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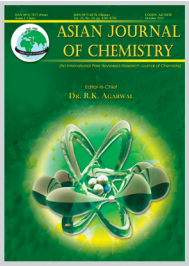

Synthesis, Anthelmintic, Insecticidal Activity of (E)-1-((1-(6-Chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(6-substituted benzo[d]thiazol-2-yl)hydrazine Derivatives

M. HIMAJA[1,*], D. MUNIRAJASEKHAR[1], ASIF KARIGAR[2], M.V. RAMANA[2] and MUKESH SIKARWAR[3]

[1]Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore-632 014, India
[2]Maratha Mandal's College of Pharmacy, Belgaum-590 016, India
[3]KLE College of Pharmacy, Ankola-518 314, India

*Corresponding author: E-mail: dr_himaja@yahoo.com

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A new series of (E)-1-((1-(6-chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(6-substitutedbenzo[d]thiazol-2-yl)hydrazine derivatives (**6a-6e**) were synthesized and the structure of all the compounds was confirmed by IR, ^{1}H NMR and mass spectral analysis. All the newly synthesized compounds were evaluated for anthelmintic activity against earthworm species (*Eudrilus eugeniae* and *Megascolex konkanensis*) and the insecticidal activity was carried out against termites (*Coptotermis formasanus*) using chloropyrifos as the standard drug.

Key Words: Benzothiazole, Pyrazole, Schiff base, Anthelmintic activity, Insecticidal activity.

INTRODUCTION

Hydrazones possessing an azomethine (-NHN=CH-) proton, constitute an important class of compounds for new drug development. Pyrazole was found to be a pharmacophoric moiety for compounds exhibiting antitumor[1], immunosuppressive[2], antibacterial[3], antitubercular[4], antiinflammatory[5], anticancer[6] activities. Benzothiazole derivatives were reported to possess broad spectrum of biological activities like antitumor[7,8], antifungal[9], antimalarial[10] and anthelmintic[11] activities. Anticipating a synergistic effect, a new series of Schiff bases were designed, which possessed all the pharmacophoric moieties, *viz*. substituted pyrazole and benzothiazole. The synthesized molecules were characterized by spectral analysis and were screened for their insecticidal and anthelmintic activities.

The precursor benzothiazole hydrazine (**2**) was synthesized by treating 6-chloro-2-aminobenzothiazole (**1**) with hydrazine hydrate in presence of ethylene glycol. The benzothiazole hydrazine was condensed with acetophenone (**3**) to form the Schiff base (**4**), which was treated with DMF/$POCl_3$ to form the aldehyde (**5**). Finally compound **5** was coupled with different substituted-hydrazines to form the title compounds **6a-f**.

EXPERIMENTAL

All the reagents were procured from SD Fine and Spectrochem Ltd., without further purification. Melting points were determined by open capillary tube and were uncorrected. Purity of the synthesized compounds was checked by TLC and column using chloroform and ethyl acetate (90/10 v/v). The IR spectra in KBr disk were recorded from 4000 to 400 cm^{-1} on Avatar 330 FTIR spectrometer equipped with DTGS detector. ^{1}H NMR spectra were recorded on GEOL-JMS D-300 (MHz) NMR using $CDCl_3$ as the solvent with trimethylsilane (TMS) as an internal standard. FAB mass spectra were recorded on a Joel Sx 102/DA-600.

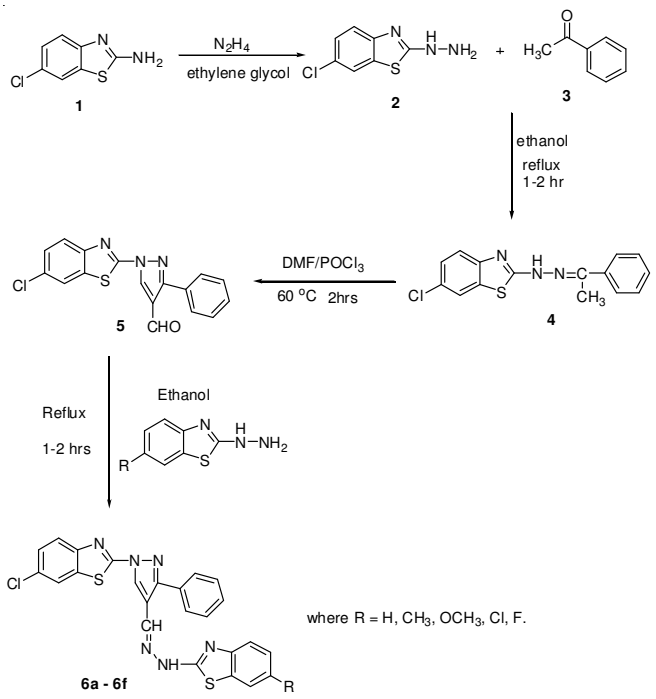
1-(6-Chlorobenzo[d]thiazol-2-yl)hydrazine (2): Conc. hydrochloric acid (2 mL) was added dropwise with stirring to hydrazine hydrate (40 mmol, 1.94 mL) at 5 ºC, followed by ethylene glycol. Then 2-amino-6-chlorobenzothiazole **1** (10 mmol) was added in portions. The reaction mixture was refluxed for 2-3 h and cooled. The fine crystalline solid separated was filtered, washed with water and recrystallize from ethanol. The title compound was obtained as white needle-like crystals with 98 % yield, m.p.: 196-198 ºC, IR (KBr, ν_{max}, cm^{-1}): 3320 (NH_2 str), 3199 (NH str), 3061 (aromatic str), 1649 (C=N str), 1447 (C-N str), 1267 (C-S str), 886, 976 (aromatic H-bending), 766 (C-Cl str). ^{1}H NMR (δ, DMSO): 5.090 (2H, s, NH_2), 7.202-7.295 (3H, m, Ar-H), 9.155 (1H, s, NH), LC-MS: m/z 200.2 (M+1).

1-(6-Chlorobenzo[d]thiazol-2-yl)-2-(1-phenylethylidene)hydrazine (4): To a mixture of compound **2** (0.597 g, 3 mmol) and acetophenone **3** (0.35 mL, 3 mmol), **5** drops of acetic acid and 10 mL of ethanol were added and refluxed

for 2 h. The crude product was separated by filtration, which was recrystallized from ethanol as white needle like crystals with 98 % yield, m.p.: 196-198 ºC, IR (KBr, ν_{max}, cm^{-1}) : 3444 (NH str), 2927 (aliphatic-CH str), 1590 (C=N stretching of benzothiazole), 1549 (C=N stretching of Schiff base), 1447 (C-N str), 1255 (C-S str), 760 (C-Cl stretching). ^{1}H NMR (δ, $CDCl_3$): 2.4 (3H, s, CH_3), 7.3-7.9 (8H, m, Ar-H), 9.3 (1H, s, NH), FAB mass: m/z 302 (M+1).

1-(6-chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde (5): To the Vilsmeier-Haack reagent prepared from DMF (10 mL) and $POCl_3$ (1.1 mL), hydrazone **4** (0.9 g, 3 mmol) was added, the reaction mixture was stirred at 60-65 ºC for 2.5 h and then it was poured into ice cold water. The solid that separated on neutralization $NaHCO_3$ was filtered, washed with water and was purified by column chromatography. The compound **5** was obtained as white needle-like crystals with 98 % yield, m.p.: 196-198 ºC, IR (KBr, ν_{max}, cm^{-1}) : 3460 (NH str), 3012 (aromatic-CH str), 1681(C=N str of benzothiazole), 1536 (C=N of pyrazoline ring), 1448 (C-N str), 1202 (C-S str), 933 (aromatic H-bending), 705 (C-Cl str). ^{1}H NMR (δ, DMSO): 7.26-7.88 (8H, m, Ar-H), 9.059 (1H, s, Pyrazoline -CH), 10.104 (1H, s, CHO). FABMass: m/z 200.2 (M+1).

(E)-1-((1-(6-Chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(6-substitutedbenzo[d]thiazol-2-yl) hydrazine (6a-6f): A mixture of compound **5** (0.341 g, 1 mmol) and different substituted benzothiazole hydrazines (1 mmol), 2-3 drops of glacial acetic acid and 10 mL of ethanol was refluxed for 1.5 h. The crude product was separated by filtration, which was recrystallized from ethanol (**Scheme-I**).



Scheme-I Synthesis of (E)-1-((1-(6-chlorobenzo [d] thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(6-substituted benzo[d]thiazol-2-yl) hydrazine derivatives

(E)-2-(benzo[d]thiazol-2-yl)-1-((1-(6-chlorobenzo[d] thiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl) methylene) hydrazine (6a): White solid, m.p: 248-250 ºC, m.w.: 486.48, m.f.: $C_{24}H_{15}N_6S_2Cl$, FTIR (KBr, ν_{max}, cm^{-1}): 3318 (NH str), 3028 (aromatic str), 2911 (aliphatic CH str), 1653 (C=N str of benzothiazole), 1565 (C=N of pyrazoline ring), 1512 (C=N str of Schiff base), (1447 C-N str), 1134 (C-S str), 784 (C-Cl str). ^{1}H NMR (δ, DMSO): 7.302-8.014 (12H, m, Ar-H), 8.235 (1H, s, -CH=N-), 8.983 (1H, s, Pyrazoline -CH), 12.316 (1H, s, NH). FAB mass m/z: 487.1 (M+1).

(E)-1-((1-(6-Chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(6-methylbenzo[d]thiazol-2-yl) hydrazine (6b): Yellow colour powder, m.p.: 210-212 ºC, m.w.: 500.06, m.f.: $C_{25}H_{17}N_6S_2Cl$, FTIR (KBr, ν_{max}, cm^{-1}): 3417 (NH str), 3067 (aromatic str), 2924 (aliphatic CH str), 1681 (C=N str of benzothiazole), 1592 (C=N of pyrazoline ring), 1506 (C=N str of Schiff base), (1435 C-N str), 1120 (C-S str), 747 (C-Cl str). ^{1}H NMR (δ, DMSO): 2.312 (3H, s, CH_3), 7.089 - 8.005 (11H, m, Ar-H), 8.189 (1H, s, -CH=N-), 8.923 (1H, s, Pyrazoline - CH), 12.126 (1H, s, NH). FAB mass m/z: 501.1 (M+1).

(E)-1-((1-(6-Chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(6-methoxybenzo[d]thiazol-2-yl) hydrazine (6c): Yellow colour powder, m.p.: 228-230 ºC, m.w.: 516.05, m.f.: $C_{25}H_{17}N_6OS_2Cl$, FTIR (KBr, ν_{max}, cm^{-1}): 3227 (NH str), 3063 (aromatic str), 2917 (aliphatic CH str), 1690 (C=N str of benzothiazole), 1613 (C=N of pyrazoline ring), 1482 (C=N str of Schiff base), (1463 C-N str), 1137 (C-S str), 779 (C-Cl str). ^{1}H NMR (δ, DMSO): 3.756 (3H, s, OCH_3), 7.345- 8.008 (11H, m, Ar-H), 8.169 (1H, s, -CH=N-), 8.918 (1H, s, Pyrazoline -CH), 12.094 (1H, s, NH). FAB mass m/z: 517.1 (M+1).

(E)-2-(6-Chlorobenzo[d]thiazol-2-yl)-1-((1-(6-chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl) methylene) hydrazine (6d): Yellow powder, m.p.: 262-264 ºC, m.w.: 520.00, m.f.: $C_{24}H_{14}N_6S_2Cl$, FTIR (KBr, ν_{max}, cm^{-1}): 3292 (NH str), 3022 (aromatic str), 2961 (aliphatic CH str), 1682 (C=N str of benzothiazole), 1513 (C=N of pyrazoline ring), 1458 (C=N str of Schiff base), (1425 C-N str), 1105 (C-S str), 720 (C-Cl str). ^{1}H NMR (δ, DMSO): 7.298-8.006 (11H, m, Ar-H), 8.216 (1H, s, -CH=N-), 8.971 (1H, s, Pyrazoline-CH), 12.273 (1H, s, NH). FAB mass m/z: 521 (M+1).

E)-1-((1-(6-Chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(6-fluorobenzo[d]thiazol-2-yl) hydrazine (6e): Yellow powder, m.p.: 260-262 ºC, m.w.: 504.03, m.f.: $C_{24}H_{14}N_6S_2Cl$, FTIR (KBr, ν_{max}, cm^{-1}): 3394 (NH str), 3013 (aromatic str), 2962 (aliphatic CH str), 1663 (C=N str of benzothiazole), 1554 (C=N of pyrazoline ring), 1516 (C=N str of Schiff base), (1447 C-N str), 1134 (C-S str), 769 (C-Cl str). ^{1}H NMR (δ, DMSO): 7.132-8.018 (11H, m, Ar-H), 8.218 (1H, s, -CH=N-), 8.987 (1H, s, Pyrazoline-CH), 12.173 (1H, s, NH). FAB mass m/z: 505 (M+1).

Anthelmintic activity: The title compounds were subjected to anthelmintic activity studies against earthworm species (*Eudrilus eugeniae*) at a concentration of 2 mg/mL using Garg and Atal's method[12]. Tween 80 (15 % solution) in distilled water used as a control and mebendazole was used as a standard drug.

Suspension of the samples was prepared by triturating the synthesized compounds (100 mg) with Tween 80 (15 % solution). The resulting mixtures were stirred for 0.5 h. The

TABLE-1
ANTHELMINTIC OF (E)-1-((1-(6-CHLOROBENZO[D]THIAZOL-2-YL)-3-PHENYL-1H-PYRAZOL-4-YL) METHYLENE)-2-(6-SUBSTITUTED BENZO[D]THIAZOL-2-YL)HYDRAZINE DERIVATIVES

Compound no	*Eudrilus eugeinae*		*Megascolex konkanensis*	
	Paralyzing time	Death time	Paralyzing time	Death time
6a	9.13 ± 0.52	9.56 ± 0.57	12.17 ± 0.37	13.24 ± 0.21
6b	10.23 ± 0.16	11.38 ± 0.34	11.42 ± 0.48	12.28 ± 0.39
6c	12.47 ± 0.23	13.11 ± 0.39	10.46 ± 0.36	11.24 ± 0.31
6d	8.47 ± 0.35	9.32 ± 0.53	13.24 ± 0.54	14.12 ± 0.32
6e	8.06 ± 0.38	8.47 ± 0.46	11.42 ± 0.31	12.35 ± 0.43
Mebendazole	7.54 ± 0.36	8.27 ± 0.34	10.51 ± 0.42	11.23 ± 0.56
Control	No effect	No effect	No effect	No effect

suspensions were diluted to contain 0.2 % (w/v) of the test samples. In a similar way, suspension of the standard reference drug mebendazole was prepared with the same concentration. 3 Sets of 5 earthworms of almost similar sizes (2" in length) were placed in 100 mL beaker containing 50 mL of suspension of the test sample. Another set of 5 earthworms was kept as a control in 50 mL of suspension of distilled water in Tween 80 (15 % solution). The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 ºC), which stimulated the movement, if the worm was alive. The anthelmintic study results are tabulated in Table-1.

Insecticidal activity: The title compounds were subjected to insecticidal activity[13] against termites-*Coptotermis formasanus*. 100 mg of each of the test compounds was dissolved in 2 mL of acetone. The solution was uniformly spread on the filter paper of diameter 4.3 cm, dried and placed in a similar sized petri-plate. The concentration of each test compound was 0.58 mg/cm^2 area. Standard drug chloropyrifos and control were maintained in a similar way. The termites were placed on the filter paper in the petri-plate and were closed with the lid containing a thin layer of wet cotton bed. The death time of the insects was observed for 3 h. No death was observed in the control even after 12 h. The results are shown Table-2.

TABLE-2
INSECTICIDAL ACTIVITY OF (E)-1-((1-(6-CHLOROBENZO [D]THIAZOL-2-YL)-3-PHENYL-1*H*-PYRAZOL-4-YL) METHYLENE)-2-(6-SUBSTITUTED BENZO[D]THIAZOL-2-YL)HYDRAZINE DERIVATIVES

Compound no	Concentration of the compound (100 mg/66.5 cm^2 area)	Death time (min)
6a	100	28
6b	100	61
6c	100	64
6d	100	15
6e	100	20
Chloropyrifos	100	22
Control	-	-

RESULTS AND DISCUSSION

The synthesis of E-1-((1-(6-chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(6-substitutedbenzo [d]thiazol-2-yl)hydrazine derivatives (**6a-6e**) involved the reaction between 1-(6-chlorobenzo[d]thiazol-2-yl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde (**5**) and 6-substituted-2-hydrazino benzothiazoles, as described in the general procedure.

IR absorption band at 1523-1458 cm^{-1} indicated the stretching vibration of -CH=N- azomethine which confirmed the formation of Schiff bases. The pyrazole moiety C=N and C-N functional groups appeared at 1613-1554 cm^{-1} and 1448-1423 cm^{-1}. The other peaks of the IR spectrum prove the structure of the formation of Schiff bases. In ^{1}H NMR spectrum protons of methyl and methoxy groups were observed at 2.312 and 3.756 ppm. One imine proton (CH=N-) at 12.094-12.316 ppm also confirmed the formation of the product. The mass spectrum of the synthesized compounds were also good in agreement the product molecular weights.

All the synthesized compounds were subjected to anthelmintic and insecticidal activities. In these series of compounds anthelmintic activity carried out against two different kind of earthworm species (*Eudrilus eugeniae* and *Megascolex konkanensis*). All the compounds showed good anthelmintic activity compared to the standard drug mebendazole. Compound **6e** showed better activity probably due to the fluorine substitution on benzothiazole moiety of the compound. Insecticidal activity was carried out against the termites *Coptotermis formasanus*. All the compounds showed significant insecticidal activity. Two compounds **6d** and **6e** showed better activity as compared to the standard drug Chloropyrifos owing to the presence of chloro, fluoro functional groups on benzothiazole moiety.

Conclusion

All the synthesized compounds were characterized by FTIR, ^{1}H NMR and mass spectral analysis and were evaluated for anthelmintic and insecticidal activity studies. In conclusion, chloro- and fluoro-substituted benzothiazoles exhibited potent anthelmintic and insecticidal activities as compared to the standard drugs mebendazole and chloropyrifos respectively.

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