keeping the temperature between 0–5°C. The mixture was allowed to stir for about 5 minutes. *N*-(6-Chloro-1, 3-benzothiazole-2-yl)acetamide (10.37 mmol) was then added to the reaction mixture and heated for 75–80°C. After completion of reaction, the reaction mixture was cooled to room temperature and then poured into crushed ice under stirred condition. A pale yellow precipitate was obtained which was filtered and dried. The crude compound was recrystallized from ethyl acetate. Melting point: 182-185°C.

(iv) Synthesis of 8-chloro-2-sulfanyl-10a-H-pyrimido [2,1-b] [1,3]benzothiazole-3-carbaldehyde (IV)

Procedure: **S**odium sulphide (1.5 mmol) was added to a solution of 2,8-dichloro-10a-H-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbaldehyde (1 mmol) in dry DMF (5 ml), and stirred for 4-5 hours at room temperature. The reaction mixture was poured into ice cold water and then acidified with acetic acid. The yellow coloured product was thus obtained which was later on filtered, washed with water, dried and recrystallized from ethyl acetate. Melting point: 270-273°C.

(v) Synthesis of Schiff's bases (imines) from carbaldehyde (V)

Procedure: A mixture of 8-chloro-2-sulfanyl-10a*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbaldehyde (0.01 mol), various anilines (0.01 mol) and glacial acetic acid (2 ml) were dissolved in ethanol. The mixture was refluxed at (90ºC) and (500 W) power in microwave synthesizer (Catalyst^{TM} System). The reaction achieved completion in 3-4 minutes, which was monitored by TLC. The reaction mixture was then cooled and poured into crushed ice to obtain the product, which was filtered, washed with water and recrystallized from ethanol. The reactions corresponding to the above synthetic procedures are depicted in (Fig 1) along with the structures of derivatives in (table 1). The physicochemical parameters of synthesized compounds are given in (table 2).

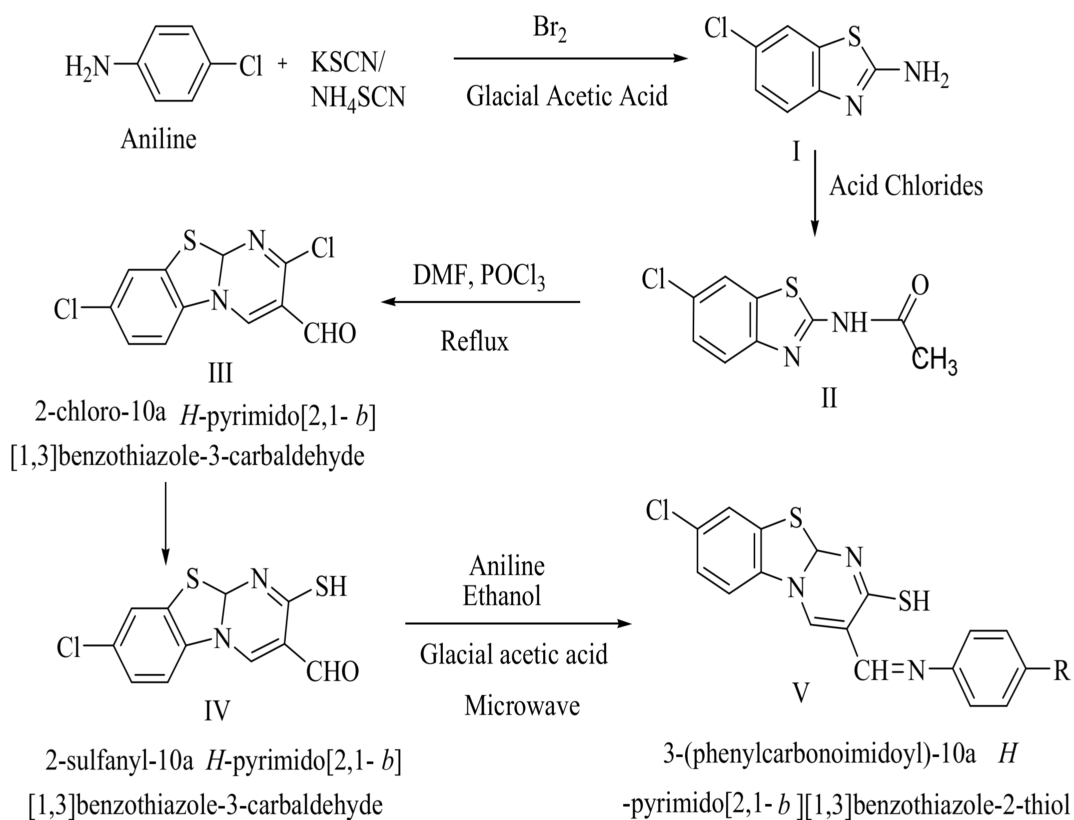

Figure 1

Synthetic scheme for synthesis of pyrimido [2,1-b] benzothiazole derivatives (Schiff' bases)

Table 1
Derivatives (R) of the pyrimido [2,1-b] benzothiazole

Sr.No.	Compound No.	R	Structure
1	III	-H	
2	IV	-H	
3	Va	-C_6H_7N	
4	Vb	-C_6H_6ClN	
5	Vc	-C_7H_9N	
6	Vd	-C_7H_9NO	
7	Ve	-$C_7H_7NO_2$	
8	Vf	-C_6H_7NO	

Table 2
Physicochemical parameters of synthesized compounds

Sr. No.	Compound Code	R	m.p. (°C)	Yield (%)	Mol. Formula	Mol. Wt.
1	III	-H	183	56.95	$C_{11}H_6N_2SOCl_2$	285
2	IV	-H	272	73.33	$C_{11}H_6N_2S_2OCl$	282.5
3	IVa	-C_6H_7N	291	62.5	$C_{17}H_{12}N_3S_2Cl$	357.5
4	Vb	-C_6H_6ClN	183	59.71	$C_{17}H_{12}N_3S_2Cl_2$	392
5	Vc	-C_7H_9N	256	64.40	$C_{18}H_{14}N_3S_2Cl$	371.5
6	Vd	-C_7H_9NO	266	63.34	$C_{18}H_{14}N_3S_2ClO$	387.5
7	Ve	-$C_7H_7NO_2$	242	77.46	$C_{18}H_{12}N_3S_2ClO_2$	401.5
8	Vf	-C_6H_7NO	265	71.86	$C_{17}H_{12}N3S_2Cl$	373.5

PHARMACOLOGICAL SCREENING

Anti-bacterial Activity [29,30]

Cup and plate method: All the test compounds were evaluated for anti-bacterial activity against *S. aureus, B. subtillus* (gram-positive), *E.coli, P.aeruginosa* (gram negative), by using the agar diffusion assay method. The solutions of test compounds were prepared by dissolving test samples (2 mg) in DMF (10ml). A reference standard was made by dissolving

accurately weighed quantity of ciprofloxacin in DMF. The nutrient agar plates were prepared by pouring 30 ml of the medium into each sterilized petridish and were allowed to set at room temperature. The cell suspension was standardized to the density of 530 nm using spectrophotometer and was inoculated over the surface of agar medium using sterile cotton swab. The cups were scooped in each plate using a sterile cork borer of 6 mm diameter. Then the solutions of test compounds (0.10 ml) were added in cups by using micropipettes and these plates were incubated at 37°C for 48 hours. The zone of inhibition was measured in mm for each bacterial strain.

Minimum inhibitory concentration method

Antimicrobial susceptibility testing was done on the same strains of bacteria by determining the minimum inhibitory concentration (MIC) using tube dilution technique. Single strength nutrient broth was prepared by dissolving 3.12 g of nutrient broth in 250 ml distilled water. The medium was boiled to aid dissolution and sterilized by autoclaving at 15 psi pressure (121˚C) for 20 minutes. 1 ml of single strength nutrient broth was added to a set of presterilized 5 test tubes (numbered from 1-5). To the first test tube, 1 ml sample solution (conc. =1000 µg/ml) was added. After thorough mixing, 1 ml of solution from test tube no.1 was transferred to test tube no.2 so as to obtain concentration of 500µg/ml. The same procedure (serial dilution) was followed for the remaining test tubes from no. 3 to no. 5 to get the concentration of samples in the range of 250µg/ml to 62.5µg/ml from 3rd to 5th test tube. From 5th test tube 1 ml of solution was discarded so as to get the equal volume in each test tube. Thus, each tube has concentration of 1000, 500, 250, 125, 62.5µg/ml. To each test tube, 20 µl of respective microorganism suspension was added (inoculation). All test tubes were incubated at 37˚C for 24 hours and observed for turbidity.

Anti-Inflammatory Activity [31,32]

Carrageenan induced paw oedema in rats (in-vivo model)

Animals were divided into control, standard and test groups comprising of five animals in each group. They were fasted overnight with free access to water before experiment. In all groups, acute inflammation was produced by sub-plantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in the right hind paw of the rats. The paw volume was measured plethysmometrically at 0 hour and 3 hours after carrageenan injection. The test compounds (25 and 50mg/kg) were administered orally, standard group was treated with diclofenac sodium (10mg/kg) orally 1 hour before the injection and control group received only vehicle. The mean difference in paw volume was measured and percentage inhibition was calculated by using formula

$$\% \text{ inhibition of oedema} = [(Vc - Vt) / Vc] \times 100$$

where Vt and Vc are the mean paw volumes of test group and control group; respectively.

RESULTS AND DISCUSSION

All the compounds were structurally elucidated by infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry. The melting points were determined by using open capillary tube method and are uncorrected. The spectral analysis data of all synthesized compounds is depicted in (table 3). The IR spectra of synthesized compounds were recorded on SHIMADZU FTIR spectrophotometer. The IR spectrum of the compounds has shown characteristic absorption bands at appropriate wavenumbers (cm^{-1}). The ^{1}HNMR spectra were analyzed on Advance 300 MHz spectrometer. The ^{1}HNMR spectrum of the compound (in DMSO-d6) has exhibited characteristic proton signals (ppm). The mass spectra of the compounds in also in agreement with the molecular weight of assigned structure. The newly synthesized compounds have been screened for their anti-bacterial and anti-inflammatory activities.

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Table 3
Spectral analysis data of the synthesized compounds

Compound Code	Spectral Analysis
III	IR= C=N (1591.94), C=O (1693.93), C-Cl (745.42), C-H (Aldehyde) (2853.31), NMR=6.2-7.4 (m,4H,Ar-H), 9.6 (s,1H,CHO)
IV	IR= C=N (1650), C=O (1695.43), C-Cl (688.59), C-H (Aldehyde) (2709.99), S-H (2550), NMR=6.2-7.4 (m,4H,Ar-H), 9.6 (s,1H,CHO), 1.6 (s,1H, SH)
Iva	IR= C=N (1680), C-Cl (690.52), S-H (2600), Ar (monosub.) (769.60), NMR= 6.2-7.3 (m,9H, Ar-H), 7.5 (s,1H, CH=NH), 1.6 (s,1H,SH)
IVb	IR= C=N (1680), C-Cl (670), S-H (2600), Ar (P-Disub.) (815.89)
IVc	IR= C=N (1678.07), C-Cl (690.52), S-H (2600), Ar (P-Disub.) (815.89), CH_3 Str. (2900)
IVd	IR= C=N (1662.64), C-Cl (688.59), S-H (2600), Ar (P-Disub.) (813.96), CH (CH_3-O) Str. (2835)
IVe	IR= C=N (1640), C-Cl (762), S-H (2550), Ar (P-Disub.) (809.25), OH (Acid) (2650), C=O (Acid) (1700.84)
IVf	IR= C=N (1678.07), C-Cl (723.20), S-H (2575), Ar (P-Disub.) (806.64), OH (Phenol) (3340.82)

Antibacterial Activity by MIC Method

MICs of test compounds were determined by tube dilution technique against gram positive (*S. aureus, B. subtillus*) and gram negative bacteria (*E. coli, P. aerugenosa*) and the results are shown in (table 4). The turbidity was measured with the help of colorimeter at about 620 nm. Thus, by colorimetry, MIC was found to be 125µg/ml. This concentration was used to study zone of inhibition of synthesized compounds.

Table 4
Antibacterial activity by MIC method

Compound No.	Concentration (µg/ml)	Transmittance (%)			
		S. aureus	***E. coli***	***B. subtillus***	***P. aeruginosa***
IV	250	90	91	89	95
IV	125	83	90	89	91
Vd	250	85	88	92	91
Vd	125	85	88	90	93

Antibacterial Activity by Cup Plate Method

All the synthesized compounds were evaluated for antibacterial activity and the result are depicted in (table 5). The concentration of test compounds which was taken for zone of inhibition was 125µg/ml. The test compounds are found to be active against both gram-positive and gram-negative organisms.

Table 5
Antibacterial activity by cup-plate method

Compound no. (125µg)	Zone of inhibition (mm)			
	S. aureus	***E. coli***	***B. subtillus***	***P. aeruginosa***
IV	15	14	12	12
Va	17	15	16	15
Vb	15	14	14	12
Vc	10	12	10	10
Vd	10	13	12	12
Ve	12	12	11	11
Vf	12	15	12	13
Ciprofloxacin	32	29	34	30

Anti-inflammatory Activity

***In-vivo* model:** Anti-inflammatory activities were determined by carrageenan-induced paw oedema in rats. The results of activity are presented in (tables 6 and 7). Compound were tested at two different concentrations as 25 mg / kg and 50 mg / kg body weight (b.w). The tested compounds show moderate anti-inflammatory activity as percentage inhibition were 50.28% and 56.52% for 25 and 50 mg/kg b.w.; respectively and that for the standard compound was found to be 65.71% for 10 mg/kg b.w. The mean difference in paw volume was evaluated statistically by student's t-test [33] and the results are found to be statistically significant at P< 0.01.

Table 6
Anti-inflammatory activity (in-vivo model) by carrageenan induced paw oedema in rats (Mean paw volume)

Compound no.	Mean Paw Volume (ml)						
	Initial	30 Min.	1 hr.	2 hr.	3 hr.	6 hr.	24 hr.
Control	0.75	0.96	1.35	1.44	1.75	1.65	1.45
IV-A	0.69	0.86	0.96	0.94	0.87	0.83	0.69
IV-B	0.71	0.87	0.9	0.84	0.76	0.75	0.73
Diclofenac	0.75	0.72	0.69	0.63	0.6	0.6	0.6

A= 25 mg / kg b.w., B= 50 mg / kg b.w.

Table 7
Anti-inflammatory activity (in-vivo model) by carrageenan induced pawo edema in rats

Compound no.	Inhibition of oedema (%)					
	30 min.	1 hr.	2 hr.	3 hr.	6 hr.	24 hr.
Control	--	--	--	--	--	--
IV-A	8.51	3.03	34.72	50.28	49.69	52.41
IV-B	9.35	33.35	41.52	56.52	54.54	50.11
Diclofenac	25	48.88	56.25	65.71	63.63	58.62

A= 25 mg / kg b.w., B= 50 mg / kg b.w., P<0.01 (t-test)

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