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Synthesis, Characterization and Pharmacological Activity Studies of Pyrazolines Containing 3, 4-Dimethoxy and 2, 4- Dinitro Compounds

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Abstract: *A series of novel pyrazoline derivatives 5-(substituted aryl)-3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazole (5a-l) were synthesized by heating 2,4-dinitrophenyl hydrazine (4) with (E)-3-(3,4-dimethoxyphenyl)-1-substituted aryl prop-2-en-1-one (3) in presence of catalytic amount of concentrated H_2SO_4. The structures of the novel derivatives were confirmed by various spectroscopic techniques i.e., IR, ^{1}H-NMR, ^{13}C- NMR and LC–MS. The novel compounds were screened for antioxidant, antimicrobial and antihelmintic activity. Some of the compounds showed significant biological activity compared to the standard drug.*

Keywords: *Chalcones, pyrazoline, antimicrobial, antioxidant and anthelmintic.*

1. INTRODUCTION

There is a substantial need for new antimicrobial agents, because of the resistance by the present antibiotics. By designing innovative agents with various modes of action may lead to solve the problem of antibiotic resistance

Many naturally occurring possess pyrazol nucleus which plays significant role and possessing with wide range of pharmacological activities such as antitumor [1]. Anti-inflammatory [2], anti-parasitic [3], anticonvulsant [4], antimicrobial effects [5], antimicrobial [6] and antifungal [7].

Similarly, propenones have been extensively studied for their broad spectrum of biological activities which exert antiparasitic [8], antimitotic [9], immunomodulatory [10], antileishmanial [11], antimalarial [12], anti-invasive [13], anti-obesity activities [14].

Encouraged from the above mentioned literature, present study has been carried out with the synthesis of novel pyrazoline derivatives (5a-l) and screened for their antimicrobial and anthelmintic activities.

2. PHARMACOLOGY

The novel 5-(substituted aryl)-3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1*H*-pyrazole (5a-l) were synthesized and evaluated for antimicrobial and anthelmintic activity studies.

2.1. Antibacterial Activity

All the newly synthesized compounds were screened for their *in vitro* antibacterial activity against the Gram positive *Staphylococcusaureus* (NCIM 2079), *Bacillus subtilis (*ATCC 6633) and gram negative *Escherichia coli (*NCIM 2931), *Klebsiellapneumoniae (*NCIM 2957) procured from National Chemical Laboratory, Pune, India

Antibacterial assay was carried out by disc diffusion method [16]. For *in vitro* antibacterial activity, 200µL of overnight grown culture of each organism was dispensed into 20 ml of sterile nutrient broth

and incubated for 4-5 hours at 37^0C to standardize the culture to 10^{-5} CFU/ml. For this, 0.1ml (10^{-5} CFU /ml) of 24 hrs old bacterial culture was placed on Muller Hinton agar medium and spread throughout the plate by spread plate technique.

The synthesized (*E*)-1-Aryl-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives dissolved in DMSO (Dimethyl sulphoxide) were loaded to the sterile discs (250µg concentration, 6mm diameter disc) purchased from HIMEDIA laboratories, individually and aseptically before screening for antibacterial activity. Antibacterial activity was recorded by measuring the diameter of zone of inhibition. Streptomycin was used as positive and DMSO as negative standard against bacterial strains respectively.

2.2. Antifungal Activity

Antifungal activities of all the synthesized compounds were tested by potato dextrose agar well diffusion method. The microorganisms used were *A.niger, Cladosporaspp, Candida albicuns,* and *T.viride*. The test was run in triplicates. Pure cultures of the organisms were inoculated onto potato dextrose agar incubated for 72hr, 37° C.

The antifungal property of the each compound was carried out by potato dextrose agar well diffusion method in order to measure the zone of inhibition [17].The compounds were dissolved in DMSO to get a concentration of 10mg/mL (1.0in DMSO). Fluconazole (5mg/mL) was used as reference standard and solvent control was also maintained throughout the experiment. The screening was initiated by inoculating the test fungi on to nutrient broth under incubation temperature of 37°C for 72h. From the broth, lawn of each test fungi was made with the help of sterile cotton swabs on nutrient agar plates. Well of 0.5 cm in diameter was punched on the plate with the help of sterile cork borer. The well was filled with varied concentrations of the compounds and the experiment was carried out in triplicate. Plates were incubated for 72h at 37°C after loading the extracts. The plates were then observed for clear zone formation around the well. Antifungal activities were expressed in millimeter.

2.3. Antihelmintic Activity

Indian adult earthworms (*Eudriluseugeniae)* collected from earthworm rearing center, Davangere (Karnataka), were washed with normal saline to remove all fecal matter and used for the anthelmintic study. The earthworms of 3-5cm in length and 0.1-0.2cm in width were used. The anthelmintic activity was evaluated on Indian adult earthworms due to its anatomical and physiological resemblance with the intestinal round worm parasites of human being. They were divided into12 groups of 6 earthworms each of approximately equal size were released into 25mL of desired dextrose solution. The experiment was carried out in one Petriplate for each group. To study anthelmintic property each petriplate was treated with one of the following, (1% normal saline), albendazole (10mg/mL), or 2% of each compounds [18]. Observations were made for the time taken to cause paralysis and death time of the individual worms. Death was concluded when the worms lost their motility followed with fading away of their body colors. The experiment was carried out in one Petriplate for each group.

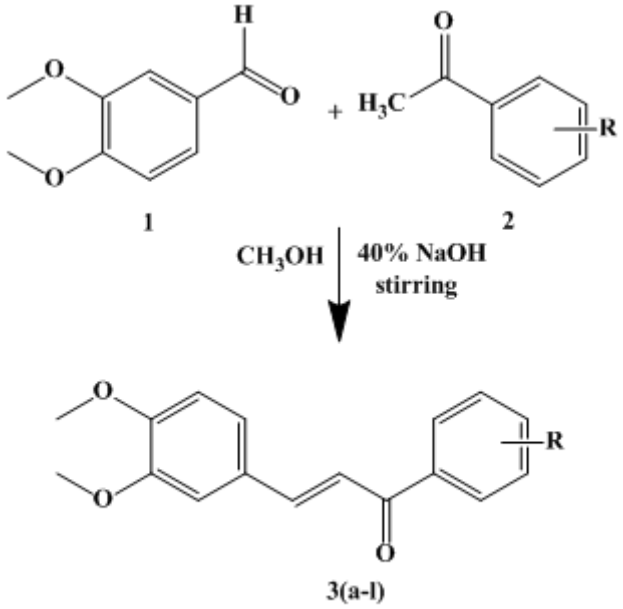
3. RESULTS AND DISCUSSION

3.1. Chemistry

The general synthetic strategy employed for the synthesis of title compounds pyrazoline derivatives are depicted in the scheme 1 and 2 according to the procedure [15]. In the first step (scheme 1), treatment of 3,4-dimethoxy benzaldehyde 1 with substituted acetophenones 2 and 40% NaOH in ethanol afforded the corresponding propenones 3 in a competitive yield *via* Claisen-Schmidt reaction. In the second step (scheme2), furnished compound 5(a-l) were synthesized by well known condensation reaction upon treating previously synthesized propenones with 2,4-dinitro phenylhydrazine 4 in refluxing glacial acetic acid containing Catalytic amount of Conc. H_2SO_4.The resulting solution was then evaporated under reduced pressure to get crude compound, which was further purified by column chromatography using petroleum ether: ethyl acetate(7:3) as eluent to get pure compound.

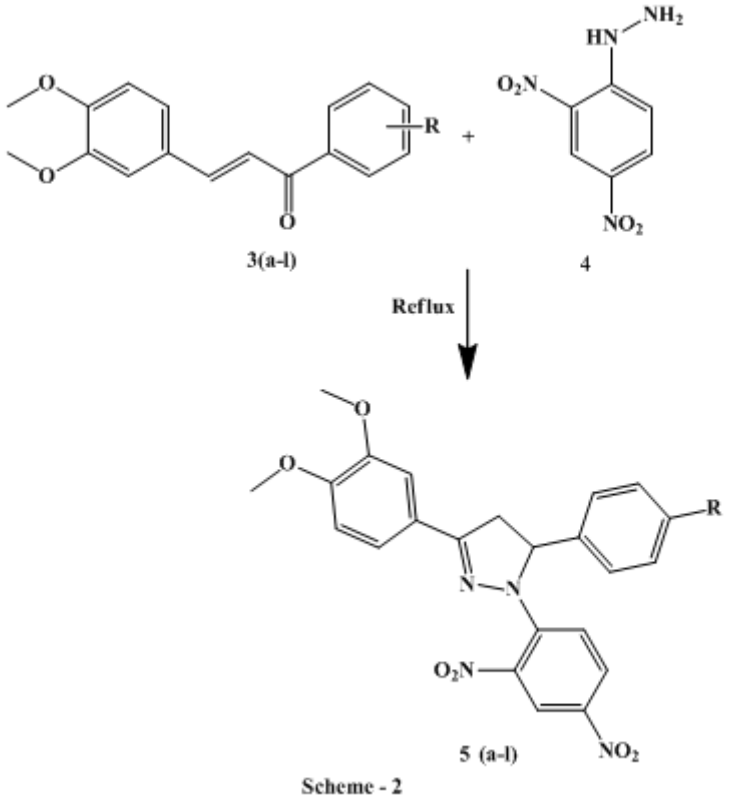
The structures of the synthesized latter compounds 5 (a-l) were confirmed on the basis of spectral analysis (IR, 1H NMR, 13C NMR and LC–MS). The target compound in general, in the IR revealed

Ar C-H, C=N and C-N peaks at 3072, 1546 and 1460, respectively. In the 400 MHz ^{1}H-NMR spectrum of the pyrazoline compounds, H_A, H_B and H_X protons of pyrazoline ring observed as doublet of doublets at δ 3.07-3.94ppm (H_A), 3.85-4.47ppm (H_B) and 5.07-5.47ppm (H_X) due to vicinal coupling with two magnetically non-equivalent protons of methylene group of the pyrazoline ring ($J_{(AB)}$ = 12.4Hz, $J_{(AX)}$ = 12.4Hz, $J_{(AB)}$ = 12.4Hz). The Dimethoxy protons of phenyl appeared as a singlet at δ 3.84. All the other aromatic protons were observed in the region of 6.75-8.45. The peaks which attributed to the carbon atoms at 41.81-43.31ppm, 62.74 - 63.79ppm and 151.63-158.23ppm confirms the pyrazoline compounds in ^{13}C- NMR shift values.



Scheme - 1

SCHEME1. *Synthesis of (E)-3-(3, 4-dimethoxyphenyl)-1-substituted aryl prop-2-en-1-one 3(a- l)*



Scheme - 2

SCHEME2. *Synthesis of 5-(substituted aryl)-3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazole 5(a-l)*

4. BIOLOGICAL ACTIVITY

For the preliminary pharmacological studies, all the synthesized novel compounds 5-(substituted aryl)-3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1*H*-pyrazole 5 (a-l) were characterised in Table 1 and evaluated for antimicrobial and anthelmintic activity studies.

4.1. Antibacterial Activity

Comparing the antibacterial activity values of compounds 5(a-l) with the standard drug Streptomycine by zone inhibition technique. The compounds 5b, 5f having substitutions 4-Br phenyl and 4-NH_2 phenyl were showing excellent activity against all the bacterial strains. Compounds 5d (3,4-dimethoxyphenyl) and 5j (2,4-difluorophenyl) showed promising activity against the bacterial strain *Staphylococus aureus* and moderate activity for all the remaining strains. Compound 5i also exhibit significant activity against the strains *Staphylococus aureus* and *Bacillus subtilis*. Especially compound 5k having 4-CH_3 phenyl exhibit excellent activity except for the bacterial strain *Klebsiella pneumonia* compared to all the remaining novel compounds 5(a-l). It is found that the compounds having bulky groups bromo and fluro substituted phenyl ring enhanced the antibacterial activity. Meanwhile, also the compounds which are attached to the electron releasing groups such as 3,4-dimethoxy, 4-methyl and 4-amino enhances significant activity. It is important to note that the incorporation of another florine atom at position 3 in the phenyl ring 5j slightly decreased the potency of the compound compared to the compound 5i. Table2.

4.2. Antifungal Activity

The in vitro antifungal activity of all the synthesized 5-(substituted aryl)-3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1*H*-pyrazole 5(a-l) with their standard drug Flucunozol by zone inhibition method. Only the compound 4-F phenyl substituted analogue 5i showed relatively significant activity for all the fungal strains compared to remaining compounds. On the other hand, compound 5b substituted with 4-Br phenyl showed promising activity against the fungal strains *C. albicans* and *C. albicans* and *Cladospora spp.* However the compound 5f having the substitution 4-NH_2 phenyl ring exhibit good activity against only *A.niger*. The remaining compounds 5a, 5c, 5d, 5e, 5g, 5h, 5j, 5k, and 5l showed moderate to least activity Table 3.

4.3. Anthelmintic Activity

Among all the newly synthesized pyrazoline derivatives, only the bulky group analogues such as compounds 5b, 5c and 5i substituted with 4-Br, 4-Cl and 4-F to the phenyl ring were displayed significant anthelmintic activity with the standard drug albendazole by showing the paralysis time in ranging from 16.13-19.24 min. The remaining compounds 5a, 5d, 5e, 5f, 5g, 5h, 5j, 5k, and 5l were showed moderate to less activity against the standard drug Table 4.

5. CONCLUSION

The *in vitro* antibacterial activity of the novel compounds 5(a-l) was studied with four pathogenic bacteria and standard drug streptomycine. Compounds 5b, 5d, 5f and 5j exhibit significant antibacterial activity against all the strains except compound 5b 5d and 5j against the strains *K.pneumonia*, *E. coli* and *B. subtilis*. The compound 5i showed moderate activity against only the strains *S. aureus* and *B. subtilis*. Compound 5k showed better inhibitory activity against only with the strain *K.pneumonia* and the remaining compounds exhibit least active than the streptomycine.

Comparing with the results obtained with Flucunozol for antifungal activity, only the compound 5i is active against all the fungal strains. The compounds 5b and 5f active only against the strains *C. albicans* and *Cladospora spp*. The pyrazoline derivatives 5b, 5c and 5i were showed promising activity with the reference drug albendazole for anthelmintic activity.

In general, the bulkier groups such as 4-bromo, 4-fluoro and 4-chloro phenyl ring substituted analogues enhances the pharmacological behavior of pyrazoline derivatives 5(a-l) in the order antibacterial > anthelmintic > antifungal activity.

The studies of these newly synthesized compounds 5-(substituted aryl)-3-(3, 4-dimethoxyphenyl)-1-(2, 4-dinitrophenyl)-4, 5-dihydro-1*H*-pyrazole 5(a-l) should be continued for further toxicity and mechanism of action.

Table1. *Characterization data of 3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-5-(substitutedaryl)-4,5-dihydro-1H-pyrazole 5(a-l)*

Compound	R	Mol. Formula	Mol. Wt.	M.p. (^{0}C)	Yield (%)
5a	OCH_3	$C_{24}H_{22}N_4O_7$	478	143-146	79
5b	Br	$C_{23}H_{19}BrN_4O_6$	527	123-126	64
5c	4-Cl	$C_{23}H_{19}ClN_4O_6$	482	187-186	71
5d	3,4-$(OCH_3)_2$	$C_{25}H_{24}N_4O_8$	508	131-134	69
5e	-	$C_{23}H_{20}N_4O_6$	448	171-174	78
5f	4-NH_2	$C_{23}H_{21}N_5O_6$	463	132-135	73
5g	2-OH	$C_{23}H_{21}N_5O_6$	464	92-95	51
5h	4-OH	$C_{23}H_{21}N_5O_6$	464	76-79	59
5i	4-F	$C_{23}H_{19}FN_4O_6$	466	139-142	62
5j	2,4-$(F)_2$	$C_{23}H_{19}F_2N_4O_6$	485	151-154	49
5k	4-CH_3	$C_{24}H_{22}N_4O_6$	462	203-206	82
5l	4-NO_2	$C_{23}H_{19}N_5O_8$	493	163-166	66

Table2. *Antibacterial activity of 3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-5-(substitutedaryl)-4,5-dihydro-1H-pyrazole 5(a-l)*

	Zone of inhibition(mm)			
Samples	***Staphylococus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Klebsiella pneumoniae***
5a	13.16±0.61	14.34±0.19	11.21±0.32	13.11±0.28
5b	21.36±0.22	18.13±0.62	16.36±0.41	12.31±0.57
5c	6.46±0.24	10±0.65	8.62±0.73	11.72±0.62
5d	19.31±0.44	17.19±0.12	-	16.36±0.51
5e	-	9.29±0.13	-	-
5f	21.31±0.46	16.63±0.24	16.29±0.33	16.01±0.13
5g	-	12.19±0.32	8.15±0.14	13.21±0.29
5h	16.12±0.57	-	-	8.17±0.32
5i	19.13±0.24	15.12±0.177	13.93±0.11	15.93±0.11
5j	17.48±0.08	11.33±0.30	15.72±0.23	16.06±0.11
5k	13.51±0.42	11.15±0.67	11.10±0.54	18.06±0.11
5l	8.26±0.32	11.83±0.64	10.33±0.72	-
Streptomycine	24.3±0.30	21.16±0.37	18.73±0.64	20.6±0.52

Table3. *Antifungal Activity of 3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-5-(substitutedaryl)-4,5-dihydro-1H-pyrazole 5(a-l)*

Samples	*C. albicans*	*Cladospora spp*	*A. niger*	*A. fumigatus*
5a	10.33±0.23	10.23±0.23	8.09±0.98	9.1±0.17
5b	21.68±0.19	17.73±0.64	13.76±0.28	-
5c	-	-	-	-
5d	-	10.13±0.25	-	-
5e	10.23±0.25	09.19±0.46	-	8.16±0.28
5f	11.65±0.75	17.20±0.34	9.23±0.25	8±0.00
5g	9.43±0.4	15.93±0.11	11.93±0.11	-
5h	15.72±0.16	12.66±0.57	-	8.25±0.29
5i	18.9±0.26	16.73±0.85	14.06±0.11	17.91±0.28
5j	16.19±0.20	-	-	-
5k	14.74±0.48	-	11.91±0.10	-

5l	-	13.74±0.38	-	-
Flucunozol	26.3±0.30	22.46±0.37	20.73±0.64	23.6±0.52

Table4. *Antihelmintic Activity of 3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-5-(substitutedaryl)-4,5-dihydro-1H-pyrazole 5(a-l) and Albendazole (Ref. Standard).*

Samples	Paralysis time in min	Death time in min
5a	26.42	31.19
5b	17.50	22.55
5c	19.24	26.02
5d	33.25	38.20
5e	49.34	54.28
5f	24.17	49.19
5g	29.18	35.23
5h	25.46	31.46
5i	16.13	22.31
5j	28.25	36.23
5k	40.59	43.18
5l	38.29	42.36
Albendazole	10.65	14.52

6. EXPERIMENTAL PROTOCOLS

The melting points of the synthesized compounds 5(a-l) were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT IR 157 spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded ($CDCl_3$/DMSO-d_6 mixture) on 400 MHz spectrometer using TMS as an internal standard. Mass spectra were recorded in Agilent Technology LC-mass spectrometer spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer using argon/xenon (6kv, 10mA). The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel plates and some of them are isolated through column chromatogram by using pet ether: methylene dichloride.

7. MATERIALS AND METHODS

7.1. Synthesis of (*E*)-3-(3,4-Dimethoxyphenyl)-1-Substituted Aryl Prop-2-En-1-One 3(A-L)

Substituted aryl ketones (0.1mol) were dissolved in methanol followed by addition of 10ml of 40% NaOH solution under stirring. Then the solution of 3,4-dimethoxy benzaldehyde (0.1mol) in methanol were added and stirring was continued for 6-8hrs. The completion of the reaction was monitored by TLC method and the reaction mixture was cooled and poured into ice cold water. The obtained precipitate was filtered, washed and recrystalized from hot ethonal.

7.2. Synthesis of 5-(substituted aryl)-3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazole 5(a-l)

Substituted propenones 3(a-l) (0.001 mol) and 2,4-dinitro phenyl hydrazine (0.001 mol) were dissolved in glacial acetic acid and added catalytic amount of conc H_2SO_4 . Then the contents were heated under reflux on oil bath 100-110 ^{o}C. Completion of the reaction was monitored by TLC method. Reaction mixture were poured into cold water and the obtained compounds were filtered, washed and recrystaized from ethanol and dimethyl formamide[18]. Some of them were isolated through column chromatography by pet ether and ethyl acetate (7:3) as eluent.

5a- IR(KBr)cm^{-1}: Aromatic 3263(C–H), 1650(C=N), 1540(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 2.49(s,3H, CH_3), 3.8 (s,6H, 3,4-$(OCH_3)_2$), 3.08 (dd, CH_A; J_{AB} = 16.4 Hz, J_{AX} = 6.9) 3.52 (dd, H_B, J_{AB} = 17.6 Hz, J_{AX} = 12.1 Hz, 1H), 6.02 (dd, Hx, J_{AX} = 12, J_{BX} = 6.2, 1H), 6.04 (d, 1H) 6.6(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 8.05(d, 1H, *J*=2.4 Hz, 2,4-$(NO_2)_2$ phenyl), 8.07(d, 1H, *J*=2.4 Hz, 2,4-dinitrophenyl), 8.06(d, 1H, *J*=2.4 Hz, 2,4 –dinitrophenyl; LCMS(m/z, %):579.74.

5b- IR(KBr)cm^{-1}: Aromatic 3263(C–H), 1666(C=N), 1547(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): 1^{1}H-NMR ($CDCl_3$)/ppm: 3.8 (s,6H, 3,4-$(OCH_3)_2$), 3.11 (dd, CH_A; J_{AB} = 17.6 Hz, J_{AX} = 6.4) 3.83 (dd, H_B, J_{AB} = 17.2 Hz, J_{AX} = 11.6 Hz, 1H), 6.11 (dd, Hx, J_{AX} = 12, J_{BX} = 6.8, 1H), 6.7 (d, 1H) 6.8(dd, 2H, *J*=2.4Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl),7.20(dd, *J*=2Hz, 4-Br-phenyl, 2H), 7.56(dd, *J*=2.4 Hz, 4-Br-phenyl, 2H), 8.07(dd, 1H, *J*=2.4 Hz, 2,4-$(NO_2)_2$ phenyl), 8.45(d, 1H, *J*=2.4 Hz, 2,4-dinitrophenyl), 8.4(d, 1H, *J*=2.8 Hz, 2,4–dinitrophenyl); LCMS(m/z, %); 526.74(M^+), 528.11(M+2).

5c- IR(KBr)cm^{-1}: Aromatic 3240(C–H), 1661(C=N), 1539(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 3.18 (dd, CH_A; J_{AB} = 3.2 Hz, J_{AX} = 16), 3.7 (s,6H, 3,4-$(OCH_3)_2$), 3.90 (dd, H_B, J_{AB} = 16 Hz, J_{AX} = 12 Hz, 1H), 5.60 (dd, Hx, J_{AX} = 12, J_{BX} = 8, 1H), 6.76 (d, 1H) 6.77(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 7.48(s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4 Hz, 2,4-dinitrophenyl), 8.32(d, 1H, *J*=2.4 Hz, 2,4 –dinitrophenyl; LCMS(m/z, %):483.15(M^+), 585.16(M+2).

5d- IR(KBr)cm^{-1}: Aromatic 3243(C–H), 1657(C=N), 1543(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 3.21 (dd, CH_A; J_{AB} = 3.9 Hz, J_{AX} = 16.4, 1H), 3.5 (s,6H, 3,4-$(OCH_3)_2$), 3.6 (s, 6H,$(OCH_3)_2$), 3.84 (dd, H_B, J_{AB} = 16.1 Hz, J_{AX} = 12.3 Hz, 1H), 5.43 (dd, H_X, J_{AX} = 12.4, J_{BX} = 8.4, 1H), 6.71 (d, 1H, 3,4-$(OCH_3)_2$ phenyl), 6.76(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.79(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl) 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 8.00(s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4 Hz, 2,4-dinitrophenyl), 8.32(d, 1H, *J*=2.4 Hz, 2,4 –dinitrophenyl); LCMS(m/z, %):508.32.

5e- IR(KBr)cm^{-1}: Aromatic 3243(C–H), 1654(C=N), 1544(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 3.5 (s,6H, 3,4-$(OCH_3)_2$), 3.21 (dd, CH_A; J_{AB} = 3.2 Hz, J_{AX} = 16) 3.90 (dd, H_B, J_{AB} = 16 Hz, J_{AX} = 12 Hz, 1H), 5.60 (dd, Hx, J_{AX} = 12, J_{BX} = 8, 1H), 6.89 (d, 1H), 6.77(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 7.4 (m, 5H, phenyl), 8.00(s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4Hz, 2,4-dinitrophenyl), 8.05(d, 1H, *J*=4 Hz, 2,4–dinitrophenyl); LCMS(m/z, %):448.11.

5f- IR(KBr)cm^{-1}: Aromatic 3242(C–H), 1658(C=N), 1537(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 3.17 (dd, CH_A; J_{AB} = 3.7 Hz, J_{AX} = 16), 3.71 (s,6H, 3,4-$(OCH_3)_2$), 3.90 (dd, H_B, J_{AB} = 16 Hz, J_{AX} = 12 Hz, 1H), 5(s, 4-NH_2, 2H),5.60 (dd, Hx, J_{AX} = 12, J_{BX} = 8, 1H), 6.76 (d, 1H,) 6.77(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 7.1(dd, 2H, *J*=2Hz, 4-NH_2 phenyl), 7.2(dd, 2H, *J*=2Hz, 4-NH_2 phenyl),7.48 (s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4 Hz, 2,4-dinitrophenyl), 8.32(d, 1H, *J*=2.4 Hz, 2,4 –dinitrophenyl; LCMS(m/z, %):463.15.

5g- IR(KBr)cm^{-1}: Aromatic 3248(C–H), 1662(C=N), 1534(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 3.17 (dd, CH_A; J_{AB} = 3.7 Hz, J_{AX} = 16), 3.71 (s,6H, 3,4-$(OCH_3)_2$), 3.90 (dd, H_B, J_{AB} = 16 Hz, J_{AX} = 12 Hz, 1H), 5.35(s, 2-OH, 1H),5.60 (dd, Hx, J_{AX} = 12, J_{BX} = 8, 1H), 6.76 (d, 1H,) 6.77(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 7.2(dd, 2H, *J*=2Hz, 4-NH_2 phenyl), 7.3(dd, 2H, *J*=2Hz, 4-NH_2 phenyl) 7.48 (s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4 Hz, 2,4-dinitrophenyl), 8.32(d, 1H, *J*=2.4 Hz, 2,4 –dinitrophenyl; LCMS(m/z, %):464.32

5h- IR(KBr)cm^{-1}: Aromatic 3238(C–H), 1645(C=N), 1541(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 3.17 (dd, CH_A; J_{AB} = 3.7 Hz, J_{AX} = 16), 3.71 (s,6H, 3,4-$(OCH_3)_2$), 3.90 (dd, H_B, J_{AB} = 16 Hz, J_{AX} = 12 Hz, 1H), 5.35(s, 2-OH, 1H),5.60 (dd, Hx, J_{AX} = 12, J_{BX} = 8, 1H), 6.76 (d, 1H,) 6.77(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 7.2(dd, 2H, *J*=2Hz, 4-NH_2 phenyl), 7.3(dd, 2H, *J*=2Hz, 4-NH_2 phenyl) 7.48 (s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4 Hz, 2,4-dinitrophenyl), 8.32(d, 1H, *J*=2.4 Hz, 2,4 –dinitrophenyl; LCMS(m/z, %):464.91.

5i- IR(KBr)cm^{-1}: Aromatic 3229(C–H), 1638(C=N), 1544(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 3.17 (dd, 1H, CH_A; J_{AB} = 3.8 Hz, J_{AX} = 16 Hz,), 3.74 (s,6H, 3,4-$(OCH_3)_2$), 3.92 (dd, 1H, H_B, J_{AB} = 16 Hz, J_{AX} = 12 Hz,), 5.60 (dd, 1H, Hx, J_{AX} = 12, J_{BX} = 8,), 6.77(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 7.2(dd, 2H, *J*=2Hz, 4-F phenyl), 7.3(dd, 2H, *J*=2Hz, 4-F phenyl) 7.48 (s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4 Hz, 2,4-dinitrophenyl), 8.32(d, 1H, *J*=2.4 Hz, 2,4 –dinitrophenyl; LCMS(m/z, %):465.11.

5j- IR(KBr)cm^{-1}: Aromatic 3236(C–H), 1647(C=N), 1539(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 3.21 (dd, 1H, CH_A; J_{AB} = 3.7 Hz, J_{AX} = 16), 3.78 (s,6H, 3,4-$(OCH_3)_2$), 3.91 (dd, 1H, H_B, J_{AB} = 16 Hz, J_{AX} = 12 Hz), 5.60 (dd, 1H, Hx, J_{AX} = 12, J_{BX} = 8), 6.77(dd, 2H,

J=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 7.2(s, 1H, 2,4-F_2 phenyl), 7.3(dd, 2H, *J*=2Hz, 2,4-F_2 phenyl), 7.48 (s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4 Hz, 2,4-dinitrophenyl), 8.32(d, 1H, *J*=2.4 Hz, 2,4 –dinitrophenyl; LCMS(m/z, %):485.09.

5k- IR(KBr)cm^{-1}: Aromatic 3239(C–H), 1646(C=N), 1543(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 2.9 (s, 3H, 4-CH_3), 3.23 (dd, 1H, CH_A; J_{AB} = 3.7 Hz, J_{AX} = 16 Hz), 3.72 (s,6H, 3,4-$(OCH_3)_2$), 3.4 (dd, 1H, H_B, J_{AB} = 16 Hz, J_{AX} = 12 Hz), 5.71 (dd, 1H, Hx, J_{AX} = 12, J_{BX} = 8), 6.77(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 7.2(s, 2H, *J*=2.4Hz, 4-CH_3 phenyl), 7.3(dd, 2H, *J*=2Hz, 2,4-F_2 phenyl), 7.48 (s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4 Hz, 2,4-dinitrophenyl), 8.32(d, 1H, *J*=2.4 Hz, 2,4–dinitrophenyl; LCMS(m/z, %):462.28.

5l- IR(KBr)cm^{-1}: Aromatic 3236(C–H), 1642(C=N), 1549(N=O); ^{1}H-NMR(400 MHz, $CDCL_3$, δ ppm): ^{1}H-NMR ($CDCl_3$)/ppm: 3.32 (dd, 1H, CH_A; J_{AB} = 4 Hz, J_{AX} = 14), 3.39 (s,6H, 3,4-$(OCH_3)_2$), 3.42 (dd, 1H, H_B, J_{AB} = 14 Hz, J_{AX} = 8 Hz), 5.63 (dd, 1H, Hx, J_{AX} = 4 Hz, J_{BX} = 16Hz), 6.77(dd, 2H, *J*=2Hz, 3,4-$(OCH_3)_2$ phenyl), 6.9 (s,1H, 3,4-$(OCH_3)_2$ phenyl), 7.2(s, 2H, *J*=2.4Hz, 4-CH_3 phenyl), 7.3(dd, 2H, *J*=2Hz, 2,4-F_2 phenyl), 7.48 (s, 1H, 2,4-$(NO_2)_2$ phenyl), 8.01(d, 1H, *J*=4 Hz, 2,4-dinitrophenyl), 8.32(d, 1H, *J*=2.4 Hz, 2,4 –dinitrophenyl; LCMS(m/z, %):493.01.

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