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Synthesis and anti-inflammatory activity of some novel derivatives of 2-amino-3-cyano-14-imino-10-methoxy-4-methylthio pyrimido [2,1-*b*] pyrazolo [4,5-*d*] pyrimido [2,1-*b*] benzothiazole

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ABSTRACT

*Novel derivatives of 2-amino-3-cyano-14-imino-10-methoxy-4-methylthio pyrimido [2,1-b] pyrazolo [4,5-d] pyrimido [2,1-b] benzothiazole **4** were synthesized from starting material 2-amino-6-methoxy benzothiazole **1** and evaluated for their anti-inflammatory activity. It was concluded that some of the compounds showed excellent anti-inflammatory activity as compared with others.*

Keywords: 2-amino-6-methoxy benzothiazole, anti-inflammatory.

INTRODUCTION

Benzothiazoles are bicyclic ring systems with multiple applications. In 1950s, a number of 2-aminobenzothiazoles have been investigated as central muscle relaxants. Since then, the quest for research on this heterocycle fused with others started with a great rigor. It has been reported that benzothiazole fused with pyrimidine and pyrazole found to possess potent wide spectrum of activities like anticonvulsant, anti-inflammatory, antibacterial, antifungal.**[1,2,3,11]** Pyrazolones and pyrazolinones rank among the more vulnerable non-steroidal anti-inflammatory agents. Singh et al.**[4]** prepared some new 2-(4-butyl-3,5-dimethyl-pyrazol-1-yl)-6-substituted benzothiazoles were display significant anti-inflammatory activity. Benzothiazole is a new profile of biological activities and much more area is yet to be explored.

In the light of these observations this prompted us to synthesize a novel derivatives of 2-amino-3-cyano-14-imino-10-methoxy-4-methylthio pyrimido [2,1-b] pyrazolo [4,5-d] pyrimido [2,1-b]

benzothiazole with hetaryl amines,evaluate them by spectral analysis (IR and MASS spectrometry) and screen for their anti- inflammatory activity **[5, 6,7,8].**

MATERIALS AND METHODS

2.1 Chemistry

The synthesis of compound 2-amino-3-cyano-14-imino-10-methoxy-4-methylthio pyrimido [2,1-b] pyrazolo [4,5-d] pyrimido [2,1-b] benzothiazole and its derivatives outlined in scheme. 1 and 2 respectively.

Compound **2** was obtained by nucleophilic displacement of one methyl thio group from bis(methylthio) methylene malononitrile reagent which was carried out in solvent DMF in presence of catalytic amount of anhydrous potassium carbonate under reflux for 6-8 hrs. which results into cyclization of 2-amino-6-methoxy benzothiazole with addition of one methyl thio group at IInd position and cyano and immino at IIIrd and IVth position respectively.

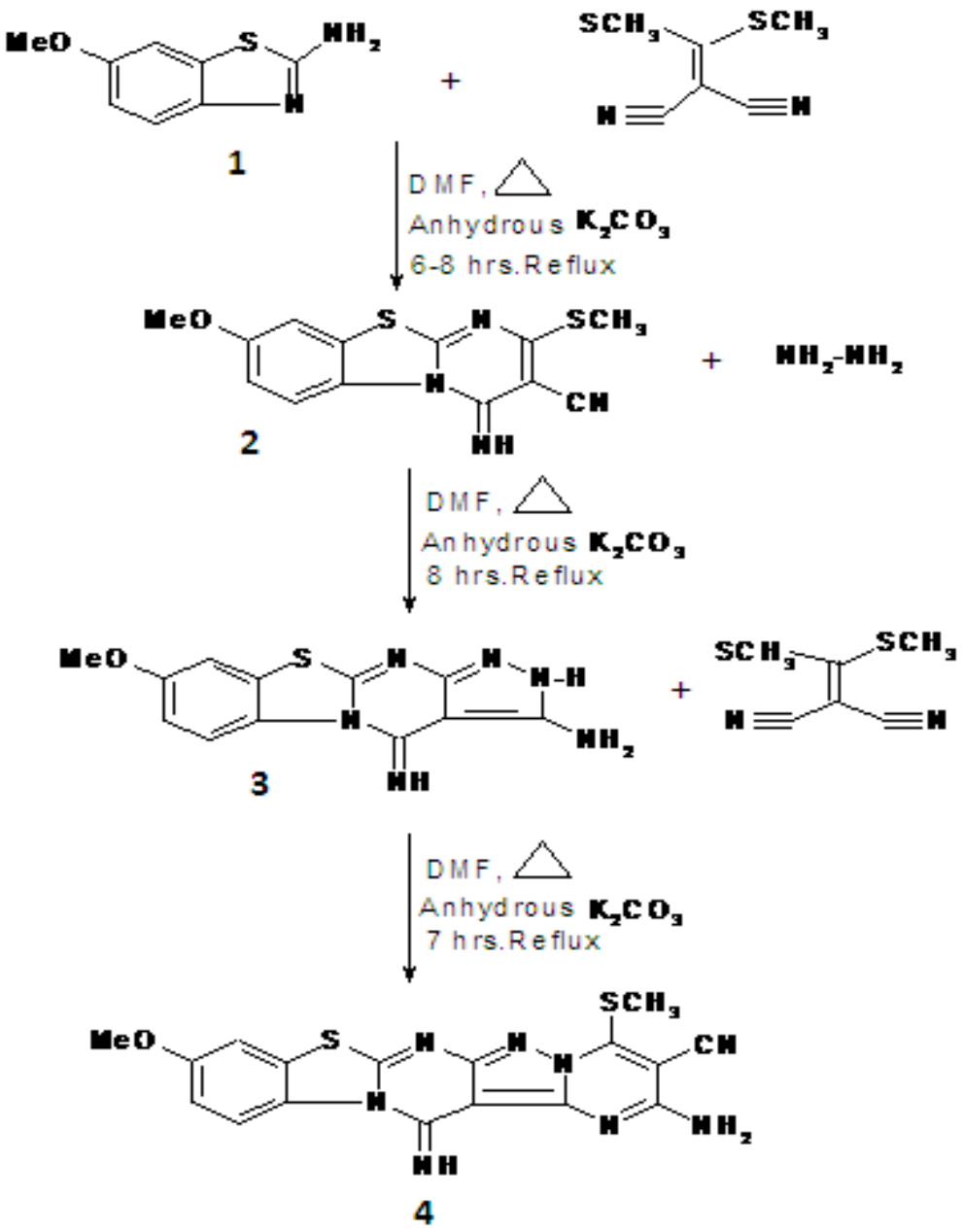
Compound **3** was obtained by nucleophillic displacement of methyl thio group of IInd with hydrazine hydrate which was carried out with same reaction conditions under reflux for 8 hrs which removed cyano, resulting into cyclization.

Compound **4** was obtained by nucleophilic displacement of one methyl thio group from bis(methylthio) methylene malononitrile reagent which was carried out in solvent DMF in presence of catalytic amount of anhydrous potassium carbonate under reflux for 7 hrs. which results into cyclization of compound **3** with addition of one methyl thio group at IVth position and cyano and amino at IIIrd and IInd position respectively. Compound **5, 6** and **7** were prepared by replacing active thiomethyl group of compound **4** at position IVth. These reactions results in the formation of above 4-substituted derivatives of compound **4**.

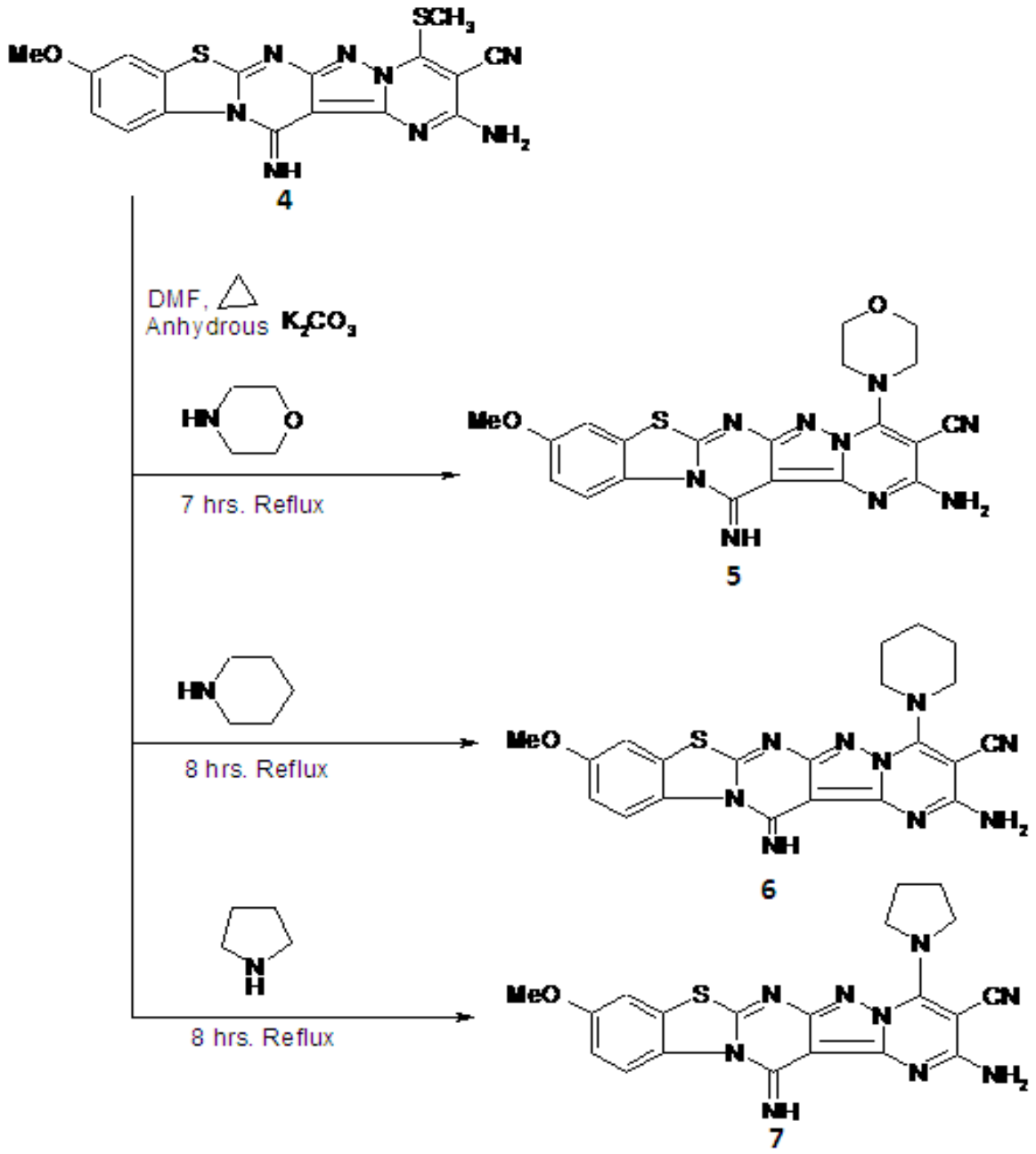
2.2 Synthesis

Melting point is valuable criteria of purity for an organic compound, as a pure crystal is having definite and sharp melting point. In our present investigation, the melting point of the organic compounds was determined by open capillary tube method and is uncorrected. Thin Layer Chromatography (TLC, Rf values) was performed on silica gel-G plates by using mobile phase benzene/methanol-4.5:0.5 respectively, benzene/methanol-7:3 respectively, visualization was effected with ultraviolet light. IR spectra were recorded on IR-200 Thermoelectron by using KBr pellet method. The Mass analysis of synthesized compounds has been done by TOF MS ES+.

6.1.1 Synthesis of 3-cyano-4-imino-8-methoxy-2-methylthio-pyrimido [2, 1-b] benzothiazole 2.
Compound 2-amino-6-methoxy benzothiazole 1 was refluxed for about 6-8 hrs with bis (methylthio) methylene malononitrile, in presence of catalytic amounts of anhydrous potassium carbonate and DMF as solvent, it was then recrystallized with ethanol and melting point was determined.
Yield-47.64% ; Melting Point - 115˚C ; IR (KBr) Vmax cm 1- : 3192 (=NH); 3101(=C-H,-CH=CH- Str. (Aromatic); 2212(-C≡N(Nitrile); 1641(>C=N-);1547(>C=C< (Aromatic); 1260(-CH- stretching (CH3-O); 1205 (C-S); MS : [M]+ at M/z=302.


MeO
S
NH2
N
1
+
SCH3
SCH3
H
H
DMF,
Anhydrous K2CO3
6-8 hrs.Reflux
MeO
S
N
SCH3
CH
NH
2
+
NH2-NH2
DMF,
Anhydrous K2CO3
8 hrs.Reflux
MeO
S
N
N
H-H
NH2
NH
3
+
SCH3
SCH3
H
H
DMF,
Anhydrous K2CO3
7 hrs.Reflux
MeO
S
N
N
N
SCH3
CH
N
NH2
NH
4

Scheme 1



Scheme 2

6.1.2 Synthesis of 3-amino-4-imino-8-methoxy-pyrazolo[4,5-d] pyrimido [2,1-b]benzothiazole 3.
Compound 2 was refluxed with hydrazine hydrate under similar weakly alkaline conditions in presence of catalytic amounts of anhydrous potassium carbonate and DMF as solvent for about 8 hrs. Solution was cooled to room temperature & ice water poured in it to get solid product. It was then recrystallized with ethanol and melting point was determined.

Yield-63.45% ; Melting Point -141˚C; IR (KBr) Vmax cm 1- : 3388 (-NH2(amine) ; 3102 (=C-H,-CH=CH- Str.(Aromatic); 1641 (>C=N-) ; 1547 (>C=C< (Aromatic) ; 1260 (-OCH3) ; 1205 (C-S) ; MS : [M]+ at M/z=286.5.

6.1.3 Synthesis of 2-amino-3-cyano-14-imino-10-methoxy-4-methylthio pyrimido [2,1-b] pyrazolo [4,5-d] pyrimido [2,1-b] benzothiazole 4.

Compound 3 was refluxed with bis (methylthio) methylene malononitrile (0.01 mol) reagent,under similar weakly alkaline conditions in presence of catalytic amounts of anhydrous potassium carbonate and DMF as solvent for about 7 hrs. Solution was cooled to room temp. & ice water poured in it to get solid product. It was then recrystallized with ethanol and melting point was recorded.

Yield- 62.20 % ; Melting Point - 120˚C; IR (KBr) Vmax cm 1- 3192 (=NH); 3364 (-NH2); 2212-(C≡N(Nitrile); 1653 (>C=N-); 1570 (>C=C< (Aromatic); 1242 (-OCH3); 1204 (C-S) MS : [M]+ at M/z= 408.5.

6.1.4 Synthesis of 2-amino-3-cyano-14-imino-10-methoxy-4-morpholino pyrimido [2,1-b] pyrazolo [4,5-d] pyrimido [2,1-b] benzothiazole 5.

Compound 4 was refluxed with morpholine under similar weakly alkaline conditions in presence of catalytic amounts of anhydrous potassium carbonate and DMF as solvent for about 7 hrs. Solution was cooled to room temp. & ice water poured in it to get solid product. It was then recrystallized with ethanol and subjected for determination of melting point.

Yield- 86.66 % ; Melting Point -130˚C; IR (KBr) Vmax cm 1- 3323 (-NH2) ; 2922(=NH) ; 2192 –(C≡N(Nitrile);1594 (>C=C< (Aromatic) ; 1263(-OCH3); 1209(C-S); MS : [M]+ at M/z= 448.

6.1.5 Synthesis of 2-amino-3-cyano-14-imino-10-methoxy-4-piperidino pyrimido [2,1-b] pyrazolo [4,5-d] pyrimido [2,1-b] benzothiazole 6.

Compound 4 was refluxed with piperidine under similar weakly alkaline conditions in presence of catalytic amounts of anhydrous potassium carbonate and DMF as solvent for about 8 hrs Solution was cooled to room temperature & ice water poured in it to get solid product. It was then recrystallized with ethanol and melting point was determined.

Yield- 78% ; Melting Point - 150˚C IR (KBr) Vmax cm 1- 3355(-NH2) ; 2928 (=NH); 2192 (-C≡N(Nitrile); 1594 (>C=C< (Aromatic); 1268 (-OCH3); 1209 (C-S) ; MS : [M]+ at M/z= 445.

6.1.6 Synthesis of 2-amino-3-cyano-14-imino-10-methoxy-4-pyrolidino pyrimido [2,1-b] pyrazolo [4,5-d] pyrimido [2,1-b] benzothiazole 7.

Compound 4 was refluxed with pyrolidine under similar weakly alkaline conditions in presence of catalytic amounts of anhydrous potassium carbonate and DMF as solvent for about 8 hrs. Solution was cooled to room temp. & ice water poured in it to get solid product. It was then recrystallized with ethanol and melting point was determined.

Yield- 75.75 %; Melting Point- 142˚C; 3342(-NH2); 2925(=NH); 2192 (-C≡N (Nitrile); 1651(-C=N); 1591(>C=C< (Aromatic); 1224(-OCH3); 1273 (C-S); MS: [M] + at M/z= 433.2.

RESULTS AND DISCUSSION

The compounds were synthesized under available laboratory conditions and were confirmed by physicochemical and spectral data. The syntheses of final compounds were carried out in mainly four steps. Since compound **4** has replaceable active thiomethyl group, the susceptibility of it towards condensation with different reagents of hetaryl amines has been investigated. These reactions results in the formation of 4-substituted derivatives of compound **4.**

3.1. Anti-Inflammatory

In-vivo study of the selected synthesized compounds were carried out for anti-inflammatory activity **[9,10]** in albino rats employing the carageenan induced rat paw edema method using digital Plethysmometer (UGO Basile 7140).

The compounds were finely powdered and suspended in 1 % w/v solution of acacia. Suspension was injected orally to the wistar albino rats. Percentage reduction in the inflammation (i.e., reduction in the volume of hind paw edema in the animals) at different time intervals after administration of carageenan was recorded and test compounds (A= 25 mg/kg and B=50 mg / kg body weight) were compared with that of the animals treated with carageenan alone and with standard diclofenac (10 mg/kg body weight). Data expressed as Mean paw volume ± SEM and analyzed by One-Way ANOVA followed by Dunnett's test to determine the significance of the difference between the control group and group treated with test compounds.

All the statistical calculations were carried out using Graph Pad® Prism 3.0 (USA) Statistical Software. **p-value<0.01 were considered significant.

The significant results of the screened compounds outlined in table 1-5. Derivatives with morpholino **5** (T1), piperidino **6** (T3) and pyrolidino **7** (T2) showed enhanced anti-inflammatory activity. Compound **6 (**T3) have excellent activity, as compared to the standard drug Diclofenac sodium after 6 hours whereas compounds **5 (**T1) and **7 (**T2) have shown moderate activity.

Table 1 : Mean paw volume (ml) and % inhibition of compounds after 30 min.

Compound codes	Mean Paw Volume (ml) ± SEM	% Inhibition of edema
Control	1.30 ± 0.023	----
T1-A	0.89± 0.065**	66.66
T1-B	0.98± 0.042**	56.66
T2-A	0.84± 0.031**	40
T2-B	1.0± 0.035**	73.33
T3-A	0.92 ± 0.042**	60
T3-B	0.87± 0.028**	86
Std- Diclofenac	0.79 ±0.045**	86.66

Table 2 : Mean paw volume (ml) and % inhibition of compounds after 1 hr.

Compound codes	Mean Paw Volume (ml) ± SEM	% Inhibition of edema
Control	1.2 ± 0.022	----
T1-A	0.85± 0.040**	73.91
T1-B	0.83± 0.056**	65.21
T2-A	0.91 ± 0.048**	34.78
T2-B	0.89± 0.078**	56.52
T3-A	0.90± 0.043**	56.52
T3-B	0.83± 0.039**	39.13
Std- Diclofenac	0.97 ± 0.033**	47.82

Table 3: Mean paw volume (ml) and % inhibition of compounds after 2 hrs.

Compound codes	Mean Paw Volume (ml) ± SEM	% Inhibition of edema
Control	1.39± 0.020	-----
T1-A	0.8900 ± 0.04336**	74.35
T1-B	0.86± 0.043**	71.79
T2-A	0.76± 0.052**	74.35
T2-B	0.93 ±0.024**	23.07
T3-A	0.87± 0.050**	39
T3-B	0.78 ±0.031**	76.92
Std- Diclofenac	0.77 ±0.044**	94.87

Table 4: Mean paw volume (ml) and % inhibition of compounds after 3 hrs.

Compound codes	Mean Paw Volume (ml) ± SEM	% Inhibition of edema
Control	1.44 ± 0.022	-----
T1-A	0.7±0.034**	97.77
T1-B	0.7 ±0.033**	80
T2-A	0.80± 0.045**	68.88
T2-B	0.81 ±0.060**	35.55
T3-A	0.83 ±0.040**	93.33
T3-B	0.74 ±0.029**	88.88
Std- Diclofenac	0.75 ±0.031**	93.33

Table 5: Mean paw volume (ml) and % inhibition of compounds after 6 hrs.

Compound codes	Mean Paw Volume (ml) ± SEM	% Inhibition of edema
Control	1.48 ±0.034	-----
T1-A	0.91 ±0.066**	75
T1-B	0.7 ±0.031**	85.41
T2-A	0.74 ±0.058**	83.33
T2-B	0.74 ±0.039**	54.16
T3-A	0.76 ±0.012**	91.66
T3-B	0.72 ±0.023**	93.75
Std- Diclofenac	0.69 ±0.033**	91.66

CONCLUSION

In the research work carried out under the title, "Synthesis and anti-inflammatory activity of some novel derivatives of 2-amino-3-cyano-14-imino-10-methoxy-4-methylthio pyrimido [2,1-b] pyrazolo[4,5-d] pyrimido [2,1-b] benzothiazole." it can be concluded that the substituted derivatives of 2-amino-3-cyano-14-imino-10-methoxy-4-methylthio pyrimido [2,1-b] pyrazolo[4,5-d] pyrimido [2,1-b] benzothiazole have proven to be effective anti-inflammatory drug candidates.

Most of the derivatives showed enhanced anti-inflammatory activity as compared to the standard drug Diclofenac sodium. Compounds **6 (**T3) have excellent activity, as compared to the standard drug after 6 hours whereas compounds **5 (**T1) and **7 (**T2) have shown moderate activity. So, compounds **6 (**T3) can serve as future therapeutic leads for the discovery of anti-inflammatory drugs.

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