Synthesis And Spectroscopic Characterization of Some Unique CNS Deppresant And Diuretic Pyrazoline Derivatives

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Abstract- *Nine novel derivatives N-{[(5-amino-2-hydroxyphenyl) (phenyl) methyl] carbamothioyl}-3-{{[substituted] carbamothioyl}amino}-5-Phenyl-4, 5-Dihydro-1H-Pyrazole-4-Carboxamide have been synthesized according to Michael addition reaction to afford a new biolologically active target compounds by refluxing novel chalcones with hydrazine hydrate in presence of ethanol in high yields. Furthermore, The structures of the newly synthesized compounds were confirmed by FT-IR, 1H-NMR and mass spectral data. The pyrazoline derivatives were evaluated for their CNS depressant and diuretic activity, the results showed sterling activity.*

Keywords- Chalcones, Pyrazolines, Michael addition reaction, CNS depressant.

I. INTRODUCTION

In recent scenario heterocycles play a considerable role in drug coalescence in that respect pyrazoline derivatives plays a vital role among other heterocycles. It has been revealed from literature survey that in recent years pyrazoline derivatives have attracted extensive interest because of their therapeutic and pharmacological properties[1]. Pyrazolines are well known heterocyclic compound and various methods have been worked out for their synthesis[2]. In various circumstances, they are used as antimicrobial[3], anti-inflammatory[4], and antidepressant[5], antitumor[6] and anti-oxidant[7] agents. In recent years, a significant portion of research in heterocyclic chemistry has been devoted to 2- pyrazolines containing different aryl groups as substituents, as evident from the literature[8]. Hence, a series of nine novel pyrazoline derivatives from 1-[(5-amino-2-hydroxy phenyl) (phenyl) methyl] thiourea has been synthesized, characterized and evaluate for CNS depressant and diuretic activity.

II. EXPERIMENTAL

Melting points were determined using an Electrothermal melting point apparatus as well as by open capillary method and are uncorrected, IR spectra were recorded on Shimadzu FTIR 8201 PC (4000-400 cm^{-1}) and Perkin Elmer Spectrum RXI (4000-450 cm^{-1}). 1H-NMR and 13C-NMR spectra were recorded on Bruker's ADVANCE –III 500 MHz FTNMR using $CDCl_3$ and DMSO as an internal solvents respectively and TMS as an internal standard and mass spectra has been evaluated in GCMS system from Agilent.

Synthesis of Pyrazolines by Modest Modification in Reported Methodologies[9-10]

I-Synthesis of 1-[(5-amino-2-hydroxy phenyl) (phenyl) methyl] thiourea (1): To a mixture of phenol (0.05mole), and benzaldehyde (0.05mole), the compounds like urea and thiourea in ethanol (0.05moles) were added in drops and the reaction mixture was stirred in hot water bath maintained at 80^0C with constant stirring for approximately 1 hr. the solid separated on cooling was washed by ether and recrystallized from ethanol.

II-Synthesisof ethyl-3-({[(5-amino-2-hydroxyphenyl)(phenyl)methyl]carbamothioyl}amino)-3-oxopropanoate (2) : Mixture of (1) and diethyl malonate were taken in a round bottom flask fitted with a refluxed condenser (18 inches) were heated for 1 hr on a water bath then condensed mixture was cooled and add 20 ml ethanol. Filter the solution and pour the filtrate over crushed ice. The solid separated was collected under suction.

III-Synthesis of N-{[(5-amino-2-hydroxyphenyl) (phenyl) methyl] carbamothioyl}-N'-{[(substituted phenyl/naphthyl) (phenyl) methyl] carbamothioyl} propanediamide (3-11): A mixture of (2) and 1-[(N-substituted) (phenyl) methyl] thiourea/urea was refluxed for 1 hour in an ethanolic medium. The condensed mixture was then cooled and the crude was diluted with 50% ethanol and transferred over crushed ice which was further recrystallised from 50% ethanol to dull brown crystallize solid.

IV-Synthesis of 2-Benzylidene N-{[(5-amino-2-hydroxyphenyl) (phenyl) methyl] carbamothioyl}-N'-{[(substituted phenyl/naphthyl) (phenyl) methyl] carbamothioyl} propanediamide (12-20): Products 3-11 and benzaldehyde were condensed in an oil bath (150-155^{0}C) for one hour in presence of 2-3 drops of conc. H_2SO_4 as a condensing agent. The reaction mixture was cooled, diluted with water and the crude product thus obtained was recrystallised from 50% ethanol. The solid thus obtained was filtered over suction.

V-Synthesis of N-{[(5-amino-2-hydroxyphenyl) (phenyl) methyl] carbamothioyl}-3- {{[(substituted phenyl/naphthyl) (phenyl) methyl] carbamothioyl}amino}-5-Phenyl-4, 5-Dihydro-1H-Pyrazole-4-Carboxamide (21-29): Products 12-20 and hydrazine hydrate were refluxed in an absolute ethanolic medium (20 ml) for one hour. The contents were cooled and transferred over the crushed ice, when the crude so separated was filtered under suction, recrystallise from ethanol (50%) to get the crystalline solid of 1H-Pyrazole-4-Carboxamide.

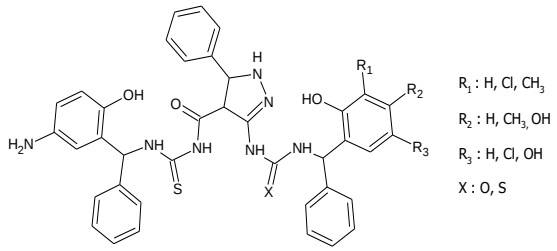

TABLE 1: PHYSICAL DATA OF SYNTHESIZED COMPOUNDS

S.No	Compound	R_1	R_2	R_3	Molecular Formula	Melting Point	% Yield
1	21	H	H	Cl	$C_{31}H_{24}ClN_5O_2S_2$	198°C	41.42%
2	22	H	CH_3	H	$C_{32}H_{27}N_5O_2S_2$	196°C	49.15%
3	23	H	OH	H	$C_{31}H_{25}N_5O_4S_2$	192°C	45.24%
4	25	Cl	H	H	$C_{31}H_{24}ClN_5O_4S$	182°C	40.53%
5	26	CH_3	H	H	$C_{32}H_{27}N_5O_4S$	199°C	48.24%
6	27	H	H	OH	$C_{31}H_{25}N_5O_5S$	280°C	45.12%
7	28	H	H	H	$C_{31}H_{25}N_5O_4S$	220°C	47.01%
8	24	Naphthyl			$C_{42}H_{27}N_5O_2S_2$	188°C	51.03%
9	29	Naphthyl			$C_{42}H_{27}N_5O_4S$	211°C	50.14%

SPECTRAL DATA[11]

Spectral Interpretation of compound (21): IR (KBr) cm^{-1}: Disubstituted phenyl ring (696.28 cm^{-1}), O-H stretching (3748.13 cm^{-1}), C-O stretching (1224.82 cm^{-1}), C=N stretching (1450.54 cm^{-1}), C=O stretching in amide (1720.34 cm^{-1}) N-H stretching for 2^0 alip. Amine (3357.57 cm^{-1}), C-N stretching 2^0 Amine (1099.80 cm^{-1}), N-H bending 2^0 amine (1608.00 cm^{-1}), O-H bending (1373.10 cm^{-1}), 3 bands for C=S in NHCS (1511.17 cm^{-1}, 1461.24 cm-^{1}, 834.07 cm^{-1}), N-H wagging (768.28 cm^{-1}). ^{1}HNMR ($CDCl_3$) ppm: 10.06 (s, 1H, C-OH), 8.56 (s, 2H, NH_2), 8.21 (d, 1H, NH Pyrazoline ring), 8.12 (d, 1H, NH), 8.11 (s, 1H, NH), 2.48 (d, 1H, CH), 4.23 (d, 1H, CH Pyrazoline ring), 3.91 (d, 1H, CH Pyrazoline ring), 7.92, 7.63, 7.56, 7.51 (m, ArH). 13CNMR (DMSO) ppm: 36.32 (2C, CH of Pyrazoline ring), 71.53(3C, CH of Pyrazoline ring), 120.80 (8C, C_6H_5), 121.21(7C, C_6H_5), 121.78 (9C, C_6H_5), 123.25 (6C, C_6H_5), 123.80(10 C, C_6H_5), 133.46 (5C, C_6H_5) 165.28 (1C, CH of Pyrazoline ring), 218.12 (4C, CO). MASS SPECTRA (m/z): 736.4 .

Spectral Interpretation of compound (24): IR (KBr) cm-^{1}: Disubstituted phenyl ring (696.28 cm-^{1}), O-H stretching (3748.13 cm-^{1}), C-O stretching (1224.82 cm-^{1}), C=N stretching (1450.54 cm-^{1}), C=O stretching in amide (1720.34 cm-^{1}) N-H stretching for 2^0 alip. amine (3357.57 cm-^{1}), C-N stretching 2^0 Amine (1099.80 cm-^{1}), N-H bending 2^0 amine (1608.00 cm-^{1}), O-H bending (1373.10 cm-^{1}), 3 bands for C=S of CSNH (1511.17 cm-^{1}, 1461.24 cm-^{1}, 834.07 cm-^{1}), N-H wagging (768.28 cm-^{1}). ^{1}HNMR ($CDCl_3$) ppm: 10.45 (s, 1H, C-OH), 8.29 (d, 1H, NH Pyrazoline ring), 8.22 (d, 1H, NH), 8.23 (s, 1H, NH), 2.52 (d, 1H, CH), 4.27 (d, 1H, CH Pyrazoline ring), 3.98 (d, 1H, CH Pyrazoline ring), 7.95, 7.61, 7.52, 7.48 (m, ArH). 13CNMR (DMSO) ppm: 36.76 (2C, CH of Pyrazoline ring), 71.98(3C, CH of Pyrazoline ring), 120.99 (8C, C_6H_5), 121.32(7C, C_6H_5), 121.54 (9C, C_6H_5), 123.89 (6C, C_6H_5), 123.97(10 C, C_6H_5), 135.21 (5C, C_6H_5) 168.21 (1C, CH of Pyrazoline ring), 216.76 (4C, CO). MASS SPECTRA (m/z) : 733.4

Spectral Interpretation of compound (26): IR (KBr) cm-^{1}: Disubstituted phenyl ring (676.12 cm-^{1}), O-H stretching (3722.15 cm-^{1}), C-O stretching (1221.23 cm-^{1}), C=N stretching (1458.23 cm-^{1}), C=O stretching in amide (1718.45 cm-^{1}) N-H stretching for 2^0 alip. amine (3312.30 cm-^{1}), C-N stretching 2^0 Amine (1097.11 cm-^{1}), N-H bending 2^0 amine (1602.01 cm-^{1}), O-H bending (1365.67 cm-^{1}), 3 bands for C=S of CSNH (1509.34 cm-^{1}, 1465.57 cm-^{1}, 842.06 cm-^{1}), N-H wagging (761.04 cm-^{1}). ^{1}HNMR ($CDCl_3$) ppm: 10.11 (s, 1H, C-OH), 8.42 (d, 1H, NH Pyrazoline ring), 8.32 (d, 1H, NH), 8.29 (s, 1H, NH), 2.43 (d, 1H, CH), 4.12 (d, 1H, CH Pyrazoline ring), 3.93 (d, 1H, CH Pyrazoline ring), 7.93, 7.65, 7.55, 7.42 (m, ArH). 13CNMR (DMSO) ppm: 36.69 (2C, CH of Pyrazoline ring), 71.02(3C, CH of Pyrazoline ring), 121.03 (8C, C_6H_5), 121.44(7C, C_6H_5), 121.66 (9C, C_6H_5), 123.11 (6C, C_6H_5), 123.76(10 C, C_6H_5), 134.23 (5C, C_6H_5) 169.67 (1C, CH of Pyrazoline ring), 213.21 (4C, CO). MASS SPECTRA (m/z) : 702.8

CNS Depressant Activity[12]: Some of the newly synthesized compounds were evaluated for CNS depressant activity by open field method. The test is based on behavioral responses to stay away from brightly illuminated areas. The animals were divided into control, standard and test group. The test groups received dose of 500 mg/kg body weight orally whereas control group receive vehicle (1% Tween 80 in water)

at 10 ml/kg body weight orally and standard group received diazepam at the dose 1 mg/kg body weight orally. One hour after oral administration (distilled water 10 ml/kg; mg/kg; and diazepam 1 mg/kg, n=7), Each mouse was gently placed at the centre of the open field and the number of square crossings, rearing, and assisted rearing (forepaws touching the walls of the apparatus) were determined for 5 min. The floor of the open field was cleaned with ethanol after each session. The present in vivo CNS depressant activity of compounds is represented in table 2.

Diuretic activity[13]: Some of the newly synthesized compounds were evaluated for diuretic activity. During the testing, animals were fasted for 18 h prior to experiment allowing only water during the fasting period and the animals were housed individually in metabolic cages. Male wistar rats (n = 6/per group) weighing 208 ± 8 g were placed in individual metabolic cages and had free access to water but no food. Test drugs at various concentrations and the reference standard, furosemide (5 mg/kg), were suspended in 0.5% w/v tween 80 and administered orally in asingle dose. Additionally, 5 ml of 0.9% NaCl solution per 100 g body weight was given by oral gavage. Urine was collected continuously up to 5 h after dosing and from then up to 24 h after dosing, and the urine volume and acidity (pH) was measured immediately after collection. pH of Urine is determined using pH meter. The present in vivo diuretic activity of compounds is represented in table 3.

TABLE 2

In Vivo CNS depressant Activity of Novel Pyrazolines

Treatment / Dose mg/ kg	Animal Mark/ Body weight	Drug Code	No. of square crossed	No. of central squares crossed	No. of Rearings	No. of assisted Rearings
Standard 1mg/kg Diazepam	Body 150gm	SD-1	8.33±2.30	2.66±0.56	2.68±0.56	3.3±1.2
Test 350 mg/kg	Tail	TD-1	13.3±1.89	2.33±1.95	2.30±0.06	5.33±1.8ᵃ
	Head	TD-2	15.6±2.30ᵃ	4.00±0.97	4.01±0.2ᵃᵃ	6.33±0.6
Control d.w. 10 ml/kg	No mark	CD-1	25.66±5.15ᵃᵃᵃ	7.33±3.36	6.338.77ᵃ	10.33±3.05ᵃᵃ

Table 3

In Vivo Diuretic Activity of Novel Pyrazolines

Group	Urine Volume (ml/ 24hr)	Normal saline intake (ml)	Urinary Excretion (%)	Diuresis	
				Diuretic Index	Lipschitz value
Normal Control (0.91% Saline)	0.96 ±0.1	3.0±0.1	32	1.00	0.35
Furosemide (20mg/kg)	2.78±0.2ᵃ	3.0±0.1	92.6	2.89	1.00
Test-I (350mg/kg)	1.01±0.1ᵃ	3.0±0.1	33.7	1.05	0.37
Test-II (350mg/kg)	1.16±.01ᵃ	3.0±0.1	38.7	1.21	0.42

III. RESULT AND DISCUSSION

Nine novel derivatives N-{[(5-amino-2-hydroxyphenyl) (phenyl) methyl] carbamothioyl}-3-{{[substituted] carbamothioyl}amino}-5-Phenyl-4, 5-Dihydro-1H-Pyrazole-4-Carboxamide have been synthesized according to Michael addition reaction in five steps starting from reaction of para amino phenol and benzaldehyde in presence of compound containg active hydrogen to produce novel thiourea derivative (1) followed by its condensation with diethyl malonoate to yield substituted 3-oxoproponoate (2) which undergoes reaction with various urea/thiourea derivates to produce disubstituted propanediamide (3-11) which on condensation with benzaldehyde forms novel chalcones (12-20) and in last step of synthesis compounds 12-20 condenses with hydrazine hydrate to yields target compounds (21-29). All the novel synthesized compounds were characterized by IR, 1HNMR, 13CNMR and Mass spectra. All the peaks were in the range and slight deviation in frequencies were justified by literature. Some of the novel pyrazoline derivatives shows better CNS activity as compare to standard drug while diuretic activity of these compounds were remarkable. They show positive dieresis but a little lesser than standard drug while higher than control.

IV. CONCLUSION

Synthesis of pyrazoline derivatives were confirmed by IR, NMR and Mass spectra. The screening results revealed that the synthesized pyrazoline shows superior CNS derpressant activity as compare to standard drug (diazepam) and shows potent diuretic activity as compare to furosemide.

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