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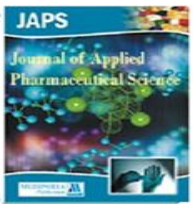

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Structure activity relationship studies of synthesised pyrazolone derivatives of imidazole, benzimidazole and benztriazole moiety for anti-inflammatory activity

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ABSTRACT

In the present investigation, a series of some synthesis and biological evaluation of some pyrazolone derivatives with imidazole, benzimidazole and benztriazole moiety were synthesized and tested for their anti-inflammatory activity in-vitro using celecoxib as a reference drug. Compound 8d was found to be the most potent derivative of the series with 75 %inhibition of inflammation.

Key words: Pyrazolone, imidazole, benzimidazole, benztriazole, %inhibition of inflammation.

INTRODUCTION

Inflammatory disease affect millions of people across the world leading to sufferings like economic loss & premature death as well as inflammatory lung diseases such as asthma, chronic obstructive pulmonary disorder & other diseases include allergic rhinitis, rheumatoid arthritis, osteoarthritis, inflammatory bowel diseases & psoriasis. Billions of dollars are being spent by pharmaceutical & biotechnology companies to identify & develop innovative therapeutics to treat such diseases. Over the last few years despite intensive global research, cures for pain & inflammation with no toxicity have still not been found. Keeping in view the potential for potent & suffer anti-inflammatory agents & in continuation of our efforts in search of bioactive molecules, it was thought of interest to design the novel new chemical entities containing heterocycle like substituted pyrazolone derivatives with imidazole, benzimidazole & benztriazole moiety (Burger et al., 1995). Inflammation is part of the body's natural defence system. It is a process whereby the body's cells & natural chemicals protect us from physical damage & infection from foreign substances such as bacteria & viruses. White blood cells or leukocytes are the body's major infection fighting cells. The primary objective of inflammation is to isolate, localized & eradicate foreign substances & repair damaged tissues. From the literature survey, in recent years pyrazole derivatives have attracted considerable interest because of their therapeutic and pharmacological properties. Several of them have been found to exhibit a wide spectrum of biological actions like anti-inflammatory, ulcerogenic, antibacterial, diuretic, analgesic, antiviral, antifungal, antimycobacterial activity etc. So it has been planned to synthesize a novel series of some pyrazolone derivatives with imidazole, benzimidazole and benztriazole moiety and to check their anti-inflammatory activity (Rainsford et al., 2001).

EXPERIMENTAL

The entire chemicals were supplied by S.D. Fine chem. (Mumbai), Finar Chem. Ltd (Ahmedabad) and LobaChemie. Pvt. Ltd. (Mumbai). Melting points were determined by open tube capillary method and were uncorrected. Purity of compounds were checked by thin layer chromatography (TLC) on silica gel G in solvent system hexane-ethyl acetate (3.5:1.5), the spots were located under iodine vapours or UV light. IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer using KBr. Mass spectra were obtained using 2010EV LCMS Shimadzu instrument. (Burger et al., 1995)

General procedure for Preparation of 1-[substituted-hydrazono-(phenyl)-methyl]-*H*-imidazole: (1b-3b)

(1g, 0.01mol) of imidazole was reacted with the mixture of 5% NaOH and (2ml, 0.01mol) benzoyl chloride and on constant shaking for 5-10 minutes produced the product. This was filtered out washed with cold water and recrystallized from ethanol. A solution of benzoylated imidazole in ethanol, hydrazine hydrate, phenyl hydrazine and semicarbazide was added dropwise. The reaction mixture was heated under reflux for 5-6h produced the product after cooling and pouring into crushed ice. The solid product was filtered and recrystallized from ethanol. (Burger et al., 1995)

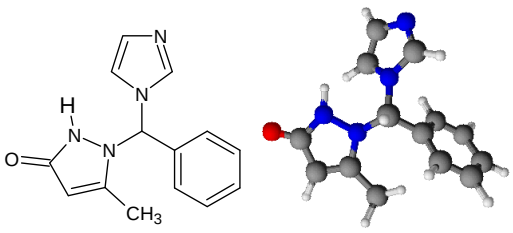
General procedure for Preparation of 1-[substituted-hydrazino-(phenyl)-methyl]-*H*-imidazole: (1c-3c)

A solution of hydrazono derivatives of 1-benzoyl-1*H*-imidazole in ethanol, amalgamated zinc and conc. Hydrochloric acid was added. The reaction mixture was heated under reflux for 8-10h and then cooled and poured onto crushed ice. The solid product was filtered and recrystallized from ethanol. (Burger et al., 1995)

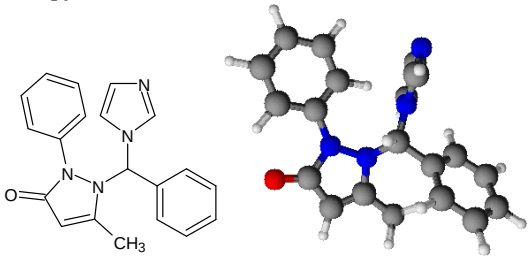
General procedure for Preparation of 1-[1*H*-imidazole-1-yl-(phenyl)-methyl]-5-methyl-1,2-dihydro-2-substituted-3*H*-pyrazol-3-one: (1d-3d)

Ethylacetoacetate (0.1mol) was added to a solution of hydrazino derivatives of 1-benzoyl-1*H*-imidazole (0.1mol) in ethanol. The reaction mixture was refluxed for 2-3h and after cooling it was poured into crushed ice. Then the separated solid mass was filtered, washed with water and crystallized from ethanol. (Burger et al., 1995)

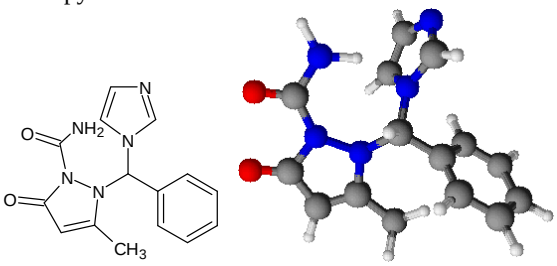
1d: 1-[1*H*-imidazol-1-yl(phenyl)methyl]-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one



2d: 1-[1*H*-imidazol-1-yl(phenyl)methyl]-5-methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one



3d: 2-[1*H*-imidazol-1-yl(phenyl)methyl]-3-methyl-5-oxo-2,5-dihydro-1*H*-pyrazole-1-carboxamide



General procedure for Preparation of 1-[substituted-hydrazono-(phenyl)-methyl]-*H*-benztriazole: (4b-6b)

(1g, 0.01mol) of benztriazole was reacted with the mixture of 5% NaOH and (2ml, 0.01mol) benzoyl chloride and on constant shaking for 5-10 minutes produced the product. This was filtered out, washed with cold water and recrystallized with ethanol. A solution of benzoylated benztriazole in ethanol, hydrazine hydrate, phenyl hydrazine and semicarbazide was added dropwise. The reaction mixture was heated under reflux for 5-6h produced the product and after cooling it was poured into crushed ice. The solid product was filtered and recrystallized from ethanol. (Burger et al., 1995)

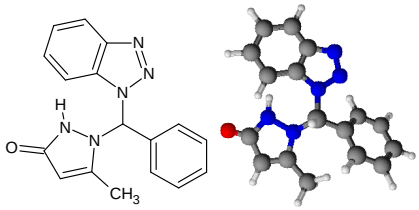
General procedure for Preparation of 1-[substituted-hydrazino-(phenyl)-methyl]-*H*-benztriazole: (4c-6c)

A solution of hydrazono derivatives of 1-benzoyl-1*H*-benztriazole in ethanol, amalgamated zinc and conc. Hydrochloric acid was added. The reaction mixture was heated under reflux for 8-10h and then cooled and poured onto crushed ice. The solid product was filtered and recrystallized from ethanol. (Burger et al., 1995)

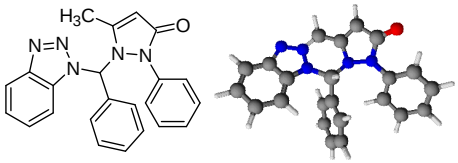
General procedure for Preparation of 1-[1*H*-benztriazole-1-yl-(phenyl)-methyl]-5-methyl-1,2-dihydro-2-substituted-3*H*-pyrazol-3-one: (4d-6d)

Ethylacetoacetate (0.1mol) was added to a solution of hydrazino derivatives of 1-benzoyl-1*H*-benztriazole (0.1mol) in ethanol. The reaction mixture was refluxed for 2-3h and after cooling it was poured onto crushed ice. Then, the separated solid mass was filtered, washed with water and crystallized from ethanol. (Burger et al., 1995)

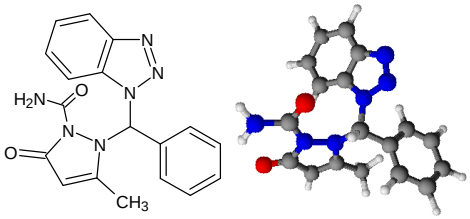
4d: 1-[1*H*-benzotriazol-1-yl(phenyl)methyl]-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one



5d: 1-[1*H*-benzotriazol-1-yl(phenyl)methyl]-4-methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one)



6d: 2-[1*H*-benzotriazol-1-yl(phenyl)methyl]-4-methyl-5-oxo-2,5-dihydro-1*H*-pyrazole-1-carboxamide



General procedure for Preparation of 1-[substituted-hydrazono-(phenyl)-methyl]-*H*-benzimidazole: (7b-9b)

(1g, 0.01 mol) of benzimidazole was reacted with the mixture of 5% NaOH and (2ml, 0.01mol) benzoyl chloride and on constant shaking for 5-10 minutes produced the product. This was filtered out washed with cold water and recrystallized with ethanol. A solution of benzoylated benzimidazole in ethanol, hydrazine hydrate, phenyl hydrazine and semicarbazide was added dropwise. The reaction mixture was heated under reflux for 5-6 produced the product which was cooled and poured into crushed ice. The solid product was filtered and recrystallized from ethanol. (Burger et al., 1995)

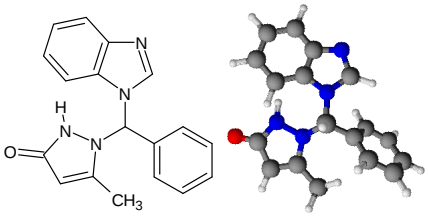
General procedure for Preparation of 1-[substituted-hydrazino-(phenyl)-methyl]-*H*-benzimidazole: (7c-9c)

A solution of hydrazono derivatives of 1-benzoyl-1*H*-imidazole in ethanol, amalgamated zinc and conc. Hydrochloric acid was added. The reaction mixture was heated under reflux for 8-10 h and then cooled and poured into crushed ice. The solid product was filtered and recrystallized from ethanol. (Burger et al., 1995)

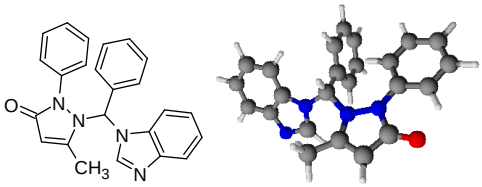
General procedure for Preparation of 1-[1*H*-benzimidazole-1-yl-(phenyl)-methyl]-5-methyl-1,2-dihydro-2 substituted-3*H*-pyrazol-3-one: (7d-9d)

Ethylacetoacetate (0.1mol) was added to a solution of hydrazino derivatives of 1-benzoyl-1*H*-benzimidazole (0.1mol) in ethanol. The reaction mixture was refluxed for 2-3 h and after cooling it was poured onto crushed ice. Then, the separated solid mass was filtered, washed with water and crystallized from ethanol. (Burger et al., 1995)

7d: 1-[1*H*-benzimidazol-1-yl(phenyl)methyl]-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one



8d: 1-[1*H*-benzimidazol-1-yl(phenyl)methyl]-5-methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one



9d: 2-[1*H*-benzimidazol-1-yl(phenyl)methyl]-3-methyl-5-oxo-2,5-dihydro-1*H*-pyrazole-1-carboxamide

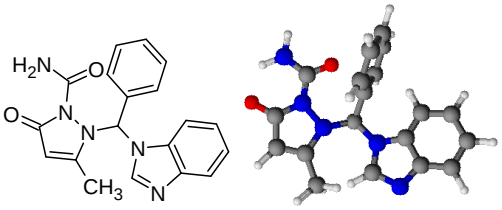


Table 1: Physical Characteristics of Synthesized Compounds

Compound Code	Molecular Formula	R	Molecular Weight (g/mol)	Melting Point (°C)	Yield (%w/w)	R_f Value
1d	$C_{14}H_{14}N_4O$	-H	254.28	180-182	72	0.50
2d	$C_{20}H_{18}N_4O$	$-C_6H_5$	330.38	158-160	73.68	0.68
3d	$C_{15}H_{15}N_5O_2$	$-CONH_2$	297.31	175-178	63	0.31

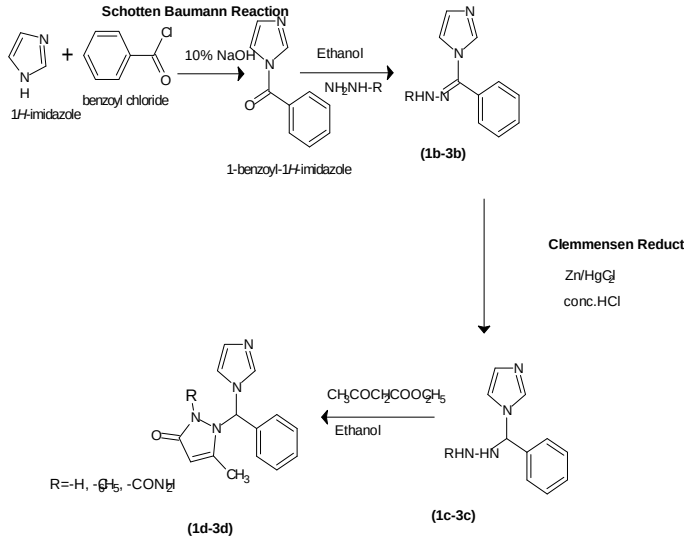
Mobile phase: ethyl acetate:hexane (1.5:3.5)

Table 2: Spectral data of synthesized compounds

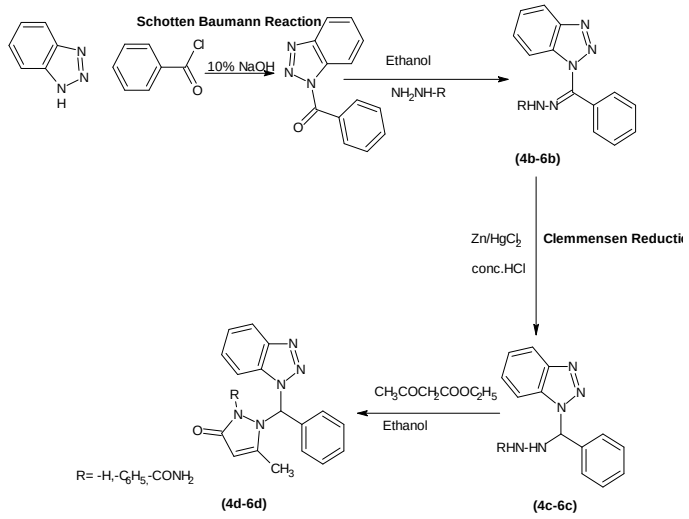
Compond code	UV (λmax, nm)	IR (υ, cm^{-1})	Mass (m/z)	NMR (δ, ppm)
1d	271.313	-NH (~3100), -C=O (1693.38)	255 (M^+)	7.4-8.02(m, 10H, Ar**H**), 2.14(s, 3H, -C**H**$_3$), 2.55(s, 1H, -C**H**Ar), 6.45(q, 1H, -CON**H**-)
2d	260.6	C-Cl (825), NH (3240), C=S (1215), NO_2 (1461, 1492)	329.8 (M^+)	7.43-7.92(m, 10H, Ar**H**), 3.32(s, 3H, -C**H**$_3$), 2.51(s, 1H, -C**H**Ar), 6.39(t, 1H, -CON**H**-)
3d	283.21	C-Cl (821, 1010), NH (3240), C=S (1207), C=N (1596)	296.5(M^+)	

Scheme of Synthesis

Scheme-1 (Furniss et al., 1998)



Scheme-2 (Sushma et al., 2007)



Anti-inflammatory activity

The anti-inflammatory activity of newly synthesized pyrazole derivatives were carried out using Carrageenan induced rat hind paw edema method (Dubois et al., 2004).

Method: Inhibition of Carrageenan induced inflammation in rat paw

Animals used: Swiss Albino Rats

No. of animals used: 6

Dose of compound: 200mg/kg

Dose of standard drug: 20mg/kg (celecoxib)

Route of administration: Oral (suspended in 1% tween-80 solution)

Mice were assigned into 11 groups of 6 animals each. They were marked with picric acid for individual animal identification. The animals were deprived of food overnight (allowed free access to water *ad libitium*) and synthetic compounds were administered once before 30 minutes the injection of Carrageenan. Dose volume not exceeding 0.5ml/100gm orally was administered. After 30 minutes of test compound administration, 0.1ml of 1%w/v of Carrageenan in normal saline was injected in to the sub-planter region of the left hind paw of mice. Immediately after the Carrageenan injection, the volume of its displacement was measured using plethysmometer. The reading was recorded at 0, ½, 1, 2, 3 hours the % inhibition of edema was calculated at the end of 3 hrs by using the formula (Winter et al., 1962).

$$\%\text{inhibition} = 100*(1-Vt/Vc)$$

Vt/Vc= edema volume in the mice treated with the test drug and control respectively.

Table 3: Physical Characteristics of Synthesized Compounds

Compound Code	Molecular Formula	R	Molecular Weight (g/mol)	Melting Point (^oC)	Yield (%w/w)	R_f Value
4d	$C_{17}H_{15}N_5O$	-H	305.33	200-202	68	0.55
5d	$C_{23}H_{21}N_5O$	$-C_6H_5$	459.54	192-194	70.48	0.67
6d	$C_{18}H_{18}N_6O_2$	$-CONH_2$	350.37	210-212	65.28	0.42

Mobile phase: ethyl acetate:hexane (1.5:3.5)

Table 4: Spectral data of synthesized compounds

Compond code	UV (λmax, nm)	IR (υ, cm^{-1})	Mass (m/z)	NMR (δ, ppm)
4d	266.4	-NH (3272.98), -C=O (1695.31)	305.1(M^+)	
5d	254.51	-NH (3261.40), -C=O (1668.31)	460.8 (M^+)	
6d	276	$-CONH_2$ (3263.33), -C=O(1647.10)	349.7(M^+)	7.67(m, 10H, Ar**H**), 7.98(m, 10H, Ar**H**), 2.54(s, 3H, $-CH_3$), 7.26(t, 2H, $-CONH_2$)

Scheme-3 (Pramila et al., 2007)

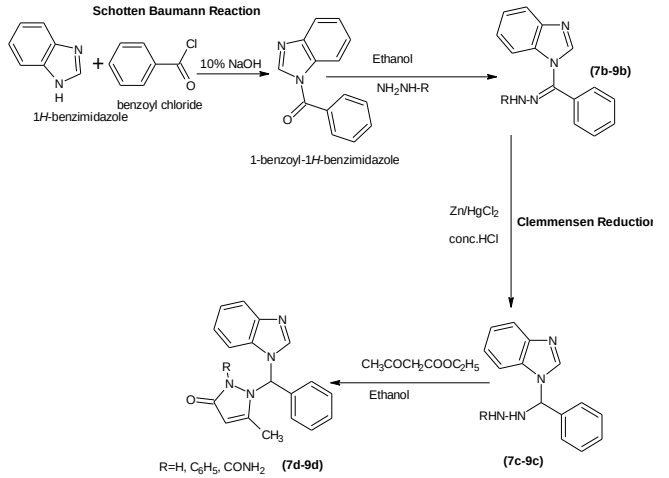


Table 5: Physical Characteristics of Synthesized Compounds

Compound Code	Molecular Formula	R	Molecular Weight (g/mol)	Melting Point (^{o}C)	Yield (%w/w)	R_f Value
7d	$C_{18}H_{16}N_4O$	-H	304.34	210-212	64.28	0.63
8d	$C_{24}H_{20}N_4O$	$-C_6H_5$	458.55	198-200	78.32	0.72
9d	$C_{19}H_{17}N_5O_2$	$-CONH_2$	347.37	218-220	63.00	0.48

Mobile phase: ethyl acetate:hexane (1.5:3.5)

Table 6: Spectral data of synthesized compounds

Compound code	UV (λmax, nm)	IR (υ, cm^{-1})	Mass (m/z)	NMR (δ, ppm)
7d	268.12	-NH (3319.26), -C=O (1670.24)	303.4(M^+)	
8d	250.78	-NH (3263.33), -C=O (1650.95),	457.6 (M^+)	8.09(m,10H,Ar**H**), 2.50(s,3H, -C**H**$_3$), 0.78-0.85(s,1H,N**H**-), 1.21(s, 1H, -N**H**-)
9d	286.35	$-CONH_2$ (3282.62), -C=O(1647.10)	348.1(M^+)	

Table 7: Screening of Anti-inflammatory activity in Albino mice (by rat paw edema method**)**

Compound Code	Inhibition of inflammation (mm)					% inhibition			
	0hr	0.5hr	1hr	2hr	3hr	0.5hr	1hr	2hr	3hr
Control	0.6	0.6	0.6	0.6	0.6	16.66	25	33.33	50
Standard (celecoxib)	0.5	0.4	0.3	0.2	0.1	33.33	50	58.66	83.33
1d	0.5	0.5	0.45	0.4	0.3	16.66	25	33.33	50
2d	0.4	0.4	0.35	0.3	0.25	33.33	41.66	50	58.33
3d	0.5	0.5	0.45	0.4	0.35	16.66	25	33.33	41.66
4d	0.5	0.5	0.5	0.5	0.5	16.66	16.66	16.66	16.66
5d	0.5	0.5	0.45	0.45	0.4	16.66	25	25	33.33
6d	0.5	0.5	0.5	0.45	0.45	16.66	16.66	25	25
7d	0.5	0.5	0.4	0.35	0.3	16.66	33.33	41.66	50
8d	0.5	0.4	0.3	0.25	0.15	33.33	50	58.33	75
9d	0.5	0.4	0.3	0.25	0.2	33.33	50	58.33	66.66

Acute toxicity studies

The acute toxicity of pyrazole derivatives was determined by using Albino Swiss mice (20-25g) before taking the anti-inflammatory activity. The animals were fasted for 24hrs prior to the experiment and up and down procedure (OECD Guideline no. 425) method of CPCSEA was adopted for acute toxicity studies (Lipnick et al., 1995). Newly synthesized compounds suspended in tween-80 was administered to the group of mice (n=3) up to dose level of 500mg/kg. Animals were placed in individual plastic cage and observed at least once daily for the first 30 minutes and periodically for 24 hours to observe for sign of toxicity (Mohan at al., 1998).

RESULTS AND DISCUSSION

The pharmacological screening of the synthesized compounds showed anti-inflammatory activity ranging from 16.66 to 75% inhibition of rat paw edema volume after 3hour, whereas the standard drug celecoxib (COX-2 inhibitor) showed 83.33% inhibition of rat paw edema volume after 3hour. The compound 8d was found to be nearly equipotent to celecoxib which is used as standard drug. Compounds 1d, 2d, 3d, 5d, 7d and 9d shown this activity but less potent than compound 8d and celecoxib.

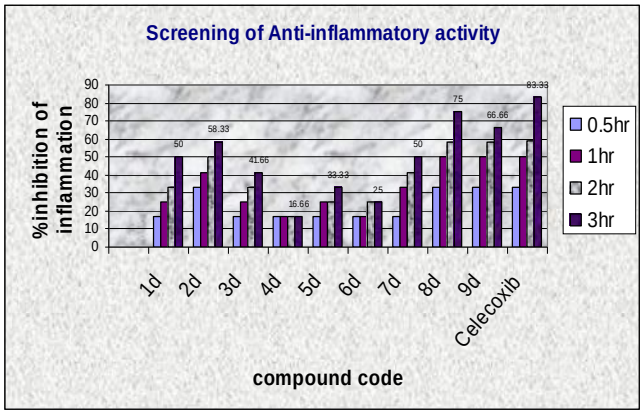
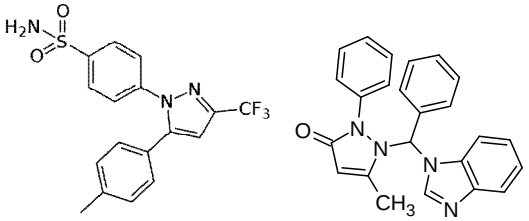


Fig. 1 Histogram of Anti-inflammatory activity

Table 8: log P of the final compounds

Test Compounds	Log P
1d	1.60
2d	1.91
3d	0.47
4d	3.34
5d	3.64
6d	2.20
7d	3.38
8d	3.69
9d	2.25
Celecoxib	3.90

Compound 4d, 6d was found to be least potent among the series. The structural similarity of the standard compound celecoxib (log P=3.90) and potent compound 8d (log P=3.69) has same pyrazole ring and methyl group (CH_3) is substituted by trifluoro methyl (CF_3). Compound 8d: 1-[1*H*-benzimidazol-1-yl(phenyl)methyl]-5-methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one showed maximum log P value among all the synthesised compounds have maximum anti-inflammatory activity compared with celecoxib as COX-2 inhibitor.



Celecoxib Compound: 8d

ACKNOWLEDGEMENT

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