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DOCKING, SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF 2-ALKYL SUBSTITUTED 1-(PYRIDIN-3-YL) METHANONE OXIME BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

A series of 2-substituted-benzimidazolyl-(pyridin-3-yl)-methanone oximes (**11-15**) were synthesized and tested for *in vivo* anti-inflammatory activity using carrageenan induced paw edema in rats. The compounds were also subjected to docking studies which indicated the importance of interactions of COX-2 receptor with synthesized derivatives. The results of docking studies and *in vivo* activity were same as and indicated compound **15** to be most active. Hence, benzimidazole nucleus can play a significant role in treatment and prevention of inflammatory disorders.

KEYWORDS: Benzimidazole; Synthesis; Anti-inflammatory; Docking; Cyclooxygenase.

INTRODUCTION

The benzimidazole ring is an important pharmacophore in modern drug discovery as it is a component of vitamin B and related to DNA base purines. The synthesis, chemistry and pharmacology of benzimidazoles have been of great interest to medicinal chemistry, because its derivatives possessed various biological activities.[1] Some of its derivatives are marketed as anti-bacterial agents (Clemizole), anti-helmintic (Mebendazole & Thiabendazole), anticancer (Pibenzimol), antiulcer (Omeprazole and Pentoprazole) and antihypertensive (Milfasartan).[2] Though a number of anti-inflammatory drugs containing benzimidazole nucleus are available yet there is an increasing demand for novel therapeutic agents with significant anti-inflammatory activity and fewer side effects.

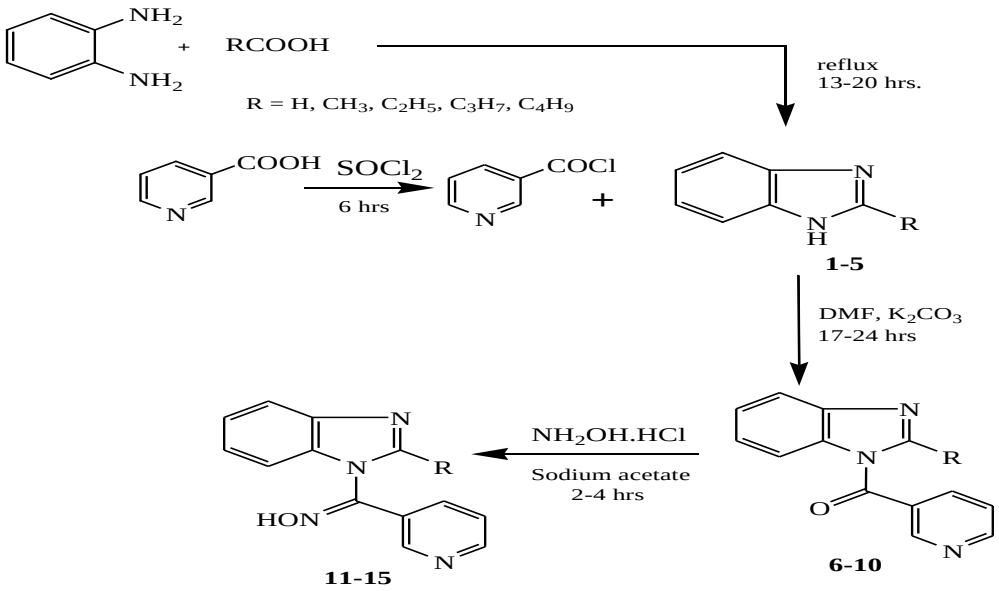
The oxime and oxime ether (RR'C=N-OR'') functional group is incorporated into many organic medicinal agents including some antibiotics (gemifloxacin mesylate, pralidoxime chloride)[3], cholinesterase reactivators (obidoxime chloride, asoxime chloride, trimedoxime), Anti-cancer agents (indirubin-3'-monoxime)[4], etc to enhance the drug action. Oximes are highly crystalline materials and oximation is very efficient method for characterization and purification of carbonyl compounds. These compounds represent a useful series of derivatives of carbonyl compounds.

Docking predicts the strength of association or binding affinity between two molecules using scoring functions and also predicts the binding orientation of small molecule drug candidates to their protein targets in order to envisage the affinity and activity of the small molecule in such a way that the free energy of the overall system is minimized.[5]

In the present study, we hereby report the synthesis, anti-inflammatory evaluation and docking studies of 2-substituted-benzimidazolyl-(pyridine-3-yl)-methanone oximes.

MATERIAL AND METHOD

The synthesis of compounds (**1-15**) is followed according to the general pathway depicted in **Scheme 1**. Melting points were determined by using open capillary method and are reported uncorrected. Reaction progress was monitored by thin layer chromatography on pre-coated plates using different solvent systems. The purity of synthesized compounds was ascertained by TLC using iodine vapours and UV chamber as visualizing agents. IR was recorded on a FT-IR Bruker (270-30) spectrophotometer. ^{1}H NMR spectra were recorded on Bruker 200 and 500 MHz spectrometers with tetramethylsilane (TMS) as the internal standard. Chemical shifts are expressed in parts per million (δ ppm); *J* values are given in Hertz.



Scheme 1. Synthesis of 2-substituted-benzimidazole, 2-substituted-benzimidazolyl-(pyridine-3-yl)-methanones and 2-substituted-benzimidazolyl-(pyridine-3-yl)-methanone oximes and their hydrochloride salts.

Synthesis of 2-substituted benzimidazoles (1-5)

Ortho-phenylenediamine (0.25 mol) and different carboxylic acid (viz. formic acid, acetic acid, propanoic acid, butyric acid and valeric acid) was placed in RBF and refluxed for 6-15 hours. The reaction mixture was cooled and basified with 20% sodium hydroxide solution. The crude product was dissolved in 95% ethanol. The mixture was digested with activated charcoal for 15-45 minutes and boiling water was then added to the filterate till slight turbidity appeared. The solution was made clear by addition of few drops of ethanol and kept for recrystallization. The product was obtained as needle shaped crystals.[6]

Synthesis of nicotinoyl chloride

Nicotinic acid (0.01 mol) was refluxed with thionyl chloride (10 mL) for 6 hours. The solvent was evaporated under reduced pressure. Needle shaped pale yellow crystals were formed and the synthesized compound was used immediately for the next step.[7]

Synthesis of 2-substituted-benzimidazolyl-(pyridin-3-yl)-methanones (6-10)

2-substituted-benzimidazole (**1-5**) (0.1 mol) was dissolved in N,N-dimethylformamide (DMF) (35.0 mL) and stirred vigorously with potassium carbonate (0.018 mol) at room temperature for 1-2 hours to get a suspension. Nicotinoyl chloride (0.1 mol) was dissolved in 20.0 mL of DMF and added drop wise to the above suspension with stirring over a period of 1 hour. The reaction was allowed to proceed further for 14-21 hours and the excess solvent was removed under vacuum. The residue was treated with 20.0 mL of dilute HCl and extracted with ethyl acetate. The organic layer was washed with brine, distilled water and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure to afford the product as a brownish amorphous solid.[8]

Synthesis of 2-substituted-benzimidazolyl-(pyridin-3-yl)-methanone oximes (11-15)

To a mixture of hydroxylamine hydrochloride (0.004 mol) and sodium acetate (0.002 mol) in 8 mL of water, 0.002 mol of (**6-10**) in 10 mL of ethanol (95%) was added in 50mL RBF. The prepared solutions were mixed and refluxed for 2-4 hours. The reaction mixture was filtered and kept on ice bath at room temperature, the crystals of oxime was obtained.

Evaluation of anti-inflammatory activity

The anti-inflammatory activity was performed by using carrageenan-induced paw edema model in rats (AEC No. LSCP/2070/866). In this method, acute inflammation was produced by the administration of 0.1 mL of 1% (w/v) of carrageenan (Sigma Co.) in the sub plantar region of left hind paw of rat. The standard drug and test compounds (40mg/kg i.p.) were administered 30 min before the carrageenan injection. In this study piroxicam was taken as standard anti-inflammatory agent.[9] The standard and test samples were dissolved in dimethylsulfoxide (DMSO) to give a concentration of 8mg/mL. The volume of the paw was measured immediately and also at the end of 3 hours and 24 hours after the administration of carrageenan.[10] The increase in paw volume was calculated using formula:

The increase in % inhibition of paw volume is calculated using formula:[11]

$$\% \text{ Inhibition} = \frac{\text{Increase in paw volume (control)} - \text{Increase in paw volume (test)}}{\text{Increase in paw volume (control)}} * 100$$

Docking studies

The original crystal structure of COX-II inhibitors (PDB code: 3HAB) was downloaded from Protein Data Bank and was prepared for docking by adding the missing hydrogens, correcting the bond orders and removing the water molecules beyond 5Å from the centroid of the

bound ligand using the protein preparation wizard of Schrodinger suite. The binding pocket of the protein was identified based upon the docked ligand. All the docking experiments were performed using Glide of Schrodinger suite on Linux based Workstation respectively. The ligand molecules were prepared using the Sketcher and Maestro windows of Schrodinger suite. In both the cases, the ligands were kept flexible and were evaluated based on the dock score and glide score (Schrodinger). The docking was done by making isomers of the title compounds and then docking scores was noted down and mean of all the isomers was calculated.

RESULTS AND DISCUSSION

Chemistry

Compounds **1-5** are the key intermediates for the synthesis of compounds **6-10**. The key intermediates, 2-substituted-benzimidazoles (**1-5**), were prepared by the condensation of o-phenylenediamine with various aliphatic acids (viz. formic acid, acetic acid, propanoic acid etc.). For the synthesis of 2-substituted-benzimidazolyl-(pyridine-3-yl)-methanones (**6-10**), the key intermediates (**1-5**) have been coupled with nicotinoyl chloride which was formed by the reaction of nicotinic acid with thionyl chloride. The next step was to convert carbonyl linker to oxime to form 2-substituted-benzimidazolyl-(pyridin-3-yl)-methanone oximes (**11-15**) in the presence of hydroxylamine hydrochloride and suitable base. The physiochemical characteristics of the synthesized compounds are presented in **Table 1**.

The structures of compounds (**1-15**) were assigned by IR and ^{1}H NMR spectroscopic data, which are consistent with the proposed molecular structures (**Table 2**). The appearance of strong out of plane deformation bands (C-C bending) at 741-752 cm^{-1} indicated the presence of 1,3-disubstituted benzene ring (benzimidazole) in compounds **1-15**. The presence of 3-substituted pyridine in structures of compounds **6-15** was confirmed by strong out of plane deformation bands (C-H bending) at 820-776 cm^{-1} which were visible from their IR spectra. Further, the appearance of strong C=O stretching bands at 1670-1630 cm^{-1} in the IR spectra of 2-substituted-benzimidazolyl-(pyridine-3-yl)-methanones (**6-10**) demonstrated the presence of tertiary amide linkage between the 3-substituted pyridine and the benzimidazole nucleus. The presence of oxime group in structures of compounds **11-15** was confirmed by a strong signal at 2248 cm^{-1} due to C=N stretch of oxime group and a broad band appears at 3600 cm^{-1} due to –OH group.

Compounds (**1-5**) showed a broad singlet at δ8.2-8.76 ppm corresponding to a proton of N-H of benzimidazole nucleus. Further, the appearance of ^{1}H NMR signals at δ7.5-8.6 ppm and δ7.21-7.64 ppm demonstrated the presence of C_4, C_7 and C_5, C_6 protons of benzimidazole nucleus.

The appearance of δ at 7.21-7.52 ppm corresponds to the benzimidazole nucleus whereas the nicotinic acid nucleus showed δ at 8.0-9.0 ppm. The absence of a broad singlet at δ8.2-8.76 ppm in ^{1}H NMR spectra of compounds (**6-15**) indicated the absence of free -NH group of benzimidazole nucleus. This confirms that compounds (**6-15**) are tertiary amides and not the physical mixture of nicotinic acid and compounds **1-5**. Therefore, this assures the reaction of nicotinoyl chloride with the secondary nitrogen of benzimidazole nucleus. Compounds **11-15** showed a singlet at δ12.0-12.3 corresponding to proton of -C=NOH linker and confirms the presence of oxime group in the structures (**11-15**).

Table 1: Physicochemical characteristics of synthesized compounds

Compound	Mol. Formula	Mol. Wt.	M.p. (^{o}C)	R_f value	Yield (%)
1	$C_7H_6N_2$	118.14	172	0.53	82
2	$C_8H_8N_2$	132.16	176	0.91	80
3	$C_9H_{10}N_2$	146.19	172	0.63	82
4	$C_{10}H_{12}N_2$	160.22	149	0.90	70
5	$C_{11}H_{14}N_2$	174.24	110	0.86	72
6	$C_{13}H_9N_3O$	223.23	136	0.90	48
7	$C_{14}H_{11}N_3O$	237.26	120	0.79	51
8	$C_{15}H_{13}N_3O$	151.11	77	0.85	43
9	$C_{16}H_{15}N_3O$	265.31	93	0.86	47
10	$C_{17}H_{17}N_3O$	279.34	72	0.85	34.5
11	$C_{13}H_{10}N_4O$	238.24	120	0.68	40
12	$C_{14}H_{12}N_4O$	252.27	108	0.79	42
13	$C_{15}H_{14}N_4O$	266.12	69	0.80	38
14	$C_{16}H_{16}N_4O$	280.32	86	0.85	39
15	$C_{17}H_{18}N_4O$	294.35	63	0.58	28

Table 2: Spectral data of synthesized compounds

Compound No.	IR (KBr pellets) cm^{-1}	^1H NMR ($CDCl_3$) δ ppm
1	3114 (N-H str., benzimidazole), 1586 (C=C str., Ar), 3062 (C-H str., Ar)	8.78 (s, 1H, NH of benzimidazole), 8.67 (s, 1H, CH of imidazole), 7.20-7.56 (m, 4H, benzimidazole)
2	3098 (N-H str., benzimidazole), 1553 (C=C str., Ar), 3059 (C-H str., Ar), 1384 (-CH_3 bending)	8.50 (s, 1H, NH of benzimidazole), 2.89 (s, 3H, methyl), 7.3-7.8 (m, 4H, benzimidazole)
3	3050 (N-H str., benzimidazole), 1542 (C=C str., Ar), 2974 (C-H str., Ar), 1379 (-CH_3 bending), 1455 (-CH_2 bending)	8.39 (s, 1H, NH of benzimidazole), 1.43 (s, 3H, methyl), 2.86 (m, 2H, -CH_2), 7.35-7.60 (m, 4H, benzimidazole)
4	3082 (N-H str., benzimidazole), 1542 (C=C str., Ar), 2980 (C-H str., Ar), 1315 (-CH_3 bending), 1452 (-CH_2 bending)	8.65 (s, 1H, NH of benzimidazole), 1.30 (s, 3H, methyl), 2.1 (m, 2H, -CH_2), 2.80 (2H, t, - CH_2), 7.18-7.40 (m, 4H, benzimidazole)
5	3080 (N-H str., benzimidazole), 1550 (C=C str., Ar), 2980 (C-H str., Ar), 1320 (-CH_3 bending), 1450 (-CH_2 bending)	8.40 (s, 1H, NH of benzimidazole), 0.9 (s, 3H, methyl), 1.8 (m, 2H, -CH_2), 2.80 (2H, t, - CH_2), 2.20 (2H, m, - CH_2), 7.1-7.52 (m, 4H, benzimidazole)
6	1586 (C=C str., Ar), 3092 (C-H str., Ar), 1680 (C=O, ter. amide), 832 (C-H, 3-sub. Pyridine)	8.09 (s, 1H, CH of imidazole), 7.21-7.52 (m, 4H, benzimidazole), 7.90-8.88 (m, 4H, pyridine ring)
7	1553 (C=C str., Ar), 3114 (C-H str., Ar), 1637 (C=O, ter. amide), 1378 (-CH_3 bending), 832 (C-H, 3-sub. Pyridine)	2.51 (s, 3H, methyl), 7.35-7.60 (m, 4H, benzimidazole), 7.8-8.86 (m, 4H, pyridine ring)
8	1588 (C=C str., Ar), 3053 (C-H str., Ar), 1637 (C=O, ter. amide), 1378 (-CH_3 bending), 1456 (-CH_2 bending) 824 (C-H, 3-sub. Pyridine)	1.43 (t, 3H, methyl), 2.52 (m, 2H, -CH_2), 7.55-7.73 (m, 4H, benzimidazole), 7.91-8.89 (m, 4H, pyridine ring)
9	1579 (C=C str., Ar), 3053 (C-H str., Ar), 1670 (C=O, ter. amide), 1378 (-CH_3 bending), 1456 (-CH_2 bending), 799 (C-H, 3-sub. Pyridine)	1.08 (t, 3H, methyl), 1.88 (m, 2H, -CH_2), 2.56 (t, 2H, -CH_2), 7.24-7.56 (m, 4H, benzimidazole), 7.92-8.89 (m, 4H, pyridine ring)
10	1550 (C=C str., Ar), 3140 (C-H str., Ar), 1670 (C=O, ter. amide), 1325 (-CH_3 bending), 1452 (-CH_2 bending) 832 (C-H, 3-sub. Pyridine)	0.91 (t, 3H, methyl), 1.43 (m, 2H, -CH_2), 1.85 (m, 2H, -CH_2), 2.97 (t, 2H, -CH_2), 7.57-7.82 (m, 4H, benzimidazole), 7.93-8.89 (m, 4H, pyridine ring)
11	1586 (C=C str., Ar), 3062 (C-H str., Ar), 2250 (C=N, oxime), 945 (N-O oxime), 3600 (-OH) 832 (C-H, 3-sub. Pyridine)	12.3 (s, 1H, -C=NOH), 8.69 (s, 1H, CH of imidazole), 7.21-7.52 (m, 4H, benzimidazole), 7.91-8.98 (m, 4H, pyridine ring)
12	1553 (C=C str., Ar), 3114 (C-H str., Ar), 1378 (-CH_3 bending), 2245 (C=N, oxime), 948 (N-O oxime), 3600 (-OH), 836 (C-H, 3-sub. Pyridine)	12.1 (s, 1H, -C=NOH), 2.89 (s, 3H, methyl), 7.35-7.60 (m, 4H, benzimidazole), 7.97-8.98 (m, 4H, pyridine ring)
13	1588 (C=C str., Ar), 3053 (C-H str., Ar), 1378 (-CH_3 bending), 1456 (-CH_2 bending), 2253 (C=N, oxime), 940 (N-O oxime), 3520 (-OH), 824 (C-H, 3-sub. Pyridine)	12.3 (s, 1H, -C=NOH), 1.43 (s, 3H, methyl), 2.97 (m, 2H, -CH_2), 7.55-7.73 (m, 4H, benzimidazole), 7.98-8.98 (m, 4H, pyridine ring)
14	1579 (C=C str., Ar), 3053 (C-H str., Ar), 1375 (-CH_3 bending), 1459 (-CH_2 bending), 2248 (C=N, oxime), 948 (N-O oxime), 3520 (-OH), 799 (C-H, 3-sub. Pyridine)	12.2 (s, 1H, -C=NOH), 1.04 (s, 3H, methyl), 1.88 (m, 2H, -CH_2), 2.92 (m, 2H, -CH_2), 7.24-7.56 (m, 4H, benzimidazole), 7.91-8.98 (m, 4H, pyridine ring)
15	1550 (C=C str., Ar), 3140 (C-H str., Ar), 1325 (-CH_3 bending), 1410 (-CH_2 bending), 2250 (C=N, oxime), 945 (N-O oxime), 3600 (-OH), 834 (C-H, 3-sub. Pyridine)	12.3 (s, 1H, -C=NOH), 0.91 (s, 3H, methyl), 1.43 (m, 2H, -CH_2), 1.85 (m, 2H, -CH_2), 2.97 (m, 2H, -CH_2), 7.57-7.82 (m, 4H, benzimidazole), 7.91-8.98 (m, 4H, pyridine ring)

Anti-inflammatory activity

The synthesized compounds were screened for their anti-inflammatory activities by using carrageenan induced rat paw edema model using Piroxicam as standard drug. The results of anti-inflammatory studies are presented in **Table 3**. In this study carrageenan significantly increased the volume of left hind paw of rats. Pretreatment was done with five test compounds (**11-15**), one compound (**15**) of which showed the significant ($p < 0.05$) preventive effect on the development of carrageenan induced edema after 3 hours and 24 hours and the compound (**12**) does not show any significant effect at 3 hours as well as 24 hours. The compounds (**11**) and (**13**) showed the significant activity after 24 hours. Piroxicam (40mg/kg; i.p.) used as a standard drug prevented the development of paw edema highly significantly ($p < 0.01$) (**Table 3**).

Table 3: Anti-inflammatory activity of title compounds (11-15)

Group	Increase in paw volume (mm)		% Inhibition	
	3 hour	24 hour	3 hour	24 hour
Control	7.19 ± 0.26	6.82 ± 0.24	-	-
Standard	3.57 ± 0.20**	2.04 ± 0.15**	50.30%	70.00%
11	6.33 ± 0.33#	5.23 ± 0.39*	11.90%	23.01%
12	6.53 ± 0.28#	5.48 ± 0.45#	9.50%	19.00%
13	6.26 ± 0.68#	5.31 ± 0.56*	12.90%	22.10%
14	6.21 ± 0.45#	5.11 ± 0.36*	13.00%	25.03%
15	4.50 ± 0.45*	3.36 ± 0.35*	37.00%	52.00%

Piroxicam used as standard drug. Values are expressed as Mean ± SEM,*p < 0.05 in comparison to control (n=6).

Docking analysis

In order to identify the binding affinity between synthesized compounds and their protein targets, docking studies of the title compounds were performed. According to docking studies of title compounds (**11-15**) as shown in **scheme 1**, compound **15** was found to be most potent as anti-inflammatory (**Table 4**). It was compared with piperidine fused benzimidazole which is already docked in receptor (3HAB). The docking score of compound **15** was less than piperidine fused benzimidazole due to the formation of hydrogen bonding between receptor and drug (shown by yellow colour). The greater the number of H-bonds more will be the binding affinity to receptor. The standard compound had formed six hydrogen bonds (**Fig. 1**) where as compound **15** was found to form only two hydrogen bonds with the receptor protein (**Fig. 2**).

Table 4: Docking scores of title compounds (11-15)

S.No.	Name of compound	Docking score
1.	Reference (piperine fused benzimidazoles)	-8.227210
2.	11	-5.402597
3.	12	4.816428
4.	13	-5.519028
5.	14	-5.581744
6.	15	-6.107430

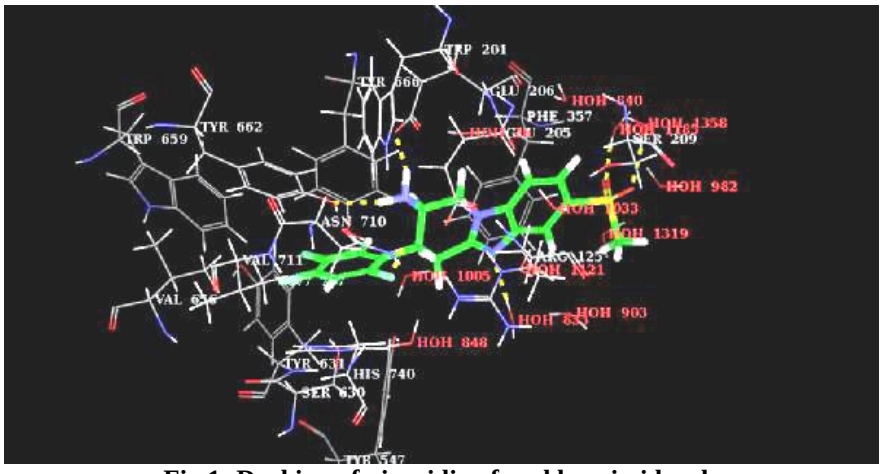


Fig 1: Docking of piperidine fused benzimidazole

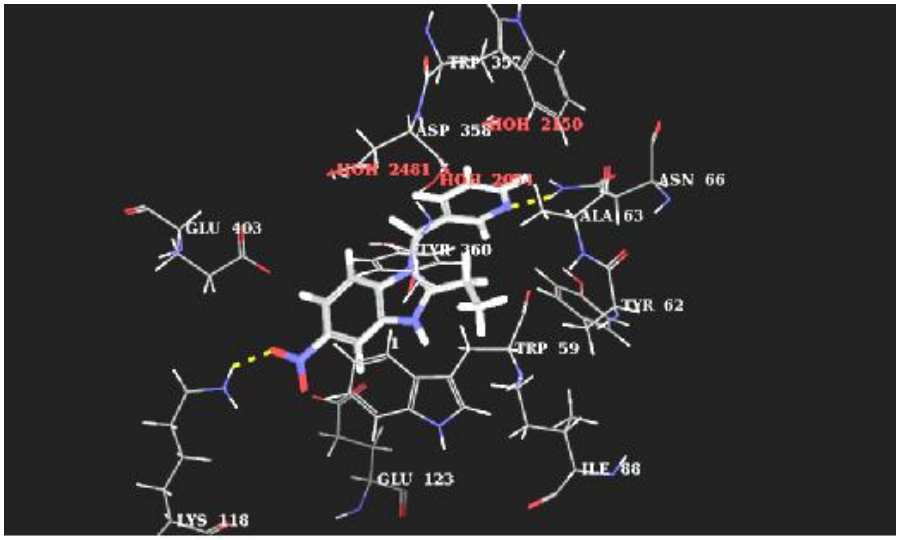


Fig 2: Docking of compound (15)

Summary

Summarizingly, a series of substituted benzimidazole derivatives (**1-15**) have been synthesized successfully in appreciable yields and screened for their anti-inflammatory activity. The result of docking studies and biological studies was found to be same i.e. the compound (**15**) was found to be most active. Thus, it may be concluded that the derivatives of benzimidazole nucleus can play a significant role in prevention and treatment of various inflammatory disorders.

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