

## CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF NEWLY SYNTHESIZED BENZIMIDAZOLYL COMPOUNDS WITH DOCKING STUDIES

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### ABSTRACT

In an effort to establish new candidates with improved antimicrobial activity, [2-(1H-benzo[d]imidazol-2-yl)-phenyl]-benzylidene amine and 2-[2-(1H-benzimidazol-2-yl)-phenylimino]-methyl phenol were synthesized, characterized and evaluated for antimicrobial activity. The desired compounds were synthesized by the condensation of benzene-1, 2-diamine with 2-aminobenzoic acid. Compounds were characterized by IR, <sup>1</sup>H-NMR, <sup>13</sup>C- NMR and elemental analysis. They were also screened for antimicrobial activity against *Staphylococcus aureus* and *Aspergillus niger*. Docking was performed by using DICLOFENAC bound to COX2 to understand the binding preference of the synthesized compounds with target protein. The compound [2-(1H-benzo[d]imidazol-2-yl)-phenyl]-benzylidene amine shows higher antimicrobial activity and is found to fit well with the binding sites of

the target protein.

**KEYWORDS:** Synthesis, Antimicrobial, Docking, benzene-1, 2-diamine, 2-aminobenzoic acid.

### INTRODUCTION

Heterocycles form by far the largest of classical divisions of organic chemistry and are of immense importance biologically and industrially. The majority of pharmaceuticals and biologically active agrochemicals are heterocyclic while countless additives and modifiers used in industrial applications ranging from cosmetics, reprography, information storage and

plastics are heterocyclic in nature. One striking structural features inherent to heterocycles, which continue to be exploited to great advantage by the drug industry, lies in their ability to manifest substituents around a core scaffold in defined three dimensional representations. For more than a century, heterocycles have constituted one the largest areas of research in organic chemistry. They have contributed to the development of society from a biological and industrial point of view as well as to the understanding of life processes and to the efforts to improve the quality of life. Among the approximately 20 million chemical compounds identified by the end of the second millennium, more than two-thirds are fully or partially aromatic and approximately half are heterocyclic.<sup>[1]</sup>

Benzimidazole nucleus is found in a variety of naturally occurring compounds such as vitamin B12 and its derivatives; it is structurally similar to purine bases. Benzimidazoles and its derivatives represent one of the most biologically active classes of compounds, possessing a wide spectrum of activities and these are well documented in the literature.<sup>[2]</sup> Several benzimidazole derivatives find applications that include antimicrobial<sup>[3]</sup>, antihypertensive<sup>[4]</sup>, anticancer<sup>[5]</sup> antiulcer, antifungal<sup>[6]</sup>, antihistamine activity<sup>[7]</sup>, herbicides, and other veterinary applications as promising drugs in different therapeutic categories. In our previous studies we reported the synthesis and antimicrobial 20–23 activities of a large series of benzimidazole derivatives. On the basis of these reports and as a continuation of our research program on benzimidazole derivatives, we report here the synthesis of novel benzimidazole derivatives to evaluate their antifungal properties.<sup>[8]</sup>

## Experimental Method

### Chemistry

All the chemicals and solvents used were of AR-grade obtained from Sigma- Aldrich, Sisco Research Laboratories, Qualingens, Hi-media, nice chemicals, Spectrochem and were used without further purification. All melting points were taken in open capillaries and are uncorrected. Elemental analysis was performed on a Perkin-Elmer analyzer. IR spectral were recorded in KBr on Shimadzu spectrometer, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR in DMSO-d6 on a Bruker AC-400 spectrometer using TMS as an internal standard. The microorganisms were obtained from National Chemical Laboratory, Pune. Thin-layer chromatography (TLC) was performed on pre-coated aluminium plates (silica gel 60F254, Merck).

### Synthesis of 2-(1H-benzo[d]imidazol-2-yl) benzene-amine

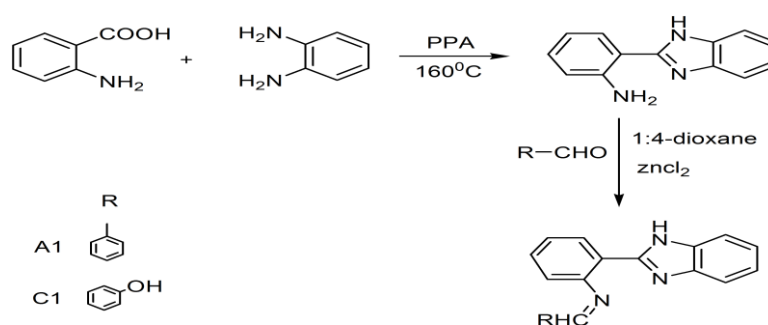
In a round bottom flask a mixture of benzene-1,2-diamine (0.01 mole, 5.4 g), 2-aminobenzoic acid (0.01 mole, 6.85 g) and polyphosphoric acid (PPA) (75ml) were heated at 160°C in an oil-bath for 3 hours. The reaction mixture was cooled to room temperature and poured into ice cold water (100 ml) and neutralized with aqueous ammonia. The separated solid was filtered, washed with water and dried to obtain the desired product. The crude product was purified by dissolving in 10% HCl and reprecipitated by the addition of aqueous ammonia. The product was purified by recrystallization from ethyl acetate.

### Synthesis of 2-(1H-benzo[d]imidazol-2-yl)-phenyl]-benzylidene-amine

In a round bottom flask, 2-(1H-benzo[d]imidazol-2-yl) benzene-amine (0.01 mole 0.90 g) was taken in 1:4-dioxane (9 ml) and benzaldehyde (0.01mole, 4 g) was added. To this mixture, a pinch of anhydrous zinc chloride was added and refluxed for about 5 hours. The solution was poured onto crushed ice. The product was filtered, washed with cold water, dried and purified by recrystallization from ethanol.

### Synthesis of 2-[2-(1H-Benzoimidazol-2-yl)-phenylimino]-methyl-phenol

In a round bottom flask, 2-(1H-benzo[d]imidazol-2-yl) benzene amine (0.01 mole, 0.3 g) was taken in 1:4-dioxane (3 ml) and Salicylaldehyde (0.01mole, 1.22 g) was added. To this mixture, a pinch of anhydrous zinc chloride was added and refluxed for about 5 hours. The solution was poured onto crushed ice. The product was filtered, washed with cold water, dried and purified by recrystallization from DMF (Scheme: 1).



## RESULTS AND DISCUSSION

### Compound 1 2-(1H-benzo[d]imidazol-2-yl) benzene-amine

Anal. Calcd. For  $\text{C}_{13}\text{H}_{11}\text{N}_3$ : C, 74.62; H, 5.30; N, 20.08; Found: C, 73.85; H, 05.78; N, 21.20; Yield % (76), ES (+) 209 (M+H); IR KBr ( $\text{cm}^{-1}$ ):  $\nu_{\text{C=N}}$ : 1662 $\text{cm}^{-1}$ ,  $\nu_{\text{C=C}}$ : 1388  $\text{cm}^{-1}$ ,  $\nu_{\text{C-NH}_2}$ : 3425  $\text{cm}^{-1}$ .

**Compound 2 2-(1H-benzo[d]imidazol-2-yl)-phenyl-benzylidene amine**

Anal. Calcd. For  $C_{20}H_{15}N_3$ : C, 80.78; H, 5.08; N, 14.13; Found: C, 80.11; H, 04.96; N, 14.01; Yield % (84), ES (+) 297 (M+H); IR KBr ( $cm^{-1}$ )  $\nu$  C-H (aryl) 3066  $cm^{-1}$ ,  $\nu$  C-N 1323  $cm^{-1}$ ,  $\nu$  N-H 3430  $cm^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.69 (Ar-H, multiplet),  $\delta$  3.4 (Ar-NH, singlet),  $\delta$  1.06 ( $-CH_3$ ).  $^{13}C$ -NMR:  $\delta$  39.55 (NH),  $\delta$  157(C=N).

**Compound 3 2-[2-(1H-Benzoimidazol-2-yl)-phenylimino] methyl-phenol**

Anal. Calcd. For  $C_{20}H_{15}N_3O$ : C, 76.66; H, 04.82; N, 13.41; O, 05.11 Found: C, 77.10; H, 04.96; N, 13.81; O, 4.98; Yield % (72), ES (+) 313 (M+H); IR KBr ( $cm^{-1}$ )  $\nu$  C-H (aryl) 2922  $cm^{-1}$ ,  $\nu$  C-N 1174  $cm^{-1}$ ,  $\nu$  N-H 3395  $cm^{-1}$ ,  $\nu$  OH 3697  $cm^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.59 (Ar-H, multiplet), 10.40 (Ar-OH, singlet),  $\delta$  3.4 (Ar-NH, singlet),  $\delta$  1.06 ( $-CH_3$ ).  $^{13}C$ -NMR:  $\delta$  39.55 (NH),  $\delta$  160 (OH),  $\delta$  152(C=N).

**Biological evaluation****Anti-microbial Activity**

The anti-microbial activity for the sample was carried out by Disc Diffusion Technique.<sup>[9]</sup>

The test microorganisms (*Staphylococcus aureus*, *Aspergillus Niger*) maintained by periodical subculturing on nutrient agar and sabouraud dextrose agar medium for bacteria and fungi respectively. The test microorganisms were obtained from National Chemical Laboratory (NCL), Pune and maintained by periodical sub culturing on nutrient agar and sabouraud dextrose agar medium for bacteria and fungi respectively. The effects produced by the sample were compared with the effect produced by the positive control (Reference standard ciprofloxacin 5  $\mu$ g/disc for bacteria; Nystatin 100 units/disc for fungi).

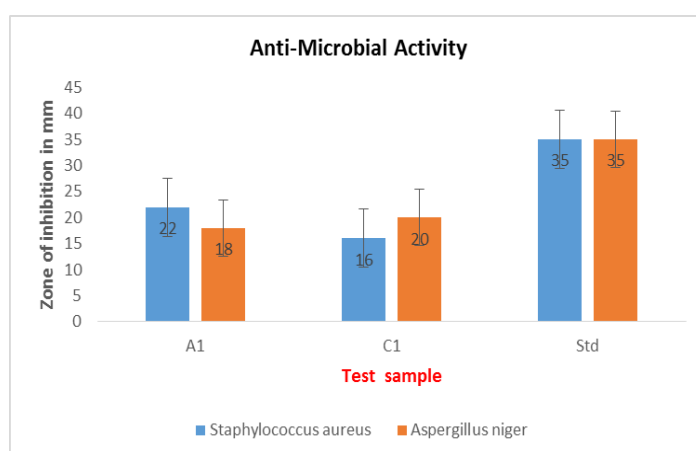
**Table 1: Anti-microbial activity of the synthesized compounds.**

S.No	Name of microorganisms	Zone of inhibition in mm			
		Test Sample		Solvent Control	Std
		A1	C1		
1	<b>Staphylococcus aureus (NCIM 2079)</b>	22	16	-	35
2	<b>Aspergillus niger (NCIM 105)</b>	18	20	-	35

Standard –Ciprofloxacin 5mg/disc for bacteria; Nystatin 100 units/disc for fungi; Solvent-DMSO.



**Fig 1&2 Zone of inhibition of synthesized benzimidazole against anti-microbial activity.**



**Fig. 3 Zone of inhibition of synthesized benzimidazole against anti-microbial activity.**

### Docking studies

Over activation of receptor tyrosine kinase (RTK) signalling pathways is strongly associated with carcinogenesis. So it is becoming increasingly clear that impaired deactivation of RTKs may be a mechanism in cancer. On this basis, we selected RTK as a biological target for docking study of synthesized compounds. The crystal structure of EGFR kinase domain (PDB ID: 1PXX) in complex with an irreversible inhibitors was obtained from the protein data bank.<sup>[10-13]</sup> The crude PDB structure of receptor was then refined by completing the incomplete residues. The crystallized ligand lying within the receptor was modified by assigning missing bond order and hybridization states. The side chain hydrogen was then added to the crystal structure and their positions were optimized up to the rms gradient 1 by aggregating the other part of the receptor.

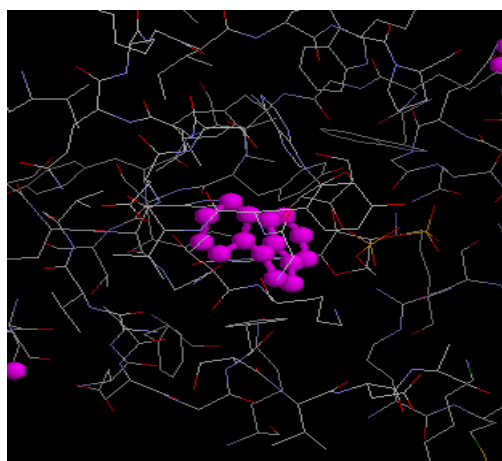
### Target Protein Structure

The structure of the target protein was downloaded from PDB.

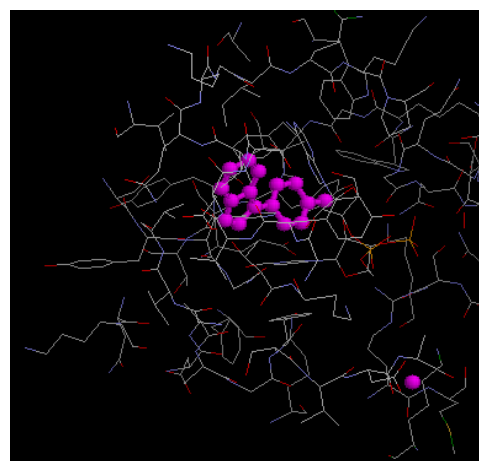
Target PDB: The crystal structure of DICLOFENAC BOUND TO COX2 PDB ID: 1PXX Structures of the compounds. The structures of the different compounds were drawn using ChemSketch software and the files were processed and saved as MOL files. The PDB structure with the ID 1FOL was loaded in to the iGEMDOCK software. The binding site for the target was prepared with the radius of 4 Å. The different ligands were drawn, prepared and uploaded into the software. The following parameters were set. Population size: 100, Generations: 50, Number of solutions: 2. the output path was set. 'Start docking' option was clicked and when docking was complete post analysis of the docked ligands was done. The predicted poses and the energy list of these poses will be outputted into the "best Pose" and "fitness.txt" of the output location, respectively. The predicted poses and scores of ligands are saved in the user defined output path. Fitness is the total energy of a predicted pose in the binding site. The empirical scoring function of iGEMDOCK is estimated as:  $\text{Fitness} = \text{vdW} + \text{Hbond} + \text{Elec}$ . Here, the vdW term is van der Waal energy. Hbond and Elec terms are hydrogen bonding energy and electro statistic energy, respectively.<sup>[14]</sup> The interaction residues and energy values of the synthesis compounds with the target.

**Table 2: Energy values of the synthesized compounds.**

S.No	Compound	Energy	VDW	HBond	Interaction
1	A1	-73.88	-71.85	-2.03	His 21, Ala54, Ser55, Tyr65
2	C1	-65.13	-54.12	-11.01	His 21, Tyr54, Ser55, Tyr65



**Fig.4 Interaction of A-1 with COX2.**



**Fig.5 Interaction of C-1 with COX2.**

## DISCUSSION

The synthesized compounds A1, C1 were prepared from anthranilic acid and *o*-phenylenediamine in the presence of polyphosphoric acid at 160°C through the formation of benzimidazoles phenylamine according to literature procedure. The structural assignments of



compounds (A1, C1) was confirmed by spectroscopic analysis. In IR spectrum of compound A1 exhibited characteristic band absorption for C-H (aryl), C-N and N-H groups at 3066, 1323 and 3430  $\text{cm}^{-1}$  respectively. The IR spectrum of compound C1 exhibited characteristic band absorption for C-H (aryl), C-N, N-H and O-H groups at 2922, 1174, 3395 and 3697  $\text{cm}^{-1}$  respectively. The  $^1\text{H}$  NMR Spectra of the synthesized compounds in this work are examined. The compound A1 shows two signals at  $\delta$  7.5 and 7.2 ppm indicating the presence of benzimidazole ring in the compound. The aromatic protons of benzene ring are confirmed by the presence of signals at  $\delta$  7.3, 7.7 and 7.4 ppm. The signals at  $\delta$  7.5 and 1.06 confirms the presence of N=CH and  $-\text{CH}_3$  groups respectively. The other compound C1 shows the following signals  $-\text{NH}$ , Ar-H, 3.4, 7.7. The signals in the range of  $\delta$  128-139 confirms the presence of aromatic carbon in the synthesized compounds. Both the compounds show moderate antibacterial activity when compared to standard drug. The compound 2-(1H-benzo[d]imidazol-2-yl)-phenyl]-benzylidene amine shows higher activity than the compound C1. From the results of docking, the compound A1 is found to fit well with the binding sites of the target protein. The compound A1 is found to have minimum energy of -73.88 respectively (Table 2). They also interacted with the residues of His 21, Ala54, Ser55 and Tyr65 of binding pocket. The compound efficiently inhibits the cyclooxygenase 2. Hence the compound can be used to treat inflammation but further research is needed to formulate it as a drug.<sup>[15]</sup>

## CONCLUSION

The present work is focussed on the synthesis characterization and antimicrobial activity of some benzimidazole compounds from o-phenylenediamine. The findings are furnished below. Two benzimidazole compounds (A1, C1) were prepared from o-phenylene diamine following this scheme (scheme: 1). both the compounds were characterized by IR spectral data. The IR spectra of the compounds provide the expected frequencies. The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR of the compounds provide the expected signals. A study of the antimicrobial activity was carried out and the results are given. Molecular docking study was also made and results are tabulated.

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