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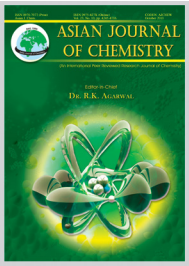

Synthesis and Pharmacological Screening of Novel Ethyl(5-Substituted Acetamido)-3-methylthio-1-(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate

S.K. Abubakkar, S.D. Minhaj and K.G. Baheti*

Department of Pharmaceutical Chemistry, Y.B. Chavan College of Pharmacy, Rauza Bagh, Aurangabad-431 001, India

*Corresponding author: Tel: +91 94223 40342, E-mail: nk_baheti@yahoo.com

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Several novel ethyl(5-substituted acetamido)-3-methylthio-1-(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate were synthesized and screened for their analgesic activity at a dose of 50 mg/kg and 10 mg/kg by acetic acid induced writhing method and hot plate method respectively. The compound **5j** and **5k** are prominent candidates, which showed good analgesic activity among the series. The compound **5j** and **5k** showed 54.20 % and 52.22 % analgesic activity to that of standard aspirin by acetic acid induced writhing method whereas 72.88 % and 69.7 % analgesic activity to that of standard pentazocine HCl by hot plate method respectively.

Key Words: Analgesic, Pyrazole, Hot plate method.

INTRODUCTION

Therapeutically active pyrazole derivatives were accidently discovered by German Chemist Ludwig Knorr in 1884 with discovery of antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), which has analgesic, antipyretic and antirheumatic activity. Aminopyrine, a more potent analogue was synthesized thereafter[1]. Then many pyrazoles were synthesized and screened. The pyrazole molecules have numerous biological activities. They were reported for analgesic[2], antiinflammatory[3], antipyretic[4], antimicrobial[5], anticonvulsant[6] and for the treatment of rheumatoid arthritis[7]. Keeping in mind the biological importance of this molecule, it was thought to synthesized the ethyl(5-substituted acetamido)-3-methylthio-1-(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate derivatives and evaluate them for analgesic activity by different models.

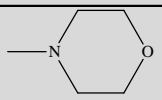
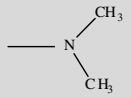
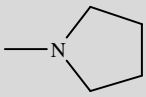
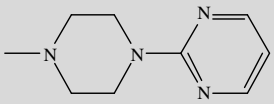
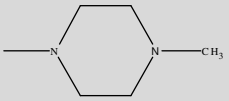
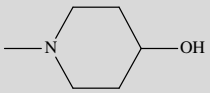
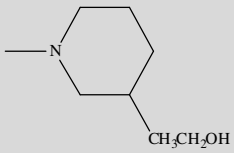
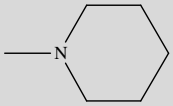
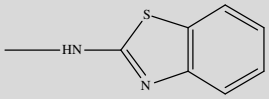
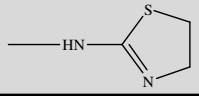
EXPERIMENTAL

Chemicals used were obtained from Merck Ltd. The reactions were carried out by conventional method. Melting points were determined in open capillaries using melting point apparatus and are uncorrected. The purity of synthesized compound was ascertained by TLC using silica gel-G plate. The structures of the synthesized compound were confirmed by IR, NMR and Mass spectral analysis. The IR spectra were recorded on JASCO FTIR-4100, NMR spectra were recorded on BRUKER AVANCE II 400 Spectrometer using $CDCl_3$ as solvent with TMS as internal standard and mass spectra were recorded on 1200 L Varian LC/MS instrument.

Synthesis of ethyl-5-amino-3-methylthio-1-(4-chlorophenyl)-2*H*-pyrazole-4-carboxylate (3): Ethyl *bis*-methylthio-2-cyanoacrylate[8] (**1**) (2.17 g, 0.01 mol) and *p*-chlorophenylhydrazine (**2**) (1.08 g, 0.01 mol) was dissolved in 15 mL of dimethylformamide in 100 mL round bottom flask. To this a catalytic amount of anhydrous K_2CO_3 was added and the reaction mixture was refluxed gently for 2 h. The reaction mixture was cooled and poured in ice cold water, the product obtained was filtered, washed with water and recrystallized from rectified spirit to yield 1.86 g (72 % w/w) of creamy colour product. m.p. 100 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 3438 (10 NH_2), 3320 (10 NH_2), 3064 (Ar-H Stretching) 1723 (C=O of ester); ^{1}H NMR ($CDCl_3$): δ 1.29 (t, 3H, -CH_3 of CH_2-CH_3), δ 2.53 (t, 3H, -SCH_3), δ 7.72 (s, 2H, NH_2), δ 7.49-7.59 (m, 4H, ClC_6H_4).

Synthesis of ethyl 5-(2-chloroacetamido)-3-methylthio-1-(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate. (4): Ethyl-5-amino-3-methylthio-1-(4-chlorophenyl)-2*H*-pyrazole)-4-carboxylate (**3**), (0.001 mol, 0.295 g), chloro acetyl chloride (0.001 mol, 0.102 mL) and 2 drops of isopropyl amine was dissolved in 10 mL of dichoromethane in 50 mL conical flask. It was stirred at room temp for 2 h. The separated crystals were filtered and recrystallized from rectified spirit to yield 0.239 g (68 % w/w) of yellow crystal product. m.p. 180 ºC, FTIR (KBr, ν_{max}, cm^{-1}): 3234 (NH-), 3064 (Ar-H Stretching) 1735(C=O of ester), 1698 (C=O of amide); ^{1}H NMR ($CDCl_3$): δ1.35 (t, 3H, -CH_3 of CH_2-CH_3), δ 2.50 (t, 3H, -SCH_3), δ 9.15 (s,1H, CONH), δ 7.50-7.79 (m, 4H, ClC_6H_4).

TABLE-1
PHYSICAL CHARACTERIZATION OF PYRAZOLE ACETAMIDO DERIVATIVES **(5a-k)**

Compound	R	Mole form	Mole (wt.)	m.p. (°C)	Yield (%)
5a		$C_{19}H_{23}N_4O_4SCl$	438	186-188	75
5b		$C_{17}H_{21}N_4O_3SCl$	396	80-82	74
5c		$C_{19}H_{24}O_3N_5SCl$	437	96-98	76
5d	$-NHCH_3$	$C_{16}H_{19}N_4O_3SCl$	382	90-92	77
5e		$C_{23}H_{26}N_7O_3SCl$	515	192-194	88
5f		$C_{20}H_{26}N_5O_3SCl$	451	126-128	74
5g		$C_{20}H_{25}N_4O_4SCl$	452	134-136	84
5h		$C_{22}H_{30}O_4N_4SCl$	481	120-122	71
5i		$C_{20}H_{25}O_3N_4SCl$	436	110-112	65
5j		$C_{22}H_{20}N_5O_3S_2Cl$	502	88-90	63
5k		$C_{18}H_{18}N_5O_3S_2Cl$	451	114-116	61

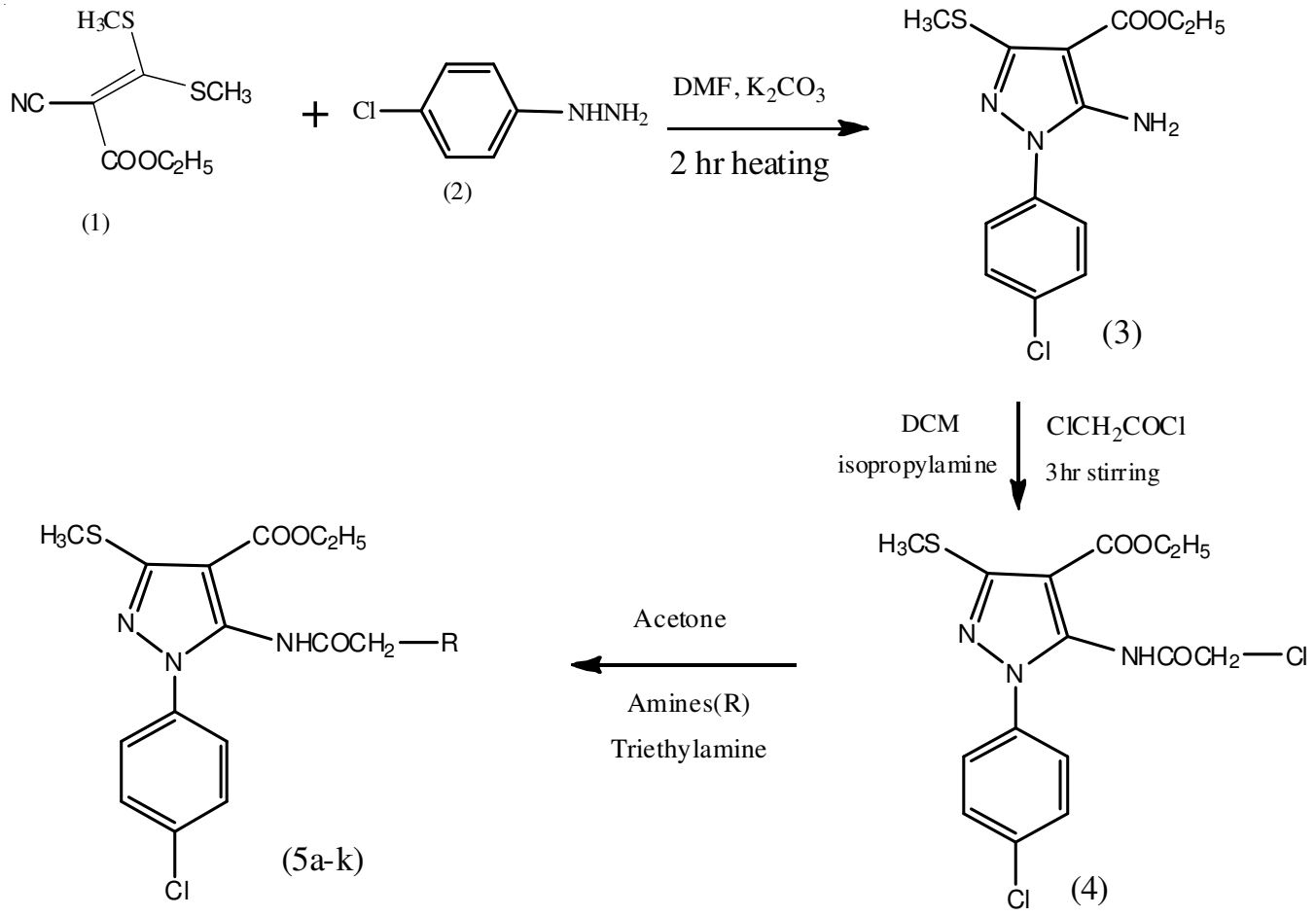
Synthesis of ethyl(5-substituted acetamido)-3-methylthio-1-(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate. (5a-k): Ethyl-5-(2-chloroacetamido)-3-methylthio-1-(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate (**4**) (0.001 mol, 0.387 g), amines (0.001 mol) and acetone (5 mL) was transferred to 25 mL conical flask, to this triethylamine (0.00125 mol, 0.126 mL) was added dropwise with stirring for 3 h, a clear solution was obtained. The reaction mixture was poured in petri dish and scratched with petroleum ether to obtain solid, which was recrystallized to yield 0.294 g of yellow colour product. The all derivatives were prepared in the same manner (Table-1).

Analgesic activity (writhing method)[9]: The analgesic activity was performed using the acetic acid induced writhing method. After 50 min of the oral administration of test sample and standard drug, each animal was injected with 3 % (w/v) acetic acid solution intraperitoneally. After 10 min of acetic acid injection, the number of muscular contractions (writhing) in mice was counted for a period of 10 min. A significant reduction in the number of writhing by any treatment as compared to control animals was considered as a positive analgesic response. The average number of writhes in each group of treated mice was compared with that of the control. The % analgesic activity was expressed according to the formula:

$$\text{Inhibition (\%)} = [n\text{-}n'/ n \times 100]$$

where; n is the number of writhes in control group of mice and n' is the number of writhes in test and standard group of mice.

Analgesic activity (hot plate method)[10]: Analgesic activity was inferred by hot plate method using albino mice as a test animals weighing 25-30 g. Mice were divided in a group of 6. The paw licking response of each animal was recorded by placing animal on a hot plate maintained at the temperature 55 ± 0.5 ºC before administration of compounds. The suspension of compound was prepared in Tween 80 (1 %) and water. The compounds were administered intraperitoneally. After 1 h interval the paw licking responses were again recorded. The increase in latency period was noted. Calculation of activity of each compounds were done with respect to the standard using the following formula:



Scheme-I

Analgesic activity (%) = Mean increase in latency period of test (sec)/Mean increase in latency period of test (sec) × 100.

RESULTS AND DISCUSSION

The synthesis of the title compound was performed as outlined in the **Scheme-I**. The desired target compounds (**5a-k**) were synthesized by reacting ethyl *bis*-methylthio-2-cyanoacrylate (**1**) and *p*-chlorophenyl hydrazine. The reaction was carried out in dimethyl formamide in presence of anhydrous K_2CO_3. The product ethyl-5-amino-3-methylthio-1-(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate (**3**) on reflux with chloroacetylchloride in dichloromethane for 2 h to yields substituted pyazolo chloro acetamide (**4**). The compounds (**4**) on treatment with various amines like morpholine, dimethylamine, methylamine, pyrrolidine, piperidine, pyrimidylpiperazine, 4-hydroxypiperidine, 4-methylpiper-azine, 3-piperidine ethanol, 2-aminothaizole, 2-aminobenzothaizole, gave the target compounds having general structure as (**5a-k**). The reactions were carried out by conventional method and the compounds were purified by recrystallization. The structures of these compounds were inferred from their spectral data. The IR spectra of compound (**5a-k**) showed the absence of 1 ºNH$_2$ group which indicate that chloroacetylation took place at -NH$_2$ group of pyrazole at 5 position. The presence of NH group at around 3240 cm^{-1}(s) as single peak was also seen in the spectra. The peak at 3000 cm^{-1}(s) due to aromatic stretching. The C=O stretching of ester appeared at about 1750 cm^{-1}(s) where as C=0 stretching of amide appeared at about 1650 cm^{-1}(s). The compound 5 g and 5 h have the characteristic peak of OH at 3500-3400 cm^{-1}(b). NMR spectra of compound (**5a-k**) showed that triplet at δ 1.24-1.29 due to CH_3 of CH_2-CH_3 at 4th position. A singlet peak was appeared at δ 2.48-2.53 due to -SCH_3. A singlet peak of -CH_2 was appeared at around δ 3.29. A quartet peak at δ 4.2-4.3 due CH_2 of CH_2-CH_3 at 4th position was appeared. Aromatic proton appeared at δ 7.49-7.57 ppm as a multiplet. A singlet peak of δ 9.7-9.9 due to NH proton of amide was identified. All the compound showed molecular ion peak exactly at molecular weight.

Analgesic activity: The analgesic activity of test compounds by acetic acid induced writhing method showed that all the compounds (**5a-k**) exhibited the activity at a dose of 50 mg/kg. The compound **5j** and **5k** showed 54 % and 52 % reduction of writhings where as standard showed 61.11 % reduction in writhing. The compound (**5a, 5b, 5c, 5d, 5e 5f, 5g, 5h, 5i**) showed less than 50 % of reduction in writhing (Table-2). Analgesic activity by hot plate method using albino mice as a test animals showed that test compound exhibited activity. The compounds **5j** and**5k** possess 72.88 % and 69.7 % activity respectively to that of pentazocine HCl at same dose level *i.e.* 10 mg/kg. Compounds (**5a, 5b, 5c, 5d, 5e, 5f, 5g, 5h, 5i**) shows less than 50 % analgesic activity (Table-3).

TABLE-2
ANALGESIC ACTIVITY OF PYRAZOLE ACETAMIDO DERIVATIVES (**5a-5k**) BY ACETIC ACID INDUCED WRITHING METHOD

Compounds	Dose (mg/kg, p.o)	No. of writhes in 10 min. after treatment (mean± SEM)	Inhibition (%)
Control	50	90 ± 1.8**	-
Std.	50	35 ± 1.12**	61.11
5a	50	55 ± 0.66**	38.88
5b	50	36 ± 1.2**	40.00
5c	50	51 ± 1.12**	43.00
5d	50	62 ± 0.72**	31.11
5e	50	60 ± 1.11**	33.33
5f	50	54 ± 0.67**	39.77
5g	50	60 ± 0.86**	33.33
5h	50	66 ± 1.08**	26.66
5i	50	51 ± 0.78**	43.33
5j	50	41 ± 0.33**	54.20
5k	50	43 ± 1.01**	52.22

Data analyzed by one way ANOVA followed by Dunnett's 't' test, n = 6, **P < 0.01 significant from control

TABLE-3
ANALGESIC ACTIVITY OF PYRAZOLE ACETAMIDO DERIVATIVES (**5a-5k**) BY HOT PLATE METHOD

Compounds	Dose (mg/kg)	Mean increase in latency period (mean ± SEM)	Analgesic activity
5a	10	4.04 ± 0.15**	41.74
5b	10	4.60 ± 0.01**	47.42
5c	10	3.26 ± 0.08**	33.65
5d	10	3.66 ± 0.10**	37.73
5e	10	4.57 ± 0.07**	47.16
5f	10	4.09 ± 0.15**	42.21
5g	10	3.24 ± 0.01**	33.40
5h	10	3.83 ± 0.02**	39.53
5i	10	2.65 ± 0.01**	27.31
5j	10	7.07 ± 0.01**	72.88
5k	10	6.76 ± 0.15**	69.70

Data analyzed by one way ANOVA followed by Dunnett's 't' test, n = 6, **P < 0.01 significant from standard; Mean increased in latency period of pentazocine HCl (Std) was 9.7 ± 0.15

Conclusion

Various ethyl(5-substituted acetamido)-3-methylthio-1-(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate derivative were synthesized and screened for analgesic activity. Most compounds exhibited analgesic activity. Among all the synthesized compounds, **5j** and **5k**, which have 2-aminobenzothiazole and 2-aminothiaozole moiety found to be most potent. These compounds **5j** and **5k** also showed significant analgesia in acetic acid induced writhing tests and Hot plate method. Further exploration of these serious of compounds may lead to potential candidate for development.

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