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Research Article

NOVEL FUSED 1H- IMIDAZO[1,2-B] PYRAZOLE ANALOGUES AS ANTI-INFLAMMATORY AND ANALGESIC AGENTS WITH THEIR MOLECULAR DOCKING STUDIES AGAINST COX-1 AND COX-2

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ABSTRACT

New series of 2,6-disubstituted-1H-imidazo [1,2-b] pyrazole analogues were synthesized structures of these compounds were predicted and confirmed by IR, ^{1}HNMR, ^{13}CNMR, Mass spectral and elemental analysis. The newly synthesised compounds were evaluated for their acute toxicity, anti-inflammatory, analgesic activities properties and in-silico molecular docking studies synthesized compounds were exhibited significant anti-inflammatory and analgesic properties. The results indicated that had good affinity to the active site residue of COX-2 and COX-1 respectively. This strongly evidences that 2,6-disubstituted-1H-imidazo [1,2-b] pyrazole analogues are anti-inflammatory and analgesic agents which was supported by both in-vivo and in-silico studies.

Keywords: 1H-imidazo[1,2-b] pyrazole, Anti-inflammatory, Analgesic activity, Acute toxicity, In-silico molecular docking studies.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) form an important class of widely used therapeutic agents due to their anti-inflammatory, analgesic and antipyretic effects. The pharmacological activity of NSAIDs is related to suppression of prostaglandin biosynthesis by inhibiting the enzyme cyclooxygenase (COX). COX is an endogenous enzyme which catalyzes the conversion of arachidonic acid into prostaglandins and thromboxanes. The enzyme exists in at least two isoforms, COX-1 and COX-2. Although both isoforms catalyze the same biochemical transformation, they are subject to a different expression regulation. COX-1 is a constitutive enzyme and is responsible for the physiological function of prostaglandins (PGs) like maintenance of the integrity of the gastric mucosa and provides adequate vascular homeostasis, whereas COX-2 is an inducible enzyme and is expressed only after an inflammatory stimulus [1]. In recent years, many heterocyclic moieties have been designed and synthesised as potent anti-inflammatory drugs. Among such scaffolds, 1H-imidazo [1,2-b] pyrazole derivatives have been reported to show various biological activities [2-8] such as anti-inflammatory, anti-allergy [8 - 10], anti-cancer agents against trypanosome eruzil [11] and also used to modulate the activity of G-protein-coupled receptor, GPR119 [12-15]. 1H-imidazo [1,2-b] pyrazole derivatives has been found to exhibit interesting biological activities such as inhibition of DNA synthesis and fMLP-induced neutrophil chemotaxis [16, 24]. Based on the above findings in quest of our work on biologically active fused heterocyclic compound, in present we synthesized 1H-imidazo [1,2-b] pyrazole ring system bridged with secondary amine such as morpholine and pyrrolidine moiety. Further these synthesised compounds were screened for in-vivo anti-inflammatory analgesic activity in-silico molecular docking studies.

MATERIALS AND METHODS

All reagents of laboratory quality were purchased from Merck and were used after purification. The melting points of the synthesized compounds are uncorrected and were recorded on a Buchi instrument using open capillaries. TLC plates were purchased from Merck silica gel 60 F254 aluminium sheets. Synthesized compounds were recrystallized using suitable solvent system. Infra-Red spectra were recorded on Nicolet-Impact-410 FT-IR spectrophotometer using KBr pellets. NMR spectra were recorded respectively on a Bruker 300 MHz spectrometer with TMS as internal standard and CDCl3 + DMSO-d6 as solvent. Chemical shifts (δ) were reported in ppm, coupling constants (J) were given in Hz. Mass spectrum was recorded on Micromass Walter instrument by the electron-impact technique, and the elemental analysis was carried out using a Heraus CHN rapid analyser.

GENERAL METHODS FOR SYNTHESIS OF TITLE COMPOUNDS

Synthesis of 3-substituted-phenyl-1H-pyrazol-5-amine: 3(a-c)

The substituted methyl ester (1) (0.05mol) was suspended in THF (15 ml) and stirred for 2 hours at -40^{0}C. In another flask n-Butyl lithium (0.05 mol) in hexane (5 ml) were added gradually with stirring to the pre cooled THF (ml) solution. Acetonitrile (0.05 mol) was added drop wise to the flask containing n-Butyl lithium over a time span of 30-45 min with stirring continued for next one hour. Pre-cooled substituted methyl ester is added to the flask over a time span of 45 min and the flasks were immersed in hot water bath and were allowed to attain 35-40 °C with occasional shaking. Thick slurry is obtained after 2-4 hour. The reaction mixture was quenched with aqueous HCl, and

diluted with ethyl acetate. The aqueous layer was extracted with chloroform and dried over $MgSO_4$, filtered. Finally the crude nitrile product (2) was obtained through rotary evaporator. The crude nitrile (2) was further used without purification for next step. Hydrazine hydrate (1 mol) in ethanol (20 ml) was added and refluxed at 80 °C for 8-10 hour. Reaction mixture was allowed to stand overnight at room temperature and next day solvent was removed under rotary evaporator. The residue was purified by column chromatography over silica gel (mesh size, column diameter) using ethyl acetatemethanol: dichloromethane (3:1:1) as elutent to give compound **3(a-e**) as brown viscous liquid.

Synthesis of 2,6-disubstituted-7H-imidazo [1,2-b] pyrazole: 5(a-f)

A mixture of 0.01mol of 3-substituted-phenyl-1H-pyrazol-5-amine (3) and 0.0lmol of bromoacetyl compound (4) was taken in round bottom flask along with ethanol (15 ml). Concentrated HCl was added drop wise and refluxed for 6-8 hour. After completion of the reaction as monitored by thin layer chromatography (TLC), the reaction mixture was cooled and diluted with water neutralized by aqueous sodium carbonate, filtered and dried. The crude product was purified by column chromatography over silica gel (mesh size, column diameter) using hexane-ethyl acetate (3:1) as eluent to give compound 5(a-f).

Synthesis of 3-(morpholinomethyl)-2,6-disubstitued- 3H-imidazo[1,2-b]pyrazole:6(a-f).

To the compound 2,6-disubstituted-7H-imidazo [1,2-b] pyrazole (0.06mol) an equimolar quantity of morpholine (0.06mol) was added with formalin (2 ml). To the same flask catalytic amount of acetic acid (1 ml) and methanol (15 ml) was added slowly along the side walls of the flask and refluxed for 4 hour. The reaction mixture was monitored by TLC. After the completion of reaction, the reaction mixture was quenched with cold water and extracted twice with chloroform and finally washed with water, dried over anhydrous sodium sulfate. The solution was evaporated in vacuo to afford the crude product 10. The solid thus obtained was recrystallized with appropriate solvent to obtain desired compound 6(a-f).

Synthesis of 2,6-disubstituted-3-(pyrrolidin-1-methyl -3H-imidazole [1,2-b]pyrazole : 7(a-f)

The procedure remains the same as is followed by replacing morpholine by pyrrolidine to obtain compound 7(a-f).

PHARMACOLOGICAL EVALUATION
Acute Toxicity Studies

The acute oral toxicity study for the test compounds 6a-f and 7a-f were evaluated according to the OECD guidelines No.420 using Swiss albino male mice weighing 25-30 g [25]. Each group consisting of 6 male mice overnight fasted, was kept in the colony cage at 25 ± 2°C with 55% relative humidity and 12 h light/dark cycles. A specified fixed dose of 250, 500, 1000 and 1500 mg kg^{-1} was selected and administered orally as a single dose as fine suspension prepared in saline using gum acacia (5%). The acute toxic symptoms and the behavioral changes produced by the test compounds were observed continuously at interval time of 4 hrs. (4^{th}, 8^{th}, 12^{th}, and 24^{th} h). Onset of toxic symptoms and gross behavioral changes were also recorded [27].

Anti-Inflammatory Activity

Carrageenan induced rat paw edema assay was carried out for all the newly synthesized compounds for testing their anti-inflammatory properties following method proposed by Winter et al [28]. Wistar albino rats of both sex weighing 200-250 g were selected, separated into various groups each containing four rats. Food was withdrawn 12 hour before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee (mention ethical clearance number). Experiment include dhenylbutazone as standard reference drug and was administered orally at concentration of 100 mg kg^{-1}, p.o. suspended in 2% gum acacia were served to the first group of rats. Other groups of the albino rats were served as control and received 0.1 ml of 1% gum acacia suspension orally. One hour after the administration of newly synthesised compounds at the dose of 100 mg kg^{-1} as suspension in gum acacia, 0.1 ml of 1% carrageenan in normal saline was given subcutaneously to the sub plantar region of right hind paw. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 0 and 3 h after carrageenan (1%, 0.1m) injection. The difference between the paw volume at 3 hour and 0hour measurement was calculated and taken as edema volume. The percentage inhibition in the paw edema was calculated by using the formula, percentage inhibition = 100 $(1 - V_t/V_c)$, where V_t = mean increase in paw volume of test, and V_c = mean increase in paw volume of control. The percentage inhibition of newly synthesised compounds was recorded.

Analgesic Activity

Analgesic activity was assessed by tail-flick method [29], Swiss albino mice of either sex weighing 20-25 g in group of five were selected by random sampling technique. The newly synthesised compounds and the anlagen as reference standard drug was orally administered at a dose level of 10 mg kg^{-1} body weight of the mice. The animals were held in position by a suitable restrained with the tail extending out and tail was then dipped in a hot water bath maintained at 52± 0.1° C. The time taken by rat to with draw the tail completely from the hot water bath is considered as the withdraw-latency. The tail-flick latency was assessed by the time taken by the rat to withdraw its tail from the hot water bath. The latent time for withdrawal of tail from hot water bath was recorded at 30, 60, 90, 120 and 300 min post drug administration and the cut-off time was 10 sec.

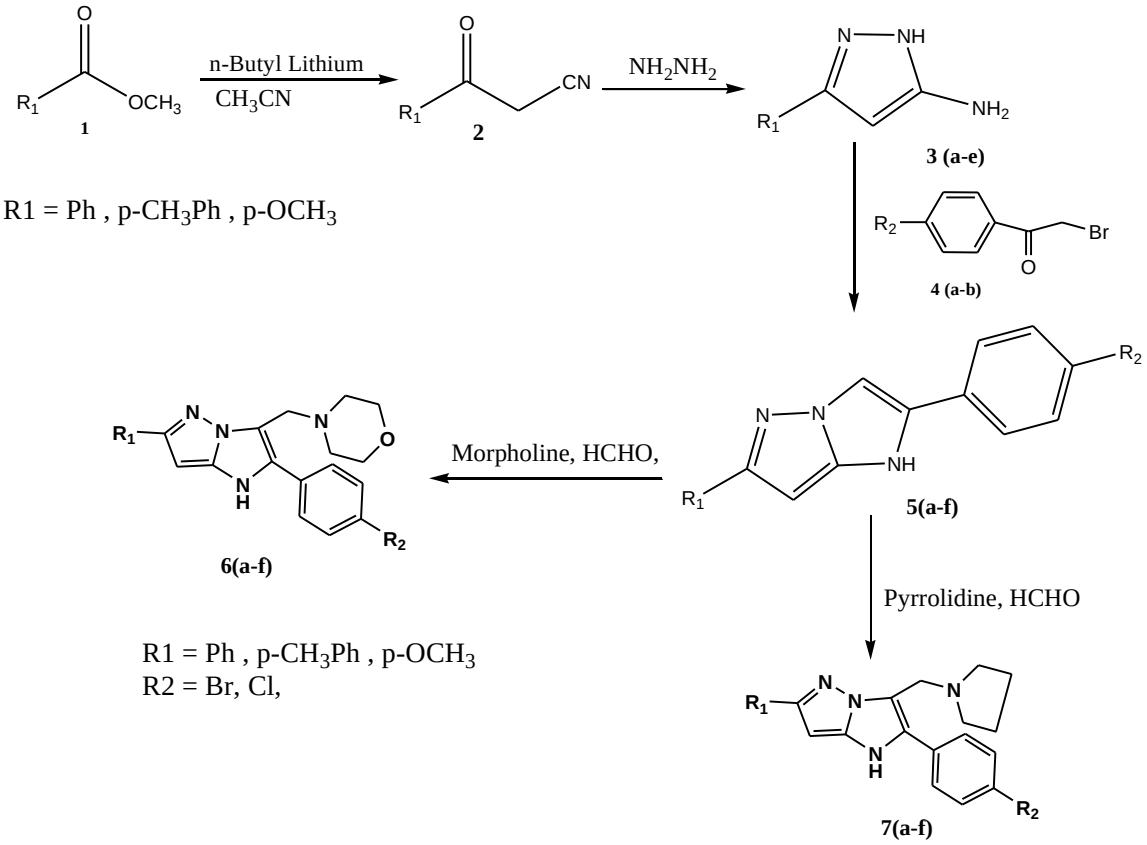
Statistical Analysis

The results were analysed as mean value ± SEM and were analysed for statistical significance using one-way ANOVA followed by Dunnet's test. A P value<0.05 was considered as statistically significant.

In-Silico Molecular Docking Studies

All the structure was predicted from the above-mentioned data strictures, kindly find the studies as mentioned here. 3D structures of all possible stereoisomer forms of synthesized compounds (ligands) were generated using the Marvin sketch [30] software package and were MM2 optimized using Discovery Studio 3.5. The bioavailability of compounds 6a, 6b, 6c, 6d, 6e, 6f, 7a, 7b, 7c, 7d, 7e and 7f was assessed using ADME (Adsorption, Distribution, Metabolism Elimination) program [31]. Lipinski´s rule-of-5 was calculated for all the compounds [31]. The 3D crystal structure of the COX-2 enzyme [1CX2] and COX-1 enzyme [1EQG] was retrieved from PDB database [32]. The COX-2 and COX-1 structure was first

relaxed by 20,000 steps of minimization and a standard relaxation procedure using restrained Molecular Dynamics [33]. The active site prediction was performed using Discovery Studio 3.5 program [34]. The molecular docking studies were carried to investigate the binding affinities and interaction modes between the inhibitors and the target proteins COX-1 and COX-2 using Lead IT program. Maximum number of fragmentation and iterations were set to 200. The docked ligand-target complexes were analyzed carefully to identify the interactions and binding affinities. The docking score was recorded and docking poses were saved for reference [32].



Scheme -1

Table 1: Screening of Anti-inflammatory activity in Albino mice (by rat paw edema method)

Compound code	Change in paw volume(ml) after ±SE #				% Inhibition of inflammation at various time intervals			
	0 hour	1hour	2 hour	3 hour	0 hour	1hour	2 hour	3 hour
6a	0.78±0.02*	0.85 ± 0.07 *	0.95±0.03*	1.03±0.08*	17.45	28.65	49.78	51.60
6b	0.92 ±0.01**	0.96 ± 0.04*	1.33 ± 0.03*	1.60± 0.06*	6.20	23.54	52.53	61.19
6c	0.82± 0.02*	0.95± 0.04*	1.02± 0.05*	1.34± 0.02*	21.33	36.25	48.67	59.45
6d	0.67±0.02*	0.69 ± 0.04*	0.85±0.06*	0.89±0.07*	5.62	6.32	7.03	10.02
6e	0.82± 0.08*	0.86 ± 0.08*	0.92± 0.02*	0.96± 0.02*	12.58	18.78	23.76	39.83
6f	0.88± 0.01**	0.92± 0.03	1.03± 0.07*	1.12± 0.04*	13.76	19.87	32.41	42.80
7a	0.83±0.03*	0.97 ± 0.08	1.03 ± 0.04*	1.24± 0.06*	4.80	14.34	36.84	50.23
7b	0.76±0.02*	0.83 ± 0.02*	0.92±0.01**	1.08±0.04*	15.35	29.57	47.89	56.29
7c	0.89±0.03	0.97 ± 0.08*	1.01 ± 0.02*	1.25± 0.05*	20.57	29.94	45.78	55.32
7d	0.56±0.04**	0.58 ± 0.06*	0.67±0.03*	0.72±0.01**	4.23	4.21	6.33	9.08
7e	0.72±0.02*	0.75 ± 0.04*	0.84±0.14**	0.94±0.03*	9.67	17.98	21.42	31.27
7f	0.78± 0.07*	0.82± 0.03*	0.93± 0.03*	1.02± 0.06*	15.30	18.65	29.76	38.65
Phenylbutazone	0.56 ±0.01**	0.76 ± 0.06	0.96 ± 0.03*	0.98 ± 0.02*	32.06	42.36	57.6	63.4
Control	0.23	0	0	0	0	0	0	0

Results are expressed in mean ± SEM (n=6) significance levels * P<0.05, ** P < 0.01 and *** P < 0.001 as compared with the respective control.

Table 2: Screening of Anti-inflammatory activity in Albino mice (by rat paw edema method)

Compound code	Change in paw volume(ml) after ±SE #				% Inhibition of inflammation at various time intervals			
	0 hour	1hour	2 hour	3 hour	0 hour	1hour	2 hour	3 hour
6a	0.78±0.02*	0.85 ± 0.07 *	0.95±0.03*	1.03±0.08*	17.45	28.65	49.78	51.60
6b	0.92 ±0.01**	0.96 ± 0.04*	1.33 ± 0.03*	1.60± 0.06*	6.20	23.54	52.53	61.19
6c	0.82± 0.02*	0.95± 0.04*	1.02± 0.05*	1.34± 0.02*	21.33	36.25	48.67	59.45
6d	0.67±0.02*	0.69 ± 0.04*	0.85±0.06*	0.89±0.07*	5.62	6.32	7.03	10.02
6e	0.82± 0.08*	0.86 ± 0.08*	0.92± 0.02*	0.96± 0.02*	12.58	18.78	23.76	39.83
6f	0.88± 0.01**	0.92± 0.03	1.03± 0.07*	1.12± 0.04*	13.76	19.87	32.41	42.80
7a	0.83±0.03*	0.97 ± 0.08	1.03 ± 0.04*	1.24± 0.06*	4.80	14.34	36.84	50.23
7b	0.76±0.02*	0.83 ± 0.02*	0.92±0.01**	1.08±0.04*	15.35	29.57	47.89	56.29
7c	0.89±0.03	0.97 ± 0.08*	1.01 ± 0.02*	1.25± 0.05*	20.57	29.94	45.78	55.32
7d	0.56±0.04**	0.58 ± 0.06*	0.67±0.03*	0.72±0.01**	4.23	4.21	6.33	9.08
7e	0.72±0.02*	0.75 ± 0.04*	0.84±0.14**	0.94±0.03*	9.67	17.98	21.42	31.27
7f	0.78± 0.07*	0.82± 0.03*	0.93± 0.03*	1.02± 0.06*	15.30	18.65	29.76	38.65
Phenylbutazone	0.56 ±0.01**	0.76 ± 0.06	0.96 ± 0.03*	0.98 ± 0.02*	32.06	42.36	57.6	63.4
Control	0.23	0	0	0	0	0	0	0

Results are expressed in mean ± SEM (n=6) significance levels * P<0.05, ** P < 0.01 and *** P < 0.001 as compared with the respective control.

Table 3a: Admet properties for synthesized compounds

Compound Name	ADMET Solubility Level	ADMET BBB Level	ADMET EXT CYP2D6	ADMET EXT Hepatotoxic	ADMET Absorption Level	ADMET EXT PPB	ADMET AlogP98	ADMET PSA 2D
6a	1	0	3.27364	-0.0936128	0	9.49581	4.975	32.771
6b	2	1	-0.549402	1.05055	0	5.28602	3.9	32.623
6c	2	1	-0.693699	3.06374	0	1.80075	3.398	41.553
6d	1	0	0.442888	-0.932894	0	6.23002	5.059	32.771
6e	2	1	-2.85131	0.616731	0	1.98318	3.984	32.623
6f	2	1	-3.08262	2.93874	0	-1.65624	3.482	41.553
7a	2	1	-0.251454	0.639236	0	3.06403	2.641	41.553
7b	2	1	-0.97103	-0.692322	0	4.85454	3.127	41.553
7c	2	2	-0.740829	1.99057	0	1.75099	2.624	50.483
7d	2	1	-2.64037	0.20542	0	-0.392957	2.725	41.553
7e	2	1	-3.35995	-1.12614	0	1.39756	3.211	41.553
7f	2	2	-3.20936	2.26513	0	-1.70599	2.708	50.483

Table 3b: Lipinksi rule of five for synthesized compounds

S. No	Compound	H-Bond Donors	H-Bond Acceptors	Molecular Weight	ALog P
1	6a	1	4	377.89	5.11
2	6b	2	4	392.924	4.035
3	6c	2	5	408.924	3.533
4	6d	1	4	422.341	5.194
5	6e	2	4	437.375	4.119
6	6f	2	5	453.375	3.617
7	7a	2	5	394.897	2.776
8	7b	2	5	408.924	3.262
9	7c	2	6	424.923	2.759
10	7d	2	5	439.348	2.86
11	7e	2	5	453.375	3.346
12	7f	2	6	469.374	2.843

Table 4: Molecular docking scores of all the compounds with COX-1 and COX-2

S.No	Compound Name	LeadIT Score	
		Cox-1	Cox -2
1	6a	-10.404	-17.231
2	**6b**	**-12.831**	**-20.096**
3	**6c**	**-12.184**	**-20.091**
4	6d	-9.774	-15.776
5	6e	-10.451	-15.602
6	6f	-10.651	-16.286
7	7a	-8.581	-16.241
8	**7b**	**-12.404**	**-18.244**
9	**7c**	**-12.448**	**-18.281**
10	7d	-9.234	-15.077
11	7e	-10.167	-12.286
12	7f	-10.07	-14.769
13	Phenylbutazone	-4.071	-8.781

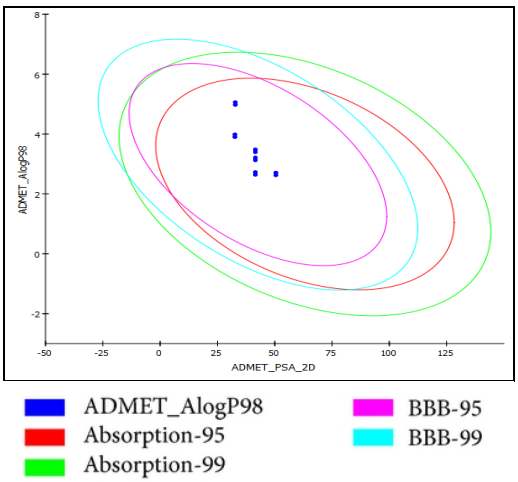
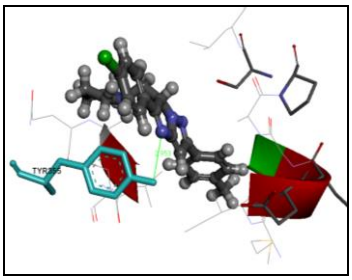
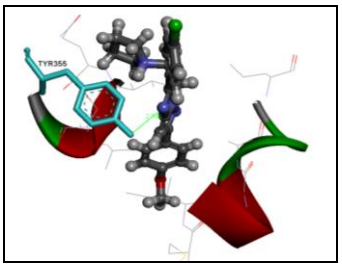


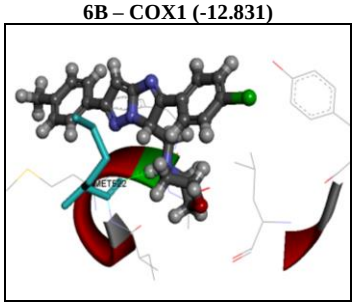
Figure 1: ADMET properties for synthesized compounds with graphical representation



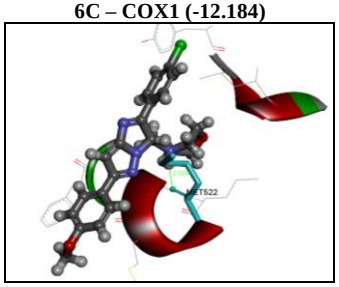
6B – COX1 (-12.831)



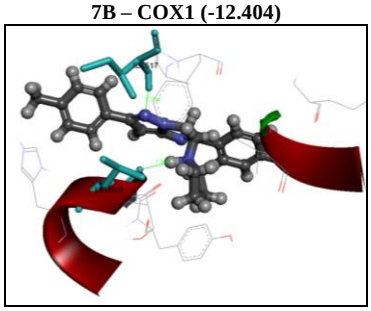
6C – COX1 (-12.184)



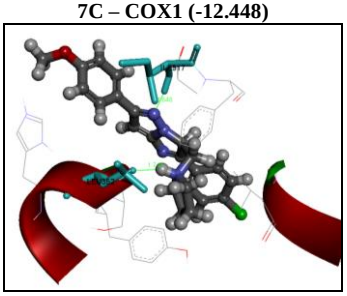
7B – COX1 (-12.404)



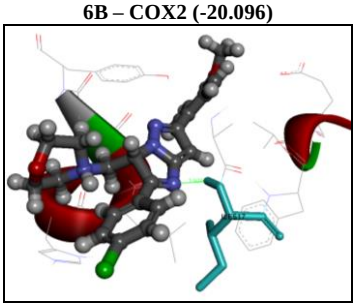
7C – COX1 (-12.448)



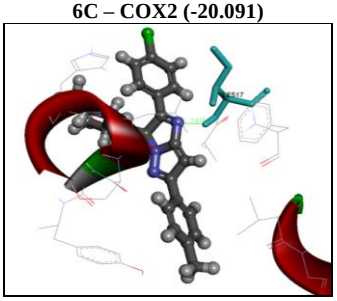
6B – COX2 (-20.096)



6C – COX2 (-20.091)



7B – COX2 (-18.244)



7C – COX2 (-18.281)

Figure 2: Binding mode of 6b, 6c, 7b, and 7c X-ray crystal structure of COX1 and COX2 enzyme
***Note: Balls and Stick model – Ligand molecule, Blue colour stick model – Interacted amino acid and Green colour dotted line – Hydrogen bond**

RESULT AND DISCUSSIONS

Chemistry

1H-imidazo [1,2-b] pyrazole have been synthesised using 5-aminopyrazole as one of the precursor or as intermediate step [**25-26**] which is also a precursor in synthesis. 2,6-disubstituted-1H-imidazo [1,2-b] pyrazole derivatives were synthesized by multi step reaction sequences by taking aryl ester which is converted to respective oxo-3-phenylpropanenitrile, which further reacts with hydrazine hydrate affording 5-amino pyrazole as a precursor. 5-amino pyrazole condenses with α-bromoarylketone to yield 1H-imidazo [1,2-b] pyrazole. 1H-imidazo [1,2-b] pyrazole in presence of catalytic amount of acetic acid and formaldehyde reacts with suitable secondary amine such as morpholine and pyrrolidine yielding corresponding Mannich base 6 and 7. The mechanism assumed to involve the attack of carbonyl carbon by the lone pair of electrons of the 5-amino pyrazole. Further the elimination of hydrogen bromide takes place when nitrogen of pyrazole ring attacks the methylene carbon followed by ring closure with a loss of water molecule leads to the 1H-imidazol [1,2-b] pyrazole moiety presented in scheme No I. Structures of all the newly synthesized compounds are well supported by spectral data such as IR, NMR, Mass and elemental analysis.

Spectral Data

The infra-red spectrum of compound 3a as an example lacks the carbonyl absorption band and showed the N-H absorption bands at 3240 cm^{-1} and 3180 cm^{-1}. Further a singlet peak at δ 13.8 along with a broad singlet peak at δ 4.3 in its ^{1}HNMR spectrum confirmed the ring closure leading to 5-amino pyrazole. In its Infra-red spectrum of compound 5a, a characteristic N-H absorption peak at the region 3240 cm^{-1} was observed. The ^{1}HNMR spectrum of 5a showed a singlet peak at δ 13.8 for N-H peak indicating the fused imidazole [1, 2-b] pyrazole ring. The ^{1}HNMR spectra of compound 6a showed two triplets at δ 2.5 and δ 3.6 along with a singlet peak for 2 H at δ 3.26, which indicated the presence of morpholine substitution with a methyl group. Mass spectrum of 6a showed molecular ion peak at m/z 392. The ^{1}HNMR spectrum of compound 7a showed a triplet at δ 2.1 for 4H and δ 1.6 for 4H which indicates for pyrrolidine ring protons. The mass spectral disintegration pattern showed the presence of isotope peaks for chlorine and bromine in the ration of 3:1 and 1:1 confirming the presence of 4-chlorophenyl and 4-bromophenyl derivatives.

PHARMACOLOGICAL EVALUATION

Acute Toxicity Studies

From the preliminary acute toxicity studies, it was observed that, the test compounds 6a, 6b, 6c, 7a and 7b, 7c have revealed good safety profile till the uppermost dose (2500 mg kg^{-1}). No mortality and behavioral changes of animals observed even after 24 h in compounds. But mortality was seen in the compounds 6d, 7d, 6e and 7e at the concentration 1000 and 1500 mg $kg^{-1}$and behavioral changes were also recorded for the same concentration of these compounds.

Anti-Inflammatory Activity

The synthesized compounds showed significant anti-inflammatory activity ranging from 9.08 to 61.19% inhibition of rat paw edema volume after 3 hour of treatment. The test compounds 6b, 7b, 6c, 7c possessing p-tolyl and 4-methoxyphenyl functional group along with 4-chlorphenyl substitution in the moiety exhibited 61.19, 56. 29, 59.45, and 55.32 % respectively, which is equipotent in comparison to the standard drug phenylbutazone exhibited inhibitions of 63.4%. Whereas, compounds 6a, 6e, 6f, 7a, 7e and 7f exhibited 51.60, 39.83, 42.80, 50.23, 31.27 and 38.65 percent of inhibition respectively, demonstrating moderate anti-inflammatory activity when compared to standard drug (phenylbutazone). The remaining compounds 6d and 7d were less potent among the series, which indicates that 4-bromphenyl substitution may reduce the activity. However, compounds 6e, 6f, 7e and 7f though contain p-tolyl and 4-methoxyphenyl substitution demonstrated moderate activity due to the presence of bromphenyl substitution. This reveals that in addition to bromphenyl substitution, pyrrolidine had less significant activity when compared to morpholine substitution at 3rd position of 1H-imidazo [1,2-b] pyrazole enhanced the activity. The percentage inhibition by newly synthesised compounds is tabulated in Table 1**.**

Analgesic Activity

According to the structure–activity relationship (SAR) studies, all newly synthesized compounds displayed moderate analgesic activity when compared with standard drug (anlagen). Interestingly, it was found that the compounds 6b, 7b with p-tolyl substitution at 6th position of pyrazole ring and compounds 6c, 7c with 4-methoxyphenyl along with 4-chlorophenyl substitution at 5th position of imidazole ring enhanced analgesic activity. But compounds having same substitution like 4-methoxyphenyl as in compound 6f, 7f and p-tolyl substitution in compounds 6e and 7e at 6th position of pyrazole ring along with 4-bromophenyl substitution at 5th position of imidazole ring showed moderate to weak analgesic property than their analogues. This indicates that bromphenyl suppressed analgesic activity of these compounds when compared to chlorphenyl substitution at 5th position of imidazole ring. Further it is observed that morpholine derivatives exerted significant activity when compared to pyrrolidine derivative. The percentage analgesia was calculated and tabulated in Table 2.

In Silico Molecular Docking Studies
Bioactivity Score Prediction

The Discovery Studio 3.5 small molecular pipeline was used to analyze drug likeness (Lipinski's Rule of Five, ADMET) of the synthesized molecules and the results obtained were tabulated in [Table 3-a, b] and [Figure 1]. It was found that all the synthesized compounds are within the acceptable range of using them as therapeutic drugs, demonstrating their potential as a drug-like molecule. All the synthesized compounds were subjected to LeadIT molecular docking studies.

Docking Studies

The molecular docking results of all the compounds are tabulated in [Table 4]. The results indicated that the compound 6b, 6c, 7b, and 7c had good affinity to the active site residue of COX-1 and COX-2 respectively, revealing their potential to inhibit these proteins. Binding mode of 6b, 6c, 7b, and 7c with crystal structure of COX-1 and COX-2 proteins is presented in [Figure 2]. Compound 6b and 6c had hydrogen bond interaction with THR355; compound 7b and 7c with MET522 with COX-1 protein, compound 6b and 6c had hydrogen bond interaction with LEU352 and ILE517 respectively; compound 7b and 7c with ILE517 interaction with COX-2.

CONCLUSION

This investigation proposes a convenient and useful method for the synthesis of biologically active 2-(4-halo aryl substituted)-3-morpholinomethyl-6-(phenyl substituted)-1H-imidazole [1,2-b]pyrazole derivatives (compound 6a-f) and 2-(4-halo aryl substituted)-3-(pyrrolidin-1-yl)methyl-6-(phenyl substituted)-1H-imidazole [1,2-b]pyrazole derivatives (compound 7a-f). Based on SAR studies, all the compounds have been evaluated for their analgesic and anti-inflammatory activities. Among the compounds synthesized, compound 6b, 6c, 7b and 7c exhibited significant anti-inflammatory and analgesic properties. They also demonstrated low toxicity and limted side effects on behaviour. Further, the in silico molecular docking studies showed that compound 6b, 6c, 7b, and 7c had good affinity to the active site residue of COX-2 and COX-1 respectively, revealing their potential to inhibit these target proteins. Thus 1H-imidazole [1,2-b]pyrazole derivatives reported through in this study are therfore expected to be of value in the treatment, alleviation and prophylaxis of a wide variety of disorders of chronic articular rheumantism.

Physical and spectral data of synthesized compounds

3-phenyl-1H-pyrazol-5-amine(3a)
Colorless crystal (ethanol); yield (86%); m.p. 172-174^{0}C; IR(KBr)υcm^{-1}: 3080 (Ar C-H), (pri N-H), 3230 (Sec N-H), 2675(C=N); ^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm: 7.4 (d, 2H, Ar H), 7.3-7.2 (m, 3H, Ar H), 8.3(bs, 1H, NH), 4.32 (bs, 2H, NH2),5.8 (s, 1H, pyrazole CH); ^{13}C NMR ($CDCl_3$)δ ppm: 156.7, 130.4,128.6, 134.2, 145.3, 90.2; Anal. Calcd for $C_9H_9N_3$, (%): C, 67.92; H, 3.47; N, 16.22; Found: C, 67.88; H, 3.2; N, 16.32.

3-p-tolyl-1H-pyrazol-5-amine(3b)
Colorless crystal (ethanol); yield (86%); m.p. 172-174^{0}C; IR(KBr)υcm^{-1}:3270, 3130, 2960, 2845,1620, 1570, 1510, 860; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm: 7.3 (d, 2H, Ar H), 7.1 (d, 2H, Ar H), 4.1(bs, 2H, NH2), 6.8 (bs, 1H, NH),5.62(s, 1H, pyrazole CH); Anal. Calcd for $C_9H_9N_3$, (%): C, 67.92; H, 3.47; N, 16.22; Found: C, 67.88; H, 3.2; N, 16.32.

3-methoxy--1H-pyrazol-5-amine (3c)
colorless crystal (ethanol); yield (86%); m.p. 172-174^{0}C; IR(KBr)υcm^{-1}: 3250, 3105, 2930, 2815, 1615, 1550, 1480; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm: 7.3(d, 2H, Ar H), 6.9(d, 2H, Ar H), 3.8(bs, 2H, NH2), 5.4(s, 1H, pyrazole CH), 6.5(bs,1H, NH);^{13}C NMR ($CDCl_3$)δ ppm: 156.7,148.4, 129.2, 114.8,126.4, 165.3, 54.3, 90.2; Anal. Calcd for $C_9H_9N_3$, (%): C, 67.92; H, 3.47; N, 16.22; Found: C, 67.88; H, 3.2; N, 16.32.

2-(4-chlorophenyl)- 6-phenyl -H-imidazo[1,2-b]pyrazole(5a)
Colorless solid (ethanol); yield (58%); m.p. 315-313^{0}C; IR (KBr) υcm^{-1}: IR(KBr)υcm^{-1}: 3230, 3180,2860, 1630,1280,740;^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm: 7.5(d, 2H, Ar H), 7.4(d, 2H, Ar H), 7.4-7.5(m, 5H, Ar H), 8.1(s, 1H, imidazole, CH),6.5(s, 1H, pyrazole CH),13.8(s,1H, NH);^{13}C NMR ($CDCl_3$)δ ppm:147.8, 141.6,136.5, 135.6, 133.4, 132.4, 129.4, 128.9, 127.8, 126.5, 96.4; Anal. Calcd for $C_{17}H_{12}N_3Cl$, (%): C,69.62; H,4.09; N,14.33; Found: C,68.35; H,4.02; N,14.07.

2-(4-chlorophenyl)- 6-p-tolyl-1H-imidazo[1,2-b]pyrazole(5b)
Colorless solid(ethanol); yield (68%); m.p. 318-316^{0}C; IR (KBr) υcm^{-1}: IR(KBr)υcm^{-1}: 3220,3160, 3060, 2845, 2245, 1620, 1270, 730;^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm: 13.3(s,1H, NH), 7.95(s, 1H, imidazole, CH), 7.5(d, 2H, Ar H), 7.4(d, 2H, Ar H), 7.3-7.1 (d, 5H, Ar H), 6.4(s, 1H, pyrazole CH), 2.34(s,3H, CH3); ^{13}C NMR ($CDCl_3$)δ ppm: 140.8,146.4, 138.5,135.8,134.8, 131.6, 130.3, 129.6, 128.6, 127.8, 126.3, 96.5, 23.4; Anal. Calcd for $C_{18}H_{14}N_3Cl$, (%): C,70.35; H,4.56; N, 13.68; Found: C, 70.14; H, 4.25; N, 13.17.

2-(4-chlorophenyl)-6-methoxy-1H-imidazo[1,2-b]pyrazole (5c)
Colorless solid(ethanol); yield (53%); m.p. 325^{0}C (dec); IR (KBr) υcm^{-1}: IR(KBr)υcm^{-1}: 3080,3280, 3080, 2860, 1520,1280, 1210, 720;^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm: 13.8(s,1H, NH), 7.8 (s, 1H, imidazole, CH),7.5(d, 2H, Ar H), 7.4(d, 2H, Ar H), 7.3(d, 2H, Ar H), 6.8(d, 2H, Ar H), 6.4(s, 1H, pyrazole CH), 3.6(s, 3H, CH3); ^{13}C NMR ($CDCl_3$)δ ppm: 160.5, 148.4, 140.3,135.8, 134.8, 131.3, 129.6, 128.6, 127.6, 125.3, 124.8, 119.6, 94.5, 60.4; Anal. Calcd for $C_{18}H_{14}N_3OCl$, (%): C, 66.87; H, 4.33; N, 13.0; Found: C,66.12; H, 4.23; N, 12.88.

2-(4-bromophenyl)-6-(4-phenyl-1H-imidazol [1,2-b]pyrazole(5d)
Brown solid (ethanol); yield (58%); m.p. 323^{0}C(dec); IR (KBr) υcm^{-1}= IR(KBr)υcm^{-1}: 3230, 3090, 3060, 2850, 1480,1280; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm13.8(s,1H, NH), 7.7(s, 1H, imidazole, CH), 7.5- 7.3(m, 5H, Ar- H), 7.3(d, 2H, Ar- H), 7.2(d, 2H, Ar- H), 6.5(s, 1H, pyrazole CH), ^{13}C NMR ($CDCl_3$)δ ppm:146.3, 142.8, 135.2, 132.8, 129.8, 127.8, 92.8,; Anal. Calcd for $C_{17}H_{12}N_3Br$, (%): C, 62.24; H, 3.45; N, 12.10; Found: C, 62.11; H, 3.23; N, 12.05.

2-(4-bromophenyl)-6-p-tolyl-1H-imidazol [1,2-b]pyrazole(5e)
Brown solid (ethanol); yield (58%); m.p. 328^{0}C(dec); IR (KBr) υcm^{-1}: IR(KBr)υcm^{-1}: 3230, 3090, 3060, 2850, 1480,1280; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm: 13.6(s,1H, NH), 7.75 (s, 1H, imidazole, CH), 7.3(d, 2H, Ar H), 7.2(d, 2H, Ar H), 7.1(d, 2H, Ar H),6.9(d, 2H, Ar H), 6.5(s, 1H, pyrazole CH), 2.34(s, 3H, CH3); ^{13}C NMR ($CDCl_3$)δ ppm: 147.3, 140.8, 133.2, 132.8, 130.4, 129.5, 127.3, 122.6, 90.8, 24.3; Anal. Calcd for $C_{17}H_{14}N_3Br$,(%): C; 61.89; H, 4.01; N, 12.03; Found: C; 61.38; H, 3.88; N, 11.92.

2-(4-bromophenyl)-6-(4-methoxyphenyl)-1H-imidazol [1,2-b]pyrazole(5f)
Brown solid (ethanol); yield (58%); m.p. 342^{0}C (dec); IR (KBr) υcm^{-1}: IR(KBr)υcm^{-1}: 3210, 3070, 3040, 2830, 1460,1280, 1210; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm: 13.4(s,1H, NH), 7.75(s, 1H, imidazole, CH), 7.3(d, 2H, Ar H), 7.2(d, 2H, Ar H), 7.1(d, 2H, Ar H), 6.8(d, 2H, Ar H), 6.5(s, 1H, pyrazole CH), 3.65(s, 3H, CH3); ^{13}C NMR ($CDCl_3$)δ ppm: 159.6, 147.8, 140.1, 134.2, 132.4, 130.4, 129.6, 127.3, 123.4, 116.5, 91.8, 60.3; Anal. Calcd for $C_{17}H_{14}N_3OBr$, (%): C, 55.89; H, 3.83; N, 11.50; Found: C, 55.25; H, 3.63; N, 11.35.

2-(4-Chlorophenyl)-3-(morpholinomethyl)-6-phenyl-1H-imidazo [1,2-b]pyrazole(6a)
Colorless solid (ethanol); yield (77%); m.p. 308-306^{0}C; IR(KBr)υcm^{-1}: 3280, 3080, 2860, 1520,1280, 780; ^{1}H NMR ($CDCl_3$, 300 MHz, TMS)δ ppm:11.5 (s, 1H, NH), 7.5-7.3 (m, 5H, ArH),7.4 (d, 2H, ArH), 7.3 (d, 2H, ArH), 6.6 (s, 1H, pyrazole CH), 2.5(t, 4H, J=4.1 Hz, C3,C5-H of morphine), 3.72 (t, 4H, J=4.1 Hz, C2, C6-H of morphine), 3.26 (s, 2H, CH2),; ^{13}C NMR ($CDCl_3$)δ ppm: 148.5, 136, 134, 131, 130, 129.3, 128.2, 127.8, 123.4, 93.7, 67.3, 53.3, 51.7; MS, m/z (%): 394(M+2, 11), 392(M^+, 33); Anal. Calcd for $C_{22}H_{21}N_4OCl$, (%): C, 67.34; H, 5.35; N, 14.28; Found: C, 67.88; H, 6.2; N, 14.32.

2-(4-chlorophenyl)-3-morpholinomethyl)-6-p-tolyl-1H-imidazo[1,2-b]pyrazole(6b)
Colorless solid (ethanol); yield (74%); m.p. 312-311^{0}C; IR(KBr) υcm^{-1}: 3215, 3040, 2845, 1510, 1270, 750; ^{1}H NMR (CDCl3, 300 MHz, TMS)δ ppm: 11.5 (s, 1H, NH), 7.5 (d, 2H, ArH), 7.4

(m, 2H, ArH), 7.3 (d, 2H ArH), 7.1 (m, 2H, ArH), 6.5 (s,1H, imidazole, CH), 3.6 (t, 4H, J=4.1 Hz, C2,C6-H of morphine), 3.5 (s, 2H, CH2), 2.3(s, 3H, CH_3),2.5 (t, 4H, J=4.1 Hz, C3, C5-H of morphine);Anal. Calcd for $C_{23}H_{23}N_4OCl$,(%): C, 67.98; H, 5.66; N, 13.79. Found: C, 67.45; H, 5.13; N, 13.56.

2-(4-Chlorophenyl)-6-(4-methoxyphenyl)-3-(morpholinomethyl)-1H-imidazo[1,2-b]pyrazole(6c)
Colorless solid; yield (73%); m.p. 321-320^{0}C; IR(KBr) υcm^{-1}: 3210, 3025, 2810, 1470, 1240, 730; ^{1}H NMR(CDCl3, 300 MHz, TMS)δ ppm: 11.5 (s, 1H, NH), 2.4 (t, 4H, J=4.1 Hz, C3,C5-H of morphine), 7.5 (d,2H, ArH),7.4 (d,2H, ArH), 7.3 (m, 2H, ArH), 6.9 (m, 2H, ArH),6.4(s,1H, pyrazole CH),3.7 (s,3H, OCH_3), 3.6 (t, 4H, J=4.1 Hz, C3,C5-H of morphine), 3.4 (s, 2H, CH2); ^{13}C NMR ($CDCl_3$) δ ppm: 162, 148.5,136, 134, 131, 130, 128, 127.7, 123.4, 113.8, 93.7, 57, 66.3, 53.8, 52.7; MS, m/z (%): 424(M+2, 13), 422(M+, 37); Anal. Calcd for $C_{23}H_{23}N_4O_2Cl$, (%): C, 67.98; H, 5.66; N, 13.79. Found: C, 67.65; H, 5.53; N, 13.76.

2-(4-bromophenyl)-3-morpholinomethyl)-6-phenyl-1H-imidazo [1,2-b]pyrazole(6d)
Brown amorphous (pet ether); yield (65%); m.p. 312-310^{0}C; IR(KBr) υcm^{-1}: 3220, 3080,1640, 1580, 530; ^{1}H NMR (CDCl3,300 MHz, TMS) δ ppm:11.3 (s, 1H, NH),7.89 (d, J = 9 Hz, 2H, ArH), 7.71 (d, J = 9 Hz, 2H, ArH), 7.5-7.3 (m, 5H, ArH),6.4 (s,1H, pyrazole CH),3.6 (t, 4H, J = 5 Hz, C2,C6-H of morphine), 3.5 (s,2H,CH_2), 2.58 (t, 4H, J = 5 Hz, C3, C5-H of morpholine); Anal. Calcd for (%):$C_{22}H_{21}N_4OBr$ C, 60.55; H, 4.81; N, 12.84. Found: C, 60.41; H, 4.26; N, 12.65.

2-(4-bromophenyl)-3-morpholinomethyl)-6-p-tolyl -1H-imidazo [1,2-b]pyrazole(6e)
Brown amorphous (pet ether); yield (58%); m.p.318-317^{0}C ; IR(KBr) υcm^{-1}: 3220 (NH str), 3080,1640, 1580; ^{1}H NMR (CDCl3, 300 MHz, TMS)δ ppm:11.3 (s, 1H, NH)2.4 (s, 3H, CH_3), 6.4 (s, 1H, pyrazole CH), 7.89 (d, J = 9 Hz, 2H, C2, C6-H, ArH),7.71(d, J = 9 Hz, 2H, C3, C5-H, ArH),7.4 (m, 2H, ArH),7.1 (m, 2H, ArH), 3.6 (t, 4H, J = 5 Hz, C2,C6-H of morphine CH2), 3.5 (s, 2H, CH_2),2.3 (t, 4H, J = 5Hz, C3,C5-H of morphine CH2); ^{13}C NMR ($CDCl_3$) δ ppm: 148.5, 139.4, 137.5, 136.2, 134.7, 132, 131, 130.3, 129.3, 127.8, 122, 93.7, 67.3, 53.3, 51.7, 23.4; Anal. Calcd for $C_{23}H_{23}N_4OBr$, (%): C, 61.33; H, 5.11; N, 12.44. Found: C, 61.34; H, 5.08; N, 12.23.

2-(4-bromophenyl)-6-(4-methoxyphenyl)-3-morpholinomethyl)-1H-imidazo[1,2-b]pyrazole(6f)
Brown amorphous (pet ether); yield(62%), m.p. 324^{0}C (dec); IR(KBr) υcm^{-1}: 3220 (N-H str), 3080,1640, 1580; 1H NMR (CDCl3, 300 MHz, TMS) δ ppm:11.4 (s, 1H, NH),7.89 (d, 2H, J = 9 Hz, C2, C6-H, ArH), 7.3 (d,2H, ArH), 7.71 (d, 2H, J = 9 Hz, C3, C5-H, ArH),6.9 (m, 2H, ArH),6.4 (s, 1H, pyrazole CH), 3.7 (s, 3H, CH_3), 3.6 (t, 4H, J = 6 Hz, C2,C6-H of morphine), 3.5 (s, 2H, CH_2),2.3 (t, 4H, J = 6 Hz, C3,C5-H of morphine CH_2),; ^{13}C NMR ($CDCl_3$) δ ppm: 157.9, 148.5, 147, 139.4, 136.2, 134.7, 132, 130.3, 128.2, 126.3, 122, 93.7, 113.8, 67.3, 53.3, 51.7, 56.8; Mass: m/z (%): 468(M+2, 21), 466(M+, 21); Anal. Calcd for $C_{23}H_{23}N_4O_2Br$,s(%): C, 59. 22; H, 4.93; N,12.0. Found: C, 59.21; H, 4.91; N, 12.03.

2-(4-chlorophenyl)-6-phenyl-3-((pyrrolidin-1-yl)methyl)-1H-imidazo [1,2-b]pyrazole(7a)
Colorless solid (chloroform); yield (72%); m.p. 306-304^{0}C; IR(KBr) υcm^{-1}: 3220, 3080,1640, 1580, 730; ^{1}H NMR (CDCl3,300 MHz, TMS) δ ppm:11.5 (s, 1H, N-H), 7.3(d, 2H, ArH), 7.4 (m, 2H, ArH),7.2-7.3 (m, 5H, ArH),6.8 (s,1H, pyrazole CH),4.1 (s, 2H, CH_2), 2.1 (t, 4H, C2, C6-H of pyrrolidine CH_2), 1.6 (t, 4H, C3, C4-H of pyrrolidine CH_2);Anal. Calcd for $C_{22}H_{21}N_4Cl$, (%): C, 70.2; H, 5.58; N, 14.89. Found: C, 70.12; H, 5.45; N, 14.78.

2-(4-chlorophenyl)-3-((pyrrolidin-1-yl)methyl)-6-p-tolyl-1H-imidazo[1,2-b]pyrazole(7b) Colorless solid (chloroform); yield (65%); m.p. 308-307^{0}C; IR(KBr) υcm^{-1}: 3220 (N-H str), 3080,1640, 1580, 730;^{1}H NMR (CDCl3,300 MHz, TMS) δ ppm:11.5 (s, 1H, NH),4.1 (s, 2H, CH_2), 7.5 (d, 2H, ArH), 7.4 (m, 2H, ArH), 7.3 (d, 2H, ArH), 7.1 (m, 2H, ArH), 6.7 (s, 1H, pyrazole CH),2.7 (t, 4H, C2, C6-H of pyrrolidine CH_2), 2.4 (s,3H, CH_3),1.5 (t, 4H, C3,C4-H of pyrrolidine CH_2);^{13}C NMR ($CDCl_3$) δ ppm: 139.4, 137.2, 148.4, 134.8, 132.4, 130.6, 129.4, 128.1, 127.4, 93.6, 52.4, 58.1, 29.3, 26.3, 23.4; Anal. Calcd for $C_{23}H_{23}N_4Cl$, (%): C, 70.40; H, 5.86; N, 14.28. Found: C, 70.32; H, 5.78; N, 14.23.

2-(4-chlorophenyl)-6-(4-methoxyphenyl)-3-((pyrrolidin-1-yl)methyl)-1H-imidazo[1,2-b]pyrazole(7c)
Colorless solid (chloroform); yield (72%); m.p. 314-312^{0}C; IR(KBr) υcm^{-1}: 3220, 3080,1640, 1580, 730; ^{1}H NMR (CDCl3,300 MHz, TMS) δ ppm:11.7 (s, 1H, NH), 7.5 (d, 2H, ArH), 7.3 (d, 2H, ArH), 7.4 (m, 2H, ArH), 6.7 (m, 2H, ArH), 6.6 (s, 1H, pyrazole CH), 4.1 (s, 2H, CH_2), 2.4 (t, 4H, C2, C6-H of pyrrolidine CH_2), 3.6 (s,3H, CH_3),1.6 (t, 4H, C3,C4-H of pyrrolidine CH_2);^{13}C NMR ($CDCl_3$) δ ppm: 158.9, 147.4, 138.4, 136.2, 134.8, 132.4, 129.2, 128.4,124.6.6, 126.1, 93.2, 56.6, 52.2, 58.4, 29.3, 26.6; Mass: 408(M+2, 13), 406(M+, 36); Anal. Calcd for $C_{23}H_{23}N_4OCl$,(%): C, 67.98; H,5.66 ; N, 13.79. Found: C, 67.75; H, 5.23; N, 13.34.

2-(4-bromophenyl)-6-phenyl-3-((pyrrolidin-1-yl) methyl)-1H-imidazo [1,2-b]pyrazole(7d)
Brown amorphous (chloroform); yield (52%); m.p. 308-306^{0}C; IR(KBr) υcm^{-1}: 3220, 3080,1640, 1580; ^{1}H NMR (CDCl3, 300 MHz, TMS) δ ppm:), 11.3 (s, 1H, N-H)7.89 (d, 2H, J = 9 Hz, C2, C6-H ArH), 7.71 (d, 2H, J = 9 Hz, C3, C5-H, ArH),7.3-7.5 (m, 5H, ArH),6.7 (s, 1H, pyrazole CH),3.7 (s, 2H, CH_2), 2.4 (t, 4H, C2, C6-H of pyrrolidine CH_2), 1.6 (t, 4H, C3,C4-H of pyrrolidine CH_2); Anal. Calcd for $C_{22}H_{21}N_4Br$, (%): C, 62.85; H,5.0; N,13.33. Found: C, 62.21; H, 4.75; N, 13.21.

2-(4-bromophenyl) -3-((pyrrolidin-1-yl) methyl)-6-p-tolyl-1H-imidazo [1,2-b]pyrazole (7e)
Brown amorphous(chloroform); yield (58%), m.p.312-311^{0}C; IR(KBr) υcm^{-1}: 3220, 3080,1640, 1580;^{1}H NMR (CDCl3,300 MHz, TMS) δ ppm:11.2 (s, 1H, NH),7.89 (d, 2H, J = 9 Hz C2, C6ArH), 7.71 (d, 2H, J = 9 Hz, C3, C5-H,ArH), 7.4 (d, 2H, ArH), 7.1 (m, 2H, ArH), 6.6 (s, 1H, pyrazole CH),3.7 (s, 2H, CH_2), 2.4 (t, 4H, C2, C6-H of pyrrolidine CH_2),1.6 (t, 4H, C3,C4-H of pyrrolidine CH_2), 2.3 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ ppm:193.2, 147.4, 145.8, 139.2, 138.4, 134.8, 136.2,.4, 130.7, 129.8, 128.9, 126.1, 51.2, 58.1, 26.4;Anal. Calcd for $C_{23}H_{23}N_4Br$, (%): C, 63.59; H, 5.29; N, 12.09. Found: C, 63.50; H, 5.23; N, 12.03.

2-(4-bromophenyl)-6-(4-methoxyphenyl)-3-((pyrrolidin-1-yl)methyl)-1H-imidazo[1,2-b]pyrazole(7f)
Brown amorphous (chloroform); yield (56%); m.p.318-316^{0}C; IR(KBr) υcm^{-1}: 3220, 3080,1640, 1580; ^{1}H NMR (CDCl3,300 MHz, TMS) δ ppm:11.3 (s, 1H, NH), 7.89 (d, J = 9 Hz, 2H, C2, C6-H, ArH),7.71 (d, J = 9 Hz, 2H, C3, C5-H, ArH),6.8 (d, 2H, ArH), 7.2 (d,2H, ArH),6.5 (s, 1H, pyrazole CH),3.7 (s, 2H, CH_2), 3.6 (s, 3H, CH_3), 2.4 (t, 4H, C2, C6-H of pyrrolidine CH_2), 1.6 (t, 4H, C3,C4-H of pyrrolidine CH_2), ^{13}C NMR($CDCl_3$) δ ppm: 161.4, 147.4, 139.2, 138.4, 136.2, 134.8,132.4, 129.3, 128.4, 125.7, 126.1, 113.7, 93.2, 58.1, 56.3, 51.2, 26.4; Mass: 452(M+2, 24), 450(M+, 24); Anal. Calcd for

C23H23N4OBr, (%): C, 61.33; H, 5.11; N, 12.44. Found: C, 61.23; H, 5.10; N, 12.32.

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