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A Novel Synthesis and Pharmacological Activity of Substituted Pyrazol Derivative

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Research Article

ABSTRACT

A new process for synthesis of 4- Hydroxyphenyl 4-(Ethoxycarbonyl)-3-(Methylthio)-1R-Pyrazol – 5 -yl Substituted Derivative by phenyl hydrazine and hydrazine hydrochloride as a new class of Pyrazol based heterocyclic compound and screened for their pharmacological activity. Some of these compounds exhibit significant analgesic anti-inflammatory and antimicrobial activity.

Key words: *Pyrazole, 5-substituted pyrazoles, analgesic, anti-inflammatory, antimicrobial*

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INTRODUCTION

5-Amino Pyrazole exhibits a variety of biological activities. 5-Acylamino pyrazoles are reported to possess tranquilizing analgesic and pharmacodynamic properties [1-4]. It was therefore thought of interest to synthesis 4-hydroxyl phenyl 4-(ethoxycarbonyl)-3-(methylthio) -1 R- pyrazol – 5 -yl substituted derivative. Pyrazole and evaluate their pharmacological activities. The synthesis of Ethyl 2-cyano-3, 3-bis (methylthio) acrylate required as a starting material was prepared by the reaction of carbon disulphide, dimethylsulphate with ethyl cyanoacetate. Different substituted hydrazinhydrate is reflux with Ethyl 2-cyano-3, 3-bis (methylthio) acrylate in the presence ethanol to give respective Ethyl 5- amino-3- (methylthio)-1R-pyrazole-4- carboxylate. Then respective pyrazole is further dissolve in minimum amount of ethyl acetate with warming and add the various aromatic carboxylic acids were dissolved in minimum amount of ethyl acetate with warming when required and reflux for few minutes. The structures of pyrazole derivative were established by IR and 1HNMR spectra data and checked the activity by different methods analgesic activity by Tail Flick Method, anti- inflammatory activity by Carrageenan– inducedrat paw edema and anti-microbial activity by Cup Plate method.

EXPERIMENTAL DESIGN

Ethyl 2-cyano- 3, 3-bis (methylthio) acrylate (A) A solution of 13.2 gm of potassium hydroxide in a minimum amount of water cooled in an ice bath at 0ºC was added 30 ml of dimethylformamide and 0.1 mole of ethyl cyanoacetate. The mixture was treated drop wise (10 sec/drop) with cooling and stirring 6 ml of carbon disulfide drop wise for at lest 1hour at 0ºC .The reaction mixture was allowed to stand at room temperature for one hour and then 25.2gm dimethyl sulphate was added (1drop/sec)at 10-15ºC. The mixture was kept for 12 hour at room temperature and poured into ice water. The solid obtained was filtered after 1 hour wash with cold water recrystallized it. This compound was obtained as a yellowish color solid crystalline m. p. 70-75ºC

I.R. (Potassium bromide): 3375.3, 2903.3, 2366.1, 1323.4 & 1108.4c.m-1 Ethyl 5-amino-3-(methylthio)-1- phenyl-1H-pyrazole-4-carboxylate A mixture of Ethyl 2-cyano-3, 3-bis (methylthio) acrylate (0.01 mole) and add phenyl hydrazine (0.01 mole) in 30 ml of ethanol was refluxed for 45- 60 min. The mixture was cooled to room temperature and poured into ice water and filtered. This was dried and recrystallized from dilute ethanol. The recrystallized product melting point is 78-80ºC white color crystalline product.

1HNMR (400MHz, CDCl3) δ: 5.3-5.5(2H, NH2), 7.2-7.7 (10H Ar-H), 7.7-7.9 (1H, CH), 6.4-6.6(1H,CH), 4.2-4.4(2H, CH2), 2.4-2.6 (3H, CH3) &1.3-1.5 (3H, CH3).
I.R. (Potassium bromide): 3328.3 cm- 1 (N-H), 3053.3 cm-1 (Ar, C-H), 2364.6 cm-:1 (CN), 1671.6 cm-1 (C=O), 1607 cm-1 (C=N) & 1534.9 cm-1 (C=C). Ethyl 5-amino-3-(methylthio)-1H- pyrazole-4-carboxylate

A mixture of Ethyl 5-amino-3-(methylthio)-1R-pyrazole-4- carboxylate (0.01 mole) and add hydrazine hydrochloride (0.01 mole) in 30 ml of ethanol was refluxed for 1-2 hour. The mixture was cooled to room temperature and poured into ice water and filtered. This was dried and recrystallized from dilute ethanol. The recrystallized product shows melting point at 65-70ºC, a white color crystalline product is obtained.

1HNMR (400MHz, CDCl3) δ: 1.33-1.36 (3H, CH3), 2.5-2.6 (3H, SH3), 2.7-2.9 (2H, NH2), 4.2-4.3 (2H, CH2) & 7.3 (1H, NH).

I.R. (KBr): 2902.6 cm-1 (N-H) δ: 2569 cm-1 (NH2), 1464 cm-1 (C=O) 1700 & 1750 cm-1 (COOC2H5) & 1698.9 cm-1 (N-H). Ethyl 5-(3-phenylpropanamido)-3(methylthio) -1-phenyl-1H-pyrazole-4-Carboxylate.(C1) This compound was obtained as a yellowish solid crystalline m. p. was 96°C

^{1}HNMR (400MHz, CDCl3) δ: 1.3-1.4 (3H, CH3), 2.4-2.6 (3H, CH3), 4.2-4.4 (2H, CH2), 8.0-8.1(1H, NH), 2.50- 2.90(4H, CH2), 7.2-7.7 (10H, Ar-H).

I.R. (Potassium bromide): 3268 cm-1 (NH), 1612.2, 1535.7, 1607.3 cm-1, (C=O, C=C) 1021 cm-1 (C-N) & 3054.4, 3329 & 3429 cm-1 (C-H). Ethyl 5-(benzamido)-3-(methylthio)-1-phenyl-1H-pyrazole-4-Carboxylate (C2) This compound was obtained as a yellowish solid crystalline, m. p. was 82°C.

^{1}HNMR (400MHz, CDCl3) δ: 1.3-1.4 (3H, CH3), 2.4-2.7 (3H, CH3), 4.2-4.5 (2H, CH2), 8.0-8.1 (1H, NH), 7.2-7.8 (8H, Ar-H), 8-8.3 (2H, Ar-H).

I.R. (Potassium bromide): 3052.4 cm- 1 (C-H), 1788.0 cm-1 (C=O), 1671, 1607, 1526 & 1452 cm-1 (C=C), 1071 cm-1 (C-N), 3291 cm-1 (NH). Ethyl5-(4-hydroxybenzamido)-3-(methylthio)-1-phenyl-1H-pyrazole-4 Carboxylate. (C3) This compound was obtained as a yellowish solid crystalline m. p. 95°C.

^{1}HNMR (400MHz, CDCl3) δ: 1.3-1.4 (3H, CH3), 2.4-2.7 (3H, SCH3), 4.2- 4.5 (2H, CH2), 8.0-8.1 (1H, NH), 6.7- 7.7 (9H, Ar-H) & 5.0-5.1 (1H, OH).

I. R. (Potassium bromide): 1669.4, 1610 & 1539.2 cm-1(C=C), 1952.8 cm- 1 (C=O), 3291 cm-1 (NH) & 3236-3429 cm-1 (C-H). Ethyl 5-(4-aminobenzamido)-3- (methylthio) -1-phenyl-1H-pyrazole-4- Carboxylate. (C4) This compound was obtained as a yellowish solid crystalline m. p. 115°C.

^{1}HNMR (400MHz, CDCl3) δ: 1.3-1.6 (3H, CH3), 2.4-2.6 (3H, SCH3), 4.2- 4.6 (2H, CH2), 8.0-8.1 (1H, NH), 6.8- 6.9 (2H, NH2) & 7.1-7.7 (9H, Ar-H).

I. R. (Potassium bromide): 1667.2, 1601.9 & 1535 cm-1 (C=C), 1021 cm-1 (C-N), 2591 cm-1 (NH), 3461-3230.1 cm-1 (C-H) & 1771 cm-1 (NH2). Ethyl 5-(3 phenylpropanamido) -3- (methylthio) -1H-pyrazole-4- Carboxylate (C5) This compound was obtained as a colorless solid crystalline m. p. 87°C.

1HNMR (400MHz, CDCl3) δ: 1.25-1.45 (3H, CH3), 2.45-2.65 (3H, CH3), 2.51- 2.65 (4H, CH2), 4.2-4.4 (2H, CH2), 6.35-6.55 (1H, CH), 7.3-7.5 (4H, Ar- H) & 7.7-7.9 (1H, NH) 13.67-13.72 (1H, NH).

I.R. (Potassium bromide): 1626, 1417.9 cm-1 (N=H, C=O), 2985.5 cm-1 (C-H) & 3297 cm-1(N-H). Ethyl 5-(benzamido)-3-(methylthio)- 1H-pyrazole-4-Carboxylate (C6) This compound was obtained as a colorless solid crystalline m. p. 65°C.

1HNMR (400MHz, CDCl3) δ: 1.25-1.45 (3H, CH3), 2.5-2.7 (3H, SCH3), 4.2- 4.5 (2H, CH2), 7.3-7.9 (5H, Ar-H) 8.2 (1H, NH), 13.3-13.5 (1H, NH).

I.R. (Potassium bromide): 3070.2 cm- 1 (N-H), 1581.6-1422.2 cm-1 (C=C, N=C), 1697 cm-1 (NH2), 3291 cm- 1 (NH). Ethyl 5-(4-hydroxy benzamido)-3- (methylthio)-1H-pyrazole-4- Carboxylate (C7) This compound was obtained as a colorless solid crystalline m. p. 110°C

1HNMR (400MHz, CDCl3) δ: 1.2-1.5 (3H, CH3), 2.4-2.9 (3H, SCH3), 4.2- 4.5 (2H, CH2), 5.3-5.6(1H, OH), 6.7- 7.9 (4H, Ar-H), 7.9 (1H, NH), 13.3- 13.6(1H, NH).

I.R. (Potassium bromide): 2989.7 cm- 1 (C-H), 2593 & 3237.1 cm-1 (C-H, N- H, S-H), 3291 cm-1 (NH), 1698.9, 1611.8 & 1578.8 cm-1 (N=C). Ethyl 5-(4-amino benzamido)-3-(methylthio)-1H-pyrazole-4-Carboxylate (C8). This compound was obtained as a colorless solid crystalline m. p. 150°C.

1HNMR (400MHz, CDCl3) δ: 1.2-1.4 (3H, CH3), 2.4-2.7 (3H, SCH3), 4.2- 4.5 (2H, CH2), 6.5-6.8 (2H, Ar-H), 5.5-5.7 (2H, NH2), 7.9 (1H, NH) &.7-7.8 (2H, Ar-H), 13.5-13.6 (1H, NH).

I.R. (Potassium bromide): 1626, 1417 cm-1 (N=C, C=O), 2984.6 cm-1 (NH2), 3229.8 cm-1 (C-H), 3363.9 cm-1 (N-H).

BIOLOGICAL EVALUATION

Analgesic activity by Tail Flick Method

Rats both male and female were selected for study. The average weight of animals was 100 to 150 g. They were divided into ten group each group have 6 rats. The first control group we gave Gum acacia normal formulation. The second group standard gave the formulation of standard drug Aspirin and all group gave the derivative of Pyrazole and gum acacia formulation. Each rat was placed in the rat holder and tail was protruded out through the slot in the lid and placed on a hot wire (resistance wire passing electric current of 3 ampere) Time taken to flick the tail known as the normal reaction time was noted down. (Table- II, and structure I)

Anti inflammatory activity by Carrageenan induced Rat paw edema Method

Albino rates weighing between 150- 200 gm body weights were selected. The rats were dived into different groups each group consisting of 6 animals. Group-I was treated as negative control (received 5% Gum acacia 5 ml/kg), group –II served as positive control (received Ibuprofen), while the other groups received synthesized compound. (Table III and structure II)

Anti-microbial activity by cup plate method

This method depend on the diffusion of an antibiotic from a vertical cylinder or a cavity through the solidify of agar layer of a Petridis or a plate to an extent such that growth of the added micro-organism is prevented entirely in a circular or zone around the cylinder or cavity containing a solution of synthesized derivative. The organism used in study was Escherichia coli, Pseudomonas auruginosa, Staphylococcus aureus and Candida albicans. (Table-III)

RESULT AND DISCUSSION

Our continuing interest to develop a general method for synthesis of 4-Hydroxyphenyl 4-(Ethoxycarbonyl)-3-(Methylthio)-1R- Pyrazol – 5 -yl Substituted Derivative was obtained from the reflux reaction with acid and Ethyl 5-amino-3-(methylthio)-1R-1H- pyrazole-4-carboxylate can be synthesis by two ways and finally, we got eight compounds (C1-8). The structure of the compound was confirmed on the basis of IR and 1HNMR spectra. The synthesized compound was checked for the analgesic activity by Tail flick method on albino rats. Aspirin was taken as a standard with gum acacia. The results reveal that the compounds C2, C5, C6 and C8 show very good analgesic activity. The anti-inflammatory activity by paw-volume method on albino rats. Ibuprofen was taken as a standard and control as a gum acacia. The results reveal that the compounds C5, C6, C7, and C8 show very good anti-inflammatory activity. For antimicrobial activity used ciprofloxacin standard for antibacterial and Fluconazole standard for antifungal activity the result show the Compound C5, C6, C7 and C8 gave good activity.

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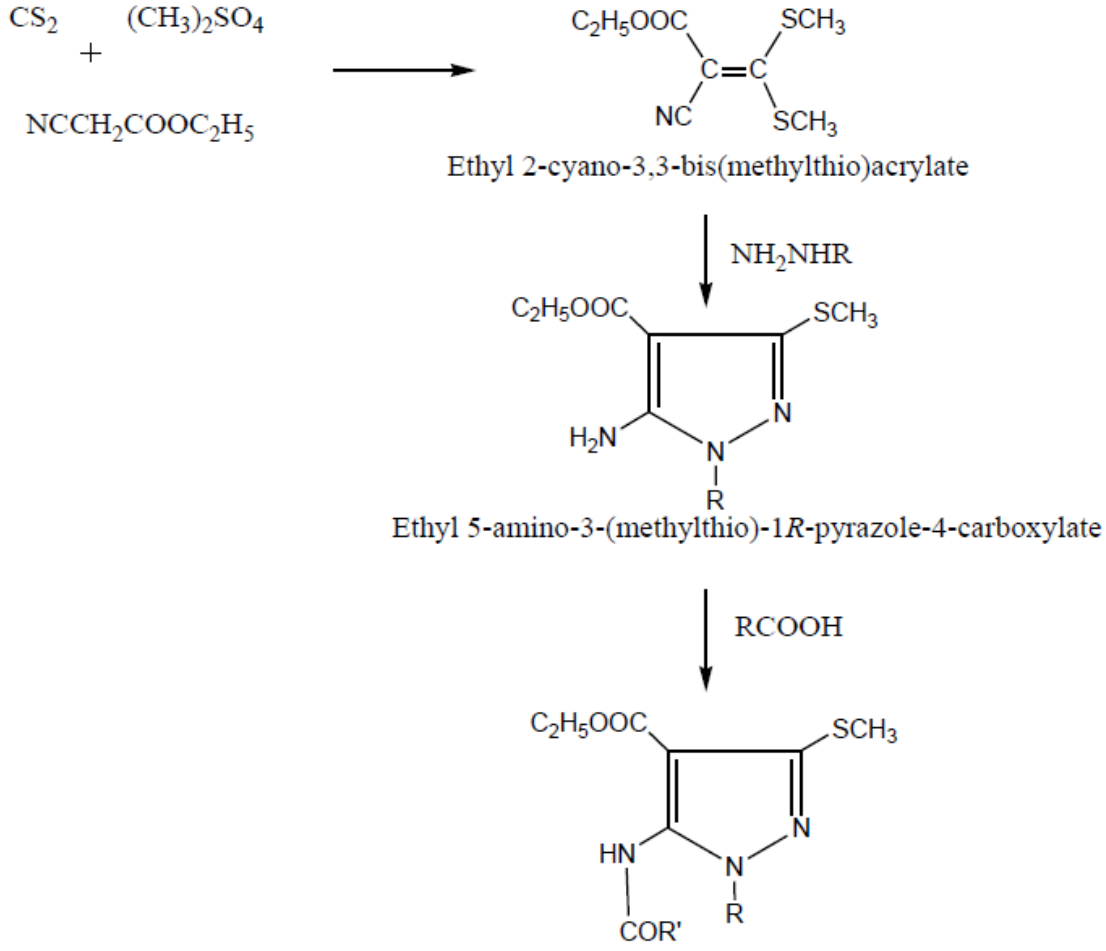
REFERENCES

1. Ghozlan S.A.S., Abdelhamid I.A., Elnagadi M.H. Gaber H.M., Studies on enaminonitriles: A new

synthesis of 1,3 substituted pyrazole -4-carbonitrile, Journal of Heterocyclic chemistry 2005, (42), 1185.

2. David B., Golic L., Golobic A., Jurij S., Branko S., Synthesis and transformations of ethyl (2E)-3-N,N-dimethylamino-2-(5-ethoxy-1-phenyl-1H-pyrazol-3-yl)propenoate Journal of Heterocyclic chemistry, 2005, (66), 207.
3. Saleh N.M., Ammar Y.A., Mikky J.A., Abbas H.A.S., El-gaby M.S.A., Novel synthesis and antimicrobial investigation of pyrazolo[1, 5-a] pyrimidine and pyrazolotriazine containing piperidinyl sulfone moiety Indian Journal of chemistry, 2004, 43(B) 2195.
4. Choudhari B.P., Mulwad V.V., Synthesis of biologically active N-[coumarin-6- yl]-3-methyl-5-(hydroxy/methyl/4- hydroxy-8-methylcoumarin-3-yl) pyrazoles and [2.3.1] bicyclo-N- [coumarin-6-yl]-1,2-diazepines, Indian Journal of chemistry, 2004, 43(B), 1544

REACTION SCHEME



4-hydroxyphenyl 4-(ethoxycarbonyl)-3-(methylthio)-1*R*-pyrazol-5-yl substituted

Table 1: % yield & melting point of synthesized derivatives of Pyrazole compound.

Compounds	R -	R'-	Yield (%)	M. P.
C_1.	C_6H_5	C_6H_5CHCH	82	96^0c
C_2.	C_6H_5	C_6H_5	84	82^0c
C_3.	C_6H_5	p-$C_6H_4(OH)$	72	95^0c
C_4.	C_6H_5	$C_6H_4(NH_2)$	77	115^0c
C_5.	H	C_6H_5CHCH	83	87^0c
C_6.	H	C_6H_5	69	65^0c
C_7.	H	p-$C_6H_4(OH)$	65	110^0c
C_8.	H	$C_6H_4(NH_2)$	71	150^0c

Table 2: Analgesic activity of synthesized derivatives of Pyrazole compound.

Formulation Used	Mean reaction time (Sec.) at deferent time intervals(min)					
	0	10	20	30	40	50
Standard Aspirin	10.91±0.22	15.4±0.23	17.35±0.22	28.8±0.42	17.42±0.29	15.27±0.15
C_1.	7.16±008	7.42±0.003	8.35±0.05	7.87±0.06	7.25±0.03	7.13±0.29
C_2.	8.46±0.03	8.68±0.24	15.87±0.32	23.27±0.14	11.95±0.48	10.48±0.26
C_3.	7.16±0.06	7.31±0.57	8.25±0.08	8.24±0.13	8.23±0.14	7.57±0.17
C_4.	7.36±0.08	7.86±0.08	12.46±0.29	10.55±0.22	9.05±0.55	7.55±0.16
C_5.	12.94±0.22	17.16±0.79	18.13±0.08	18.76±0.16	18.34±0.33	13.54±0.24
C_6.	16.31±0.14	17.23±0.06	19.31±0.02	16.56±0.24	16.17±0.14	15.35±0.23
C_7.	12.43±0.28	13.28±0.01	14.12±0.07	14.42±0.20	13.26±0.02	13.10±0.06
C_8.	11.46±0.09	11.68±0.22	25.64±0.22	26.47±0.25	23.33±0.28	17.39±0.20

Table 3: Anti-inflammatory activity of synthesized derivatives of Pyrazole compound

Compound	Initial mean Paw volume±SEM	1st hr mean paw volume± SEM	3rd hr mean paw volume± SEM	5th hr mean paw volume± SEM
Gumaccasia 5% control	0.035±0.005	0.040±0.001	0.048±0.001	0.040±0.001
Standard Ibuprofen	0.031±0.001	0.034±0.001	0.038±0.001	0.035±0.008
C_1	0.044±0.001	0.067±0.001	0.080±0.003	0.075±0.001
C_2	0.038±0.001	0.052±0.002	0.051±0.005	0.041±0.001
C_3	0.041±0.001	0.061±0.001	0.061±0.001	0.043±0.001
C_4	0.032±0.009	0.039±0.001	0.045±0.001	0.046±0.001
C_5	0.030±0.012	0.038±0.001	0.037±0.002	0.040±0.003
C_6	0.032±0.002	0.036±0.002	0.043±0.009	0.041±0.002
C_7	0.033±0.002	0.041±0.002	0.049±0.002	0.041±0.002
C_8	0.035±0.002	0.039±0.001	0.044±0.014	0.043±0.001

Table 4: Antimicrobial activity of synthesized derivatives of Pyrazole compound

	Diameter of zone of inhibition in mm concentration on 100 µg			
Compound	***Escherichia coli*** **(ATCC No. 8739)**	***Pseudomonas aeruginosa*** **(ATCC No. 9027)**	***Staphylococcus aureus*** **(ATCC No. 6538)**	***Candida albicans*** **(ATCC No. 10231)**
Standard Ciprofloxacin	18	20	19	--
Standard Fluconzole	--	--	--	9.5
C_1	04	09	11	15
C_2	00	00	08	16
C_3	08	09	12	11
C_4	00	07	16	19
C_5	14	18	16	10
C_6	20	14	12	14
C_7	10	10	00	15
C_8	00	12	19	15

Fig. I- Analgesic activity of synthesized derivatives of Pyrazole compound

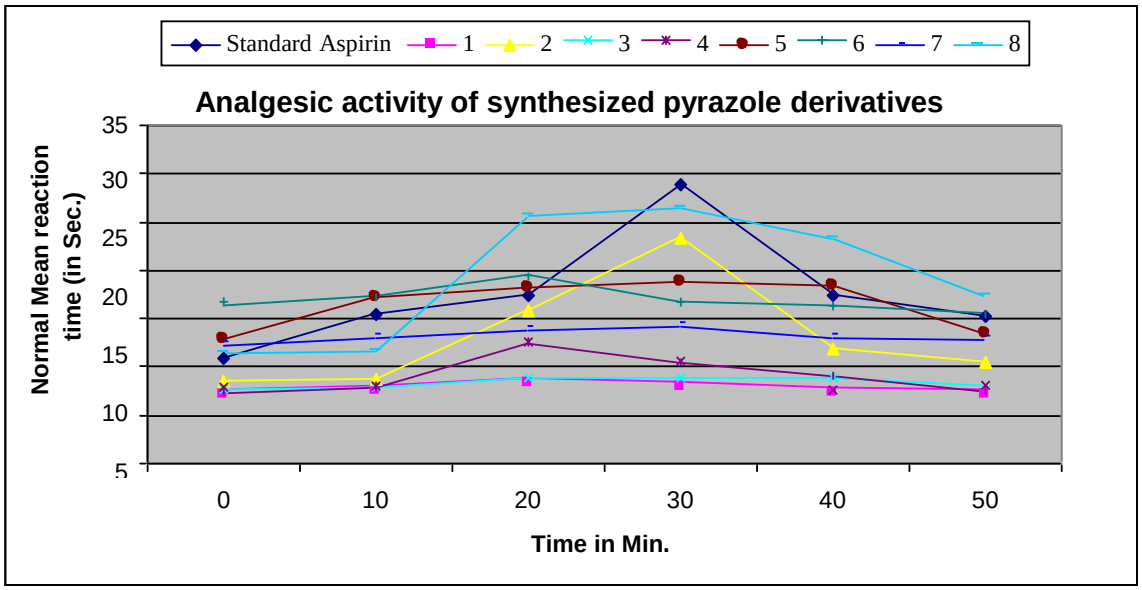


Fig. II- Anti-inflammatory activity of synthesized derivatives of Pyrazole compound