Synthesis of Benzimidazole Derivatives and their Biological Investigation

A dissertation submitted to the Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka in the partial fulfillment of the requirements for the degree of M. Phil

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Saikat Ranjan Paul

Author

Dedicated

To

My beloved Grandfather

Late Jagadish Chandra Paul

Contents

List of Figures

List of tables

Abstract

The study was designed to synthesize substituted benzimidazole derivatives from reactions between o-phenylenediamine and various aldehyde derivatives using water as reaction medium employing several surface active agents as catalyst at room temperature. At the same time to assume the biological activity of the synthesized molecules through molecular docking on Acetylcholine esterase enzyme model.

The current synthetic procedure was preceded by initial optimization of reaction condition for the synthesis of substituted benzimidazole derivatives. For this purpose we utilized o-phenylenediamine as starting and 4-Cl-benzaldehyde as aldehyde prototype, varying the equivalent mmol ratios such as 1:1 and 1:2 keeping different types of surfactants like such as benzalkonium chloride (BKC), sodium dodecyl sulfate (SDS), Tetra-n-butylammonium bromide (TBAB) and Tetra-n-butylammonium iodide (TBAI) in a fixed mmol equivalent proportion. All reactions are carried out at room temperature using water as reaction medium. With optimized reaction condition then several reactions were carried out between o-phenylenediamine and various aldehyde derivatives. Furthermore synthesized substituted benzimidazole derivatives were screened through molecular docking on Acetylcholine esterase enzyme to recognize the binding site and affinities for better inhibition with reference to the standard inhibitor pralidoxime.

All surfactants were found to be effective catalyst at room temperature providing good to moderate yields at varying ratios of o-phenylenediamine and 4-Cl benzaldehyde. We observed that at 1:1 ratio of reactants 2-substituted benzimidazole (2-(4-chlorophenyl)-1H-benzo[d]imidazole) is prominent product obtained. But at 1:2 ratios reaction condition favors 1,2-disubstituted benzimidazole (1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole). Among the four surfactants, Benzalkonium chloride shows excellent affinity to produce 2-substituted benzimidazole at both 1:1 and 1:2 ratios. Total of six benzimidazole derivative namely-2-(4-chlorophenyl)-1H-benzo[d]imidazole (Code: MS4ClB) and 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole (Code: DS4ClB); 4-(1H-benzo[d]imidazol-2-yl) phenol (Code: MSB4OH) and 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol (Code: DSB4OH); 2-phenyl-1H-benzo[d]imidazole (Code: MSBA) and 1-benzyl-2-phenyl-1H-benzo[d]imidazole (Code: DSBA) were obtained with good to moderate yield. All the products are confirmed by TLC checking with in house reference product and ^{1}H-NMR.

Binding affinity of 2-(4-chlorophenyl)-1H-benzo[d]imidazole to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -9 compared to -5.7 of pralidoxime. The product MS4ClB tends to interact with five amino acid residues present at the binding site namely val294, Tyr341, Tyr337, Tyr124 and Trp86. This interaction is due to pi to pi bond interaction with tryptophan and tyrosine residues, alkyl and alkyl to pi interaction with Valine residue at the binding site. This interaction is not sufficiently strong for prominent inhibitory effect on the enzyme. This binding interaction is also affected by the molecular orientation at the binding site. Docking study reveals that the molecule is oriented to the edge of the binding site where the aromatic system of the interacting amino acids faces in slightly different angle rendering the interaction much weaker. The binding site of the enzyme contains both H-bond donor and acceptor groups. The chloride substitution on the side chain renders the molecule to interact with the H-bonding accepting group at binding site. But bond distances are much longer to create a strong bonding. The molecules also possess bulkier chlorophenyl side chain which can interact with hydrophobic interaction at hydrophobic region of the binding site.

Binding affinity of 2-(4-chlorophenyl)-1H-benzo[d]imidazole to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -8.9 compared to -5.7 of pralidoxime. Docking of this molecule at binding site reveals the possible pi-anion interaction with Glu 292 residue, pi-pi stacked interaction with Tyr341 and Trp286 through benzyl side chain of the substitution. Docking study reveals that the benzyl side chain substituted at 1-possition is oriented to amino acid residue face as well as to H-bonding accepting site which enables this molecule to exhibit greater affinity than its mono substituted congener. But bond distance is much longer to create a strong bonding. The molecule also possesses bulkier chlorophenyl side chain which can interact with hydrophobic interaction at hydrophobic region of the binding site.

Binding affinity of 4-(1H-benzo[d]imidazol-2-yl) phenol to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -8.3 compared to -5.7 of pralidoxime. Molecular Docking reveals that molecule MSB4OH is oriented at the edge surfaces of most of the interacting amino acid residues and is oriented far from the H-bond accepting site of the enzyme. The molecule interacts with pi-donor H bond with Tyr 124 and tyr337. The molecule possesses hydroxyl group at side chain rendering weak interaction with hydrophobic region of the binding site.

Binding affinity of 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -8.3 compared to -5.7 of pralidoxime. Molecular Docking reveals that molecule DSB4OH is oriented at the edge

surfaces of most of the interacting amino acid residues and is oriented to the H-bond accepting site of the enzyme which promotes conventional H-bonding with Glu313 residue at binding pocket. The molecule possesses hydroxyl groups at side chain rendering weak interaction with hydrophobic region of the binding site.

Binding affinity of 2-phenyl-1H-benzo[d]imidazole to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -10.8 compared to -5.7 of pralidoxime. Molecular Docking reveals that this smaller molecule MSBA is oriented at the face surfaces of most of the interacting amino acid residues and is oriented to the H-bond donor site of the enzyme which promotes conventional H-bonding with Arg296 residue at binding pocket.

Binding affinity of 1-benzyl-2-phenyl-1H-benzo[d]imidazole to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -9 compared to -5.7 of pralidoxime. Molecular Docking reveals that this smaller molecule DSBA is oriented at the edge surfaces of most of the interacting amino acid residues and is not oriented to the H-bond donor site of the enzyme. Hence this molecule forms pi-pi weak interaction with amino acid residue like Trp86 and Tyr124 and Tyr337.this molecule possesses weak interpolated charge and much oriented to hydrophobic interaction.

Thus benzalkonium chloride is effective and environmentally benign alternative for synthetic purpose with easy workup procedure. Molecular docking reveals good binding affinities for the candidates synthesized in this study. There is lacking of formation of strong classical H-bonding among the candidates with the amino acid residues at the binding site of Acetylcholine esterase enzyme. Hydrogen bonding as well as hydrophobicity can be improved by substituting different groups on the benzene ring system on both benzimidazole heterocycles and on the side chain to render the candidates as good irreversible Acetylcholine esterase enzyme inhibitor of clinical importance.

Chapter-1

General Introduction

1.1. Introduction

Heterocyclic moieties are well known for their role in drug discovery, since the majority of therapeutic drugs contain a heterocyclic unit. Within the vast range of heterocycles, benzimidazoles were found to be prevalent structures employed in several areas such as materials science, for example, in fuel cells (Asensio, J. A. *et al*., 2010), or in the pharmaceutical industry. A successful example is Nexium (esomeprazole), a proton pump inhibitor used to treat peptic ulcers and gastroesophageal reflux disease, which became one of the most widely prescribed drugs (M. M. B. Marques *et al.*, 2011). In this chapter we will discuss about the basic chemistry of benzimidazole heterocyclic structure, synthetic methods most importantly very recent approaches using water as reaction media which is of great importance from green chemistry perspective, as well as their biological importance. At last we will review some synthetic approaches to find the rational for our current study.

1.2. Basic chemistry of benzimidazole heterocyclic structure

The ring system in which a benzene ring is fused to the 4,5-positions of imidazole is designated as benzimidazole. Although benzimidazole is the commonest name of the parent compound of the series, other names such as benzimidazole and 1,3-benzodiazole are often used. (Salahuddin *et al*., 2012). The various positions on the benzimidazole ring are numbered in the manner indicated with the imino function as number one (Figure-1.1). The benzimidazoles possessing free imino hydrogen are tautomeric systems. The two possible tautomeric forms (Figure-1.2) of benzimidazole (and of those of its derivatives possessing a plane of symmetry) are identical and a definite assignment of structure is possible (A.C Dash *et al*., 1998, R. Rastogi *et al*., 1979, S. Abuzar *et al*., 1980, B. A. Reddy, 2010).

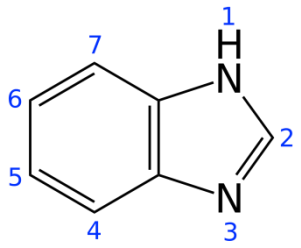


Figure- 1.1- 1*H*-benzimidazole

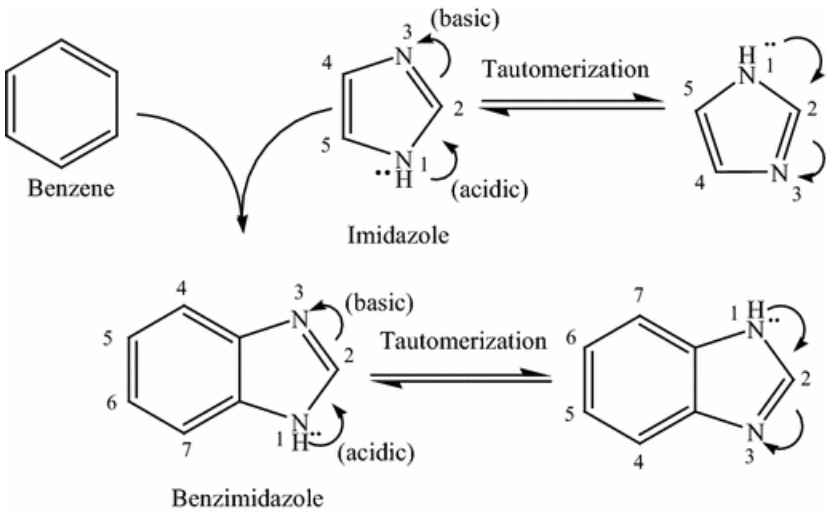


Figure-1.2: Tautomerism of benzimidazole

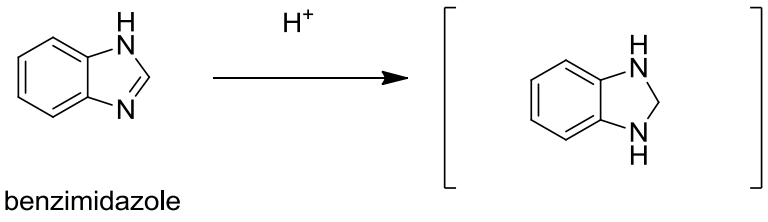


Figure- 1.3: Benzimidazole accepting proton

The benzimidazoles are predominantly basic compounds having the ability to form salts with acids (Figure- 1.3). Benzimidazole (pKa 5.5) is a basic considerably weaker than the imidazole (pKa 7.0). This difference in the basic strength is a reflection of the conjugation between the imidazole and benzene rings. Conjugation increases the number of contributing states in the resonance sense, thus enhancing the chemical stability of the molecule. A to G (Figure-1.4) represent the major contributions to the state of benzimidazole system. Structure D, E and G depicts the conjugation between the imidazole and benzene portions which may be responsible for the difference in basic strength between imidazole and benzimidazole.

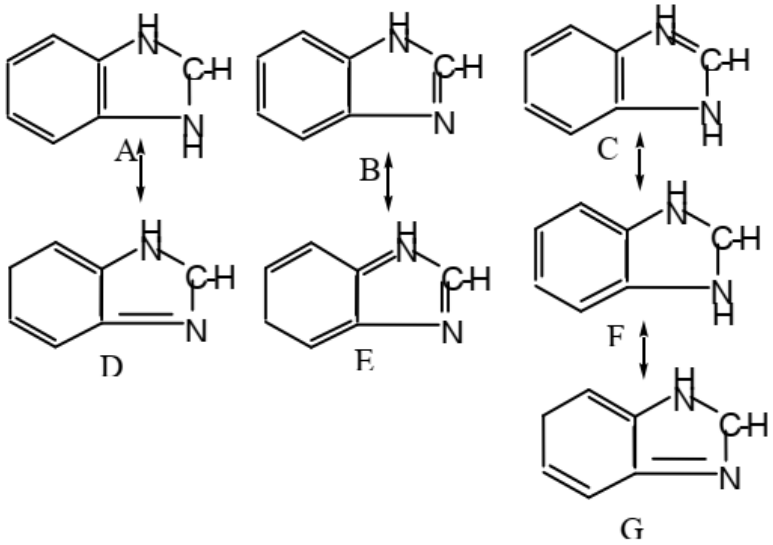


Figure-1.4: Major contributions to the state of benzimidazole system

1.3. Biological importance of substituted Benzimidazole derivatives

The benzimidazole core is classified by medicinal chemists as one of the 'privileged sub-structures' for drug design. Benzimidazole derivatives with substituents particularly at N-1 and/or C-2 positions have received paramount interest in recent times because of their broad range of biological functions and pharmacological applications.

Synthesis and biological analysis of assorted 2-substituted benzimidazole derivatives have resulted within the discovery of gastric antacid, acid, rabeprazole, and pantoprazole. In recent years, the synthesis of novel benzimidazole derivatives remains a focus of clinical analysis and notably attention has progressively been given to the synthesis of 2-substituted benzimidazole derivatives (Keri RS *et al*., 2015). The 2-subtitued benzimidazole scaffold is useful for the development of recent subjects of pharmaceutical or biological interest (Alamgir M. *et al*., 2007). The optimization of benzimidazole-based structures has resulted in numerous medicines that area unit presently within the market, like gastric antacid (proton pump inhibitor), pimobendan (Ion dilator) and vermifuge, albendazole, flubendazole (anthelmintic) area unit shown in Figure-1.5 (Mamedov VA, 2016).

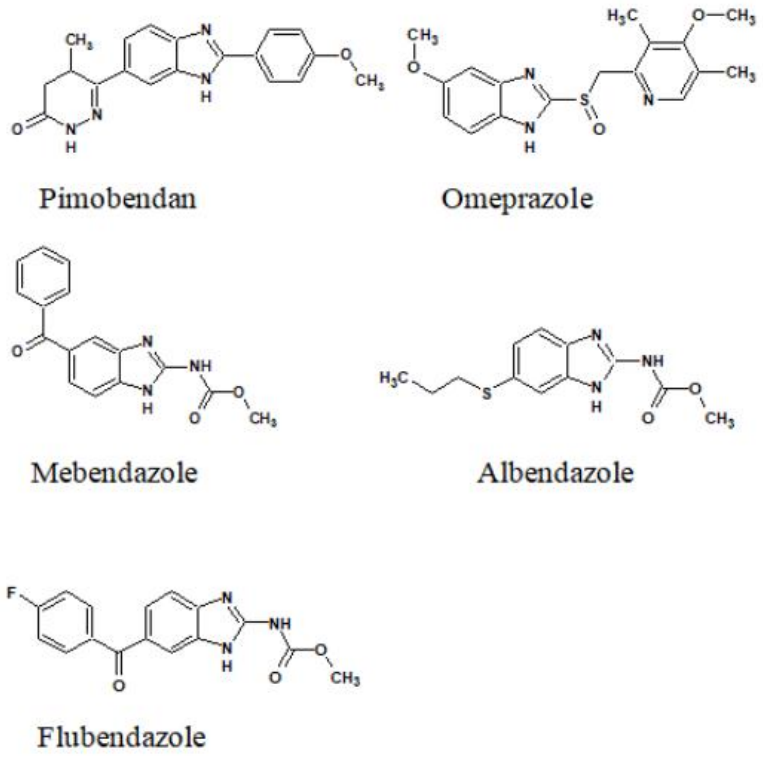


Figure- 1.5: Representative 2-Substituted Benzimidazole derivatives as some marketed drugs for different therapeutic categories.

1, 2-Disubstituted benzimidazoles and their derivatives represent an important branch of this family. These structures were reported as valuable bioactive structures, such as specific angiotensin II receptor type 1 selective antagonists (P. Naik *et al*., 2010, V. K. Vyas *et al*., 2010), or hepatitis C virus NS5B polymerase inhibitors (T. Ishida *et al*., 2006). Furthermore, they exhibit several other pharmacological activities including antidiabetic (Q. Dang *et al*., 2010), antihistamine (L. Bielory *et al*., 2005), analgesic (M. Gaba *et al*., 2010), antiviral (J. F. Miller *et al*., 2010), chemotherapeutic (M. Boiani *et al*., 2005), antifungal (C. Chen *et al*., 2009), and antiparasitic (J. P rez-Villanueva *et al*., 2011) applications. The relevance of these compounds can be demonstrated by the profusion of pharmaceutical products in the market, for example, the antihypertensives Micardis (telmisartan) and Atacand (candesartan), or Bilaxten (bilastine), a histamine H1 receptor antagonist for the oral treatment of allergic rhinitis and chronic idiopathic urticaria.

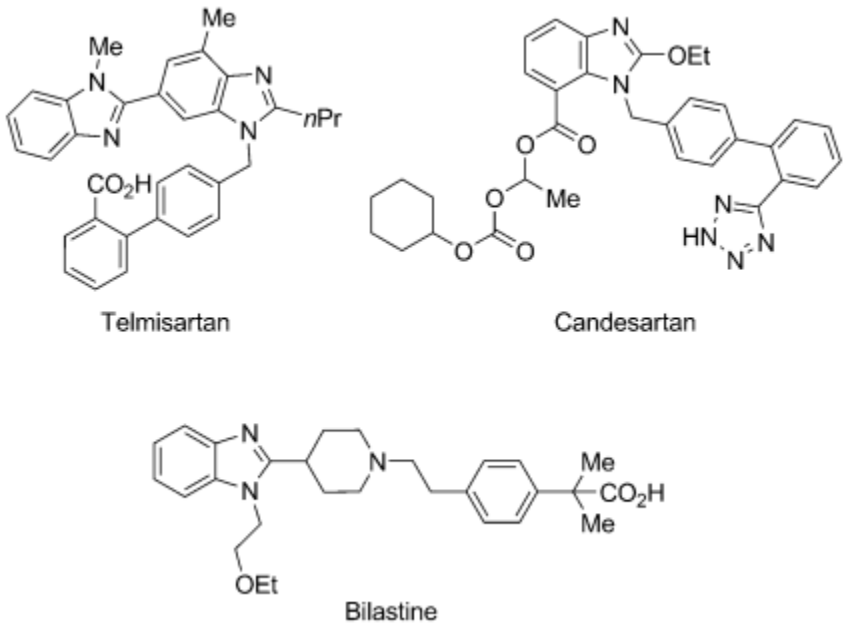


Figure- 1.6: Representative 1, 2-disubstituted Benzimidazole derivatives as some marketed drugs for different therapeutic categories.

1.4. Synthesis of substituted benzimidazoles

Almost all syntheses of benzimidazoles start with benzene derivatives possessing nitrogen-containing functions ortho to each other (Figure- 1.7) that is, the starting material possesses the function designated by formula many methods have been reported for the synthesis of benzimidazoles. Most of these methods involve the condensation of o-phenylenediamine, and its derivatives with carboxylic acids (scheme-1.1), or aldehydes (scheme-1.2) (S.I. Alaqeel, 2016).

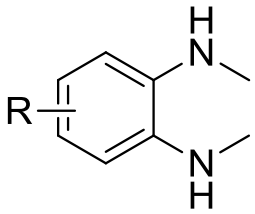
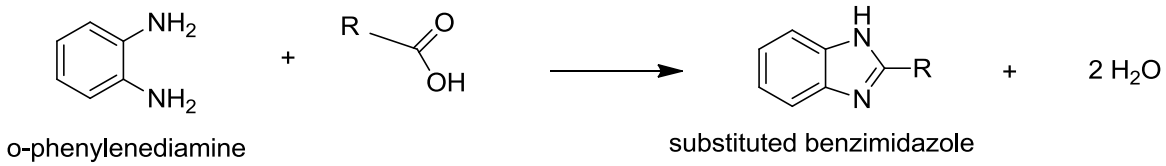
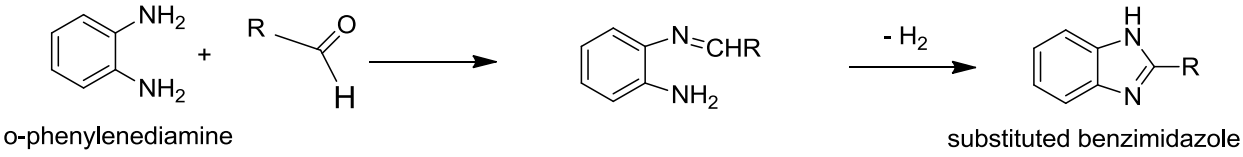


Figure-1.7: Ortho di nitrogen compounds



Scheme-1.1: Reaction of o-phenylenediamine with carboxylic acids to give 2-substituted benzimidazoles.



Scheme-1.2: Reaction of o-phenylenediamines with aldehydes to give 2-substituted benzimidazoles.

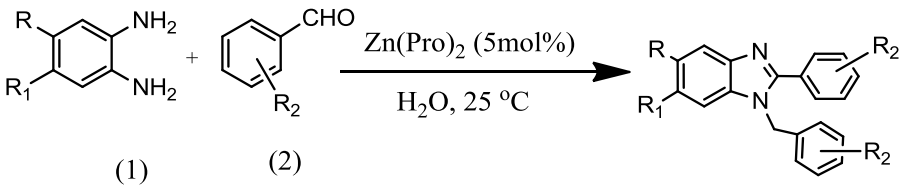
Various catalyzed synthesis of benzimidazole derivatives are known condensation of o-phenylenediamine with orthoesters in the presence of various lewis acid catalyst is also known such as $ZrCl_4$, $SnCl_4$, $TiCl_4$, $ZrOCl_2 9H_2O$ and $HFCl_4$. Several procedures have been reported for the synthesis of substituted benzimidazoles from condensation of o-phenylenediamines with carboxylic acids, acid chlorides, nitriles, imidates and orthoesters under strong acidic conditions (Lu, J. *et al*., 2012, Geratz, J.D. *et al*., 1979, Tidwell, R.R. *et al*., 1978, Fairley, T.A. *et al*., 1993), oxidative cyclodehydrogenation of o-phenylenediamine and aldehydes in the presence of different oxidants (Riadi, Y. *et al*., 2011, Bachhav, H.M. *et al*., 2011, Blacker, A.J. *et al*., 2009, Patil, V.D. *et al*., 2011), transition-metal-catalyzed intramolecular cyclization of 2-haloanilides (Saha, P. *et al*., 2009, Evindar, G. *et al*., 2006, Yang, D. *et al*., 2008), condensation reactions of o-phenylenediamine with β-ketonitriles, β-ketoesters, or β-diketones under microwave radiation and high temperature conditions or in the presence of a catalyst (Kamila, S. *et al*., 2006, Kamila, S. *et al.*, 2005, Cai, L. *et al*., 2011, Wang, Z.-X. *et al*., 2005).

Since these methods are widely used, but to improve the chemo selectivity, lowering the chances of formation of side products, to ease the workup procedure, to avoid toxic and hazardous chemicals, moreover, to employ economical and ecofriendly approaches for benzimidazole synthesis, water emerged as a useful alternative solvent to the synthetic chemist for several years. As water has several potential advantages such as safety, economy, readily available and nontoxic, utility of reactions in water have been reflected by the many studies to discover new processes with which they can be performed catalytically and chemo selectively (Azizollah H. *et al*., 2015).

1.5. Literature review:

Here a number of different synthetic methods for benzimidazoles derivatives in aqueous medium have been grouped according to the starting material of o-phenylene diamines.

Ravi Varala *et al*, 2007, reported an efficient procedure for the selective synthesis of 1,2-disubstituted benzimidazole derivatives (3) from wide range of substituted o-phenylenediamines (1) (1 mmol) and aldehydes (2) (2 mmol) in moderate to excellent isolated yields (42—92%) using 5 mol% of Zn(proline)$_2$-complex as catalyst in water as solvent at ambient temperature (Scheme-1.3).

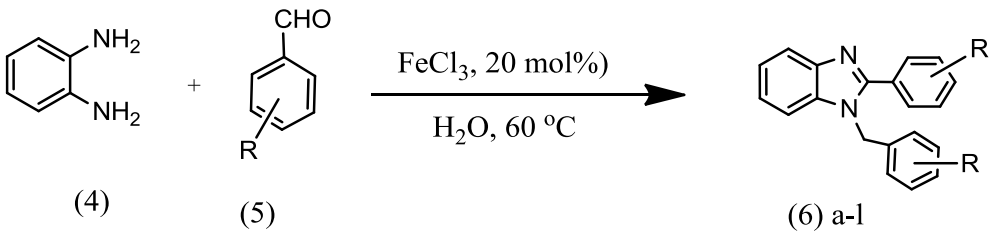


(1) (2) (3) formed exclusively

R, R_1=H
R=CH3, R_1=CH3
R=Cl, R_1=Cl
R2 = various substitution on phenyl ring

Scheme-1.3: Synthesis of 1, 2-disubstituted benzimidazole using Zn-proline as catalyst

Yunyun *et al.*, 2012, developed a reaction system to synthesize 1, 2-disubstituted benzimidazoles (6) from using o-phenylenediamine (4) with aromatic aldehydes (5) in 1:2 mmol equivalent ratio and have found good to excellent yields (63-90%) under mild reaction conditions by using water as the reaction medium in the presence of 20 mol% $FeCl_3$ (Scheme-1.4). This method possesses advantages such as clean reactions system, low cost and potentially recyclable catalyst as well as good substrate tolerance.

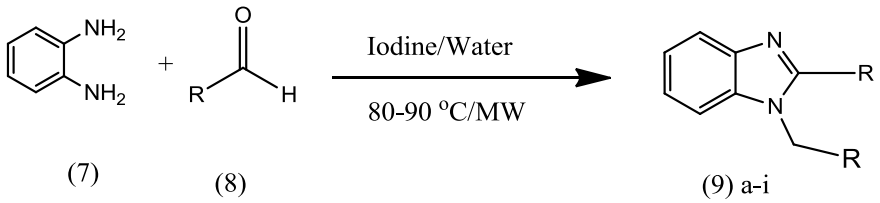


(4) (5) (6) a-l

R=H (6a), 4-Me (6b), 4-F (6c), 4-Cl (6d), 4-Br (6e), 4-NO_2 (6f), 4-OMe (6g), 2-OMe (6i), 3-NO_2 (6j), 2-OH (6k), 2-Cl (6l)

Scheme-1.4: Synthesis of 1, 2-disubstituted benzimidazole using 20 mol % $FeCl_3$ as catalyst

P. P. Sun *et al*, 2006, used catalytic amount of iodine (0.02 mmol), in THF–H2O (1:1 v/v) for the condensation of aldehydes (8) with 1, 2-phenylenediamine (7) in 1:1 mmol equivalent to give the benzimidazole derivatives under room temperature in good yields. The method can be used for the synthesis of 2-substituted benzimidazoles or 1, 2-disubstituted benzimidazoles (9). In 2015, Aniket P. Sarkate *et al.,* demonstrated iodine catalyzed synthesis of 2-Aryl-1-arylmethyl-1H-benzimidazoles (9) by using o-phenylenediamine (7) and aldehydes (8) in 1:2 mmol equivalent amount at 80-90°C or at 70°C under microwave in aqueous media. This newer approach is promising and gives moderate yields (84-95%) with high purity and selectively single product in aqueous media (Scheme-1.5).

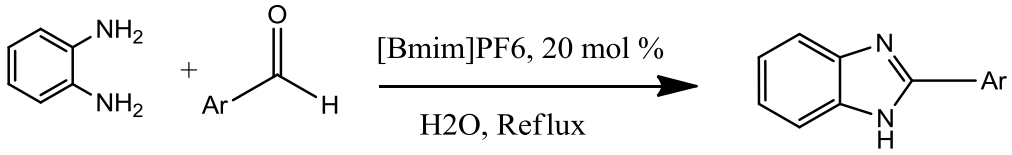


(7) (8) (9) a-i

R= Ph (9a), 4-ClC_6H_4 (9b), 3-$O_2NC_6H_4$ (9c), 4- MeC_6H_4 (9d), 4- FC_6H_4 (9e), 4-CNC_6H_4 (9f), 4-$MeOC_6H_4$ (9g), 4-$Me_2NC_6H_4$ (9h), 2-furyl (9i)

Scheme-1.5: Synthesis of 1, 2-disubstituted benzimidazole using I_2 as catalyst

Aniruddh Bhavsar *et al.*, 2016, demonstrated a novel and efficient route to synthesize 2-substituted benzimidazole derivatives (12) in good to excellent yield (87-96%) starting from o-phenylenediamine (10) and aryl aldehydes (11) in 1:1 mmol equivalent ratio in an aqueous media at reflux condition in the presence of ionic liquid [Bmim]PF6 (20 mol %) (Scheme-1.6). This ionic liquid can be reused for further reactions after simple distillation to remove water and drying the remaining ionic liquids under vacuum.

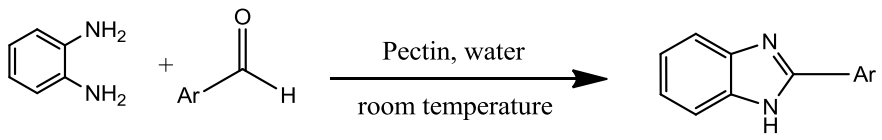


(10) (11) (12) a-h

Ar = -C_6H_5 (12a), 4-OH-C_6H_4 (12b), 4-OCH_3-C_6H_4 (12c), 4-Cl-C_6H_4 (12d), 4-Br-C_6H_4 (12e), 4-CH_3-C_6H_4 (12f), 4-C_2H_5-C_6H_4 (12g), 3-OCH_3-4-OH-C_6H_3 (12h)

Scheme-1.6: Synthesis of 2-substituted benzimidazole using ionic liquid [Bmim]PF6 (20 mol %) as catalyst

Agrwal Akansha *et al.*, 2014, developed a green procedure for synthesizing 2-substituted benzimidazole (15) by using various aromatic aldehydes (14) and o-phenylenediamine (13) (1:1 mol equivalent ratio) using pectin, a hetero polysaccharide as a catalyst and water as a solvent at room temperature (Scheme-1.7). The key advantages of this procedure were cost effectiveness of catalyst, easy workup and purification of product by non-chromatographic methods and excellent yield up to 91% depending upon various substitutions.

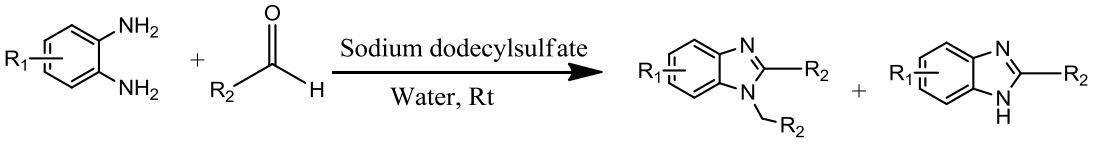


(13) (14) (15) a-k

Ar = -C_6H_5 (15a), 3-(NO_2)-C_6H_4 (15b), 4-(NO_2)-C_6H_4 (15c)
3-(OCH_3)-C_6H_4 (15d), 4-(OCH_3)-C_6H_4 (15e), 4-(Cl)-C_6H_4 (15f),
4-(OH)-C_6H_4 (15g), 3-(OH)-C_6H_4 (15h), 4-(F)-C_6H_4 (15i),
3-(Br)-C_6H_4 (15j), 4-(CH_3)-C_6H_4 (15k)

Scheme-1.7: Synthesis of 2-substituted benzimidazole by using using pectin, a hetero polysaccharide as a catalyst and water as a solvent at room temperature

S. D. Pardeshi *et al.,* 2015, developed a convenient procedure for the synthesis of 2-Aryl benzimidazoles (19) by reacting o-phenylenediamine (16) with aromatic aldehydes (17) (1:1 mol equivalent) in presence of 10 mol% sodium dodecyl sulphate in aqueous medium at room temperature in open air atmospheric condition with and without use of sonication. In 2011, P. Ghosh *et al.,* synthesized 1, 2-disubstituted benzimidazole (18) as predominant product using 1 mmol of anhydrous SDS in the reaction mixture of o-phenylenediamine (16) and benzaldehyde (17) (1:2 mol equivalent) under same reaction condition at room temperature (Scheme-1.8).

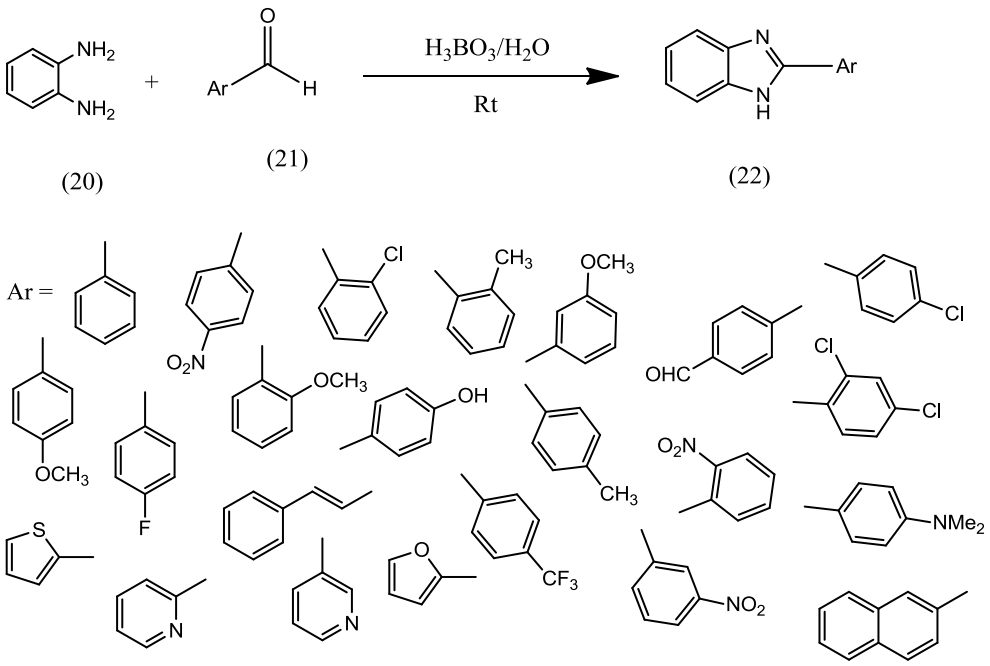


(16) (17) (18) (19)

R1 = H, 5-Cl, 3-CH3, 3-benzoyl
R2 = various aromatic substitution

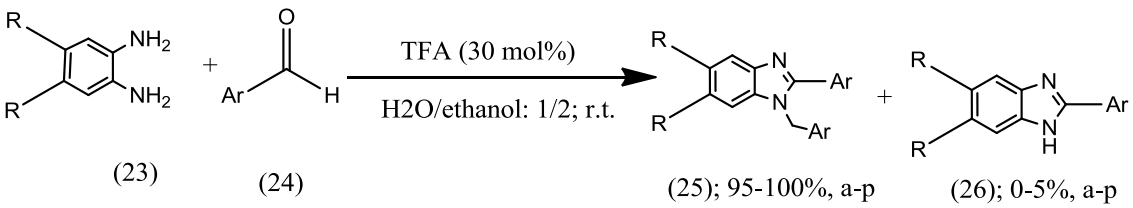
Scheme-1.8: Substituted benzimidazole using anhydrous SDS at room temperature

Zahed Karimi-Jaberi *et al.*, 2011 and M. R. Poor Heravi *et al.*, 2013, demonstrated a very simple, environmentally benign and efficient method for the synthesis of 2-substituted benzimidazoles (22) with boric acid (H_3BO_3) as catalyst in aqueous media, from o-phenylenediamine (20) and aromatic aldehydes (21), under room temperature in excellent yield (85-95%) (Scheme-1.9). The product is applicable to aryl and heteroaryl aldehydes. The main features of this procedure were mild reaction conditions, a wide range of functional groups can be tolerated and easy separation of products from the reaction mixture.



Scheme-1.9: 2-substituted benzimidazole using boric acid (H_3BO_3) as catalyst in aqueous media at room temperature

M. R. Mohammadizadeh *et al.*, 2010, introduced a selective and ecocompatible synthesis of 2-aryl-1-arylmethyl-1H-1,3-benzimidazoles (25) via condensation reaction of o-phenylenediamine (23) derivatives and aromatic aldehydes (24) in ethanol/water at room temperature in presence of an organocatalyst, Trifluoroacetic acid (TFA), 30 mol% with an excellent yield of 68-97% yield (Scheme-1.10).

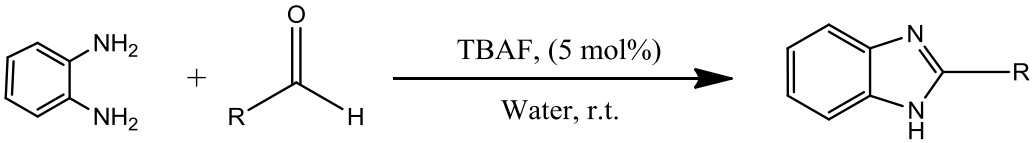


(23) (24) (25); 95-100%, a-p (26); 0-5%, a-p

R=H, Ar=Ph **(a)**; R=H, Ar = 4-Me-Ph **(b)**; R=H, Ar = 4-MeO-Ph **(c)**; R=H, Ar= 4-Cl-Ph **(d)**; R=H, Ar = 4-Me_2N-Ph **(e)**; R=H, Ar= 4-F-Ph **(f)**; R=H, Ar= 4-NC-Ph **(g)**; R= H, Ar= 4-O_2N-Ph **(h)**; R= H, Ar = 4-HO-Ph **(i)**; R= H, Ar= 3-HO-Ph **(j)**; R= H, Ar= 3,4-di(MeO)-Ph **(k)**; R=H, Ar= 2-Furyl **(l)**; R= Me, Ar= 4-iso-propyl-Ph **(m)**; R=Me, Ar= 4-Cl-Ph **(n)**; R= Me, Ar = 4-Me-Ph **(o)**; R=Cl, Ar = Ph **(p)**

Scheme-1.10: Substituted benzimidazole in presence of an organocatalyst, Trifluoroacetic acid (TFA)

Joshi *et al., 2010*, developed one-pot multistep reactions for the synthesis of 2-Arylbenzimidazole (29) by reaction of substituted aldehyde (28) with o-phenylenediamine (27) in water under ultrasonic irradiation at ambient temperature using 5 mol% of tetra-n-butylammonium fluoride (TBAF) furnish the desired product in good to excellent yield (82-94%). (Scheme-1.11). The process is green, mild, inexpensive, excellent chemo selectivity, and excellent yields are the main advantages of this procedure.

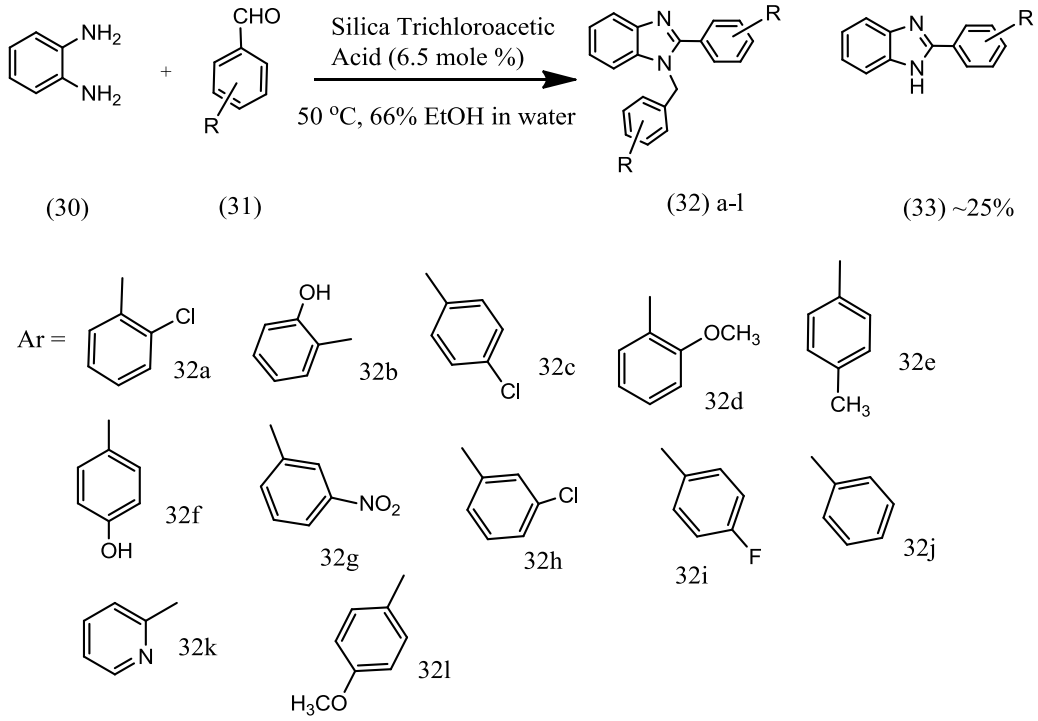


(27) (28) (29) a-m

Aldehydes (28) : Benzaldehyde **(29a)**; Anisaldehyde **(29b)**; 4-methyl benzaldehyde **(29c);** 4-chlorobezaldehyde **(29d)**; 4-flurobenzaldehyde **(29e)**; 3-bromobenzaldehyde **(29f)**; Furan-2carbaldehyde **(29g)**; Piconaldehyde **(29h)**; Nicotinaldehyde **(29i)**; 4-(1H-1,2,4-trizol-1-yl)benzaldehyde **(29j)**; Napthaldehyde **(29k);** Cinnamaldehyde **(29l)**; 3-nitrobanzaldehyde **(29m)**

Scheme-1.11: Substituted benzimidazole using tetra-n-butylammonium fluoride (TBAF) as catalyst

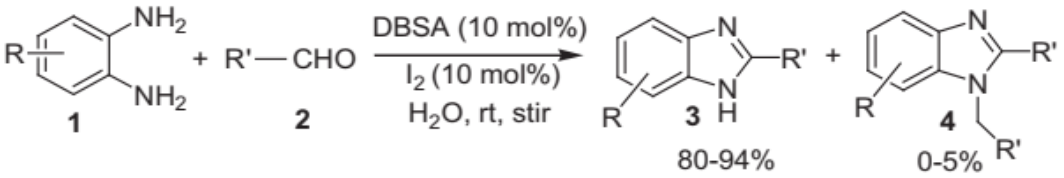
Brajesh Kumar *et al.*, 2014, introduced an efficient one-pot synthesis technique 2-aryl-1-arylmethyl-1H- benzimidazole derivatives from o- phenylenediamine and aromatic aldehyde in the presence of silica gel supported trichloroacetic acid (SiTCA) with excellent yields at 50◦C in aqueous medium by ultrasonic irradiation (Scheme-1.12). This method provided several advantages such as green solvent, inexpensive catalyst, simple experimental methodology, shorter reaction time and higher yield.



Scheme-1.12: Sonochemical synthesis of 1, 2-disubstituted benzimidazole derivatives in the presence of silica gel supported trichloroacetic acid (SiTCA)

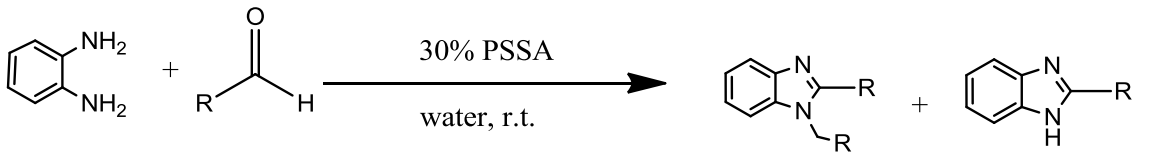
V. Kumar *et al.*, 2013, developed an efficient synthetic method for the facile synthesis of 2-substituted benzimidazoles from o-phenylenediamine and various aldehydes in aqueous media in the presence of a surfactant 10 mol% Dodecylbenzenesulfonic acid (DBSA) as

catalyst and I_2 (10 mol%) as co-catalyst (Scheme-1.13). The method described has the advantages of operational simplicity, excellent yields, high chemoselectivity, and clean and green reaction profile.



Scheme-1.13: DBSA catalyzed synthesis of 2-substituted benzimidazoles.

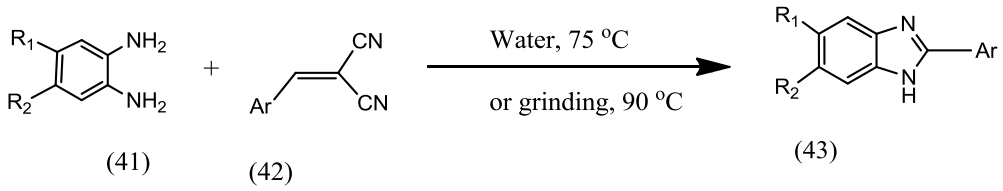
Madhukar B. Deshmukh *et al.*, 2011, incorporated polymer supported Polystyrene sulfonic acid (PSSA) at 30% w/v, as catalyst rendering excellent chemoselectivity in the synthesis of 2-Aryl-1-Arylmethyl-1H-Benzimidazoles from reaction of o-phenylene diamine with several aryl aldehydes (Scheme-1.14). The captivating aspects of this method are the greenness of water mediated system and efficient (yield 85-90%), selective achievement of desired product within 30 - 40 min.



R = Ph **(39a)**, 2-ClC_6H_4 (**39b**), 4-ClC_6H_4 (**39c**), 4-$NO_2C_6H_4$ (**39d**), 3-$NO_2C_6H_4$ (**39e**), 4-$MeOC_6H_4$ (**39f**), 4-OHC_6H_4 (**39g**), 4-$Me_2NC_6H_4$ (**39h**), 6-methoxy-2-chloro quinoline (**39j**)

Scheme-1.14: Synthesis of 2-aryl-1-arylmethyl-1H- benzimidazole derivatives using polymer supported Polystyrene sulfonic acid (PSSA) at 30% w/v, as catalyst.

A. Habibi *et al.*, 2015, reported a fast, high efficiency and environmentally friendly procedure for the synthesis of 2-aryl benzimidazole derivatives from reaction between 1, 2-phenylenediamine derivatives and arylidene malononitrile under aqueous media generates 2-aryl benzimidazole derivatives with a high yield (83-91%) (Scheme-1.15)

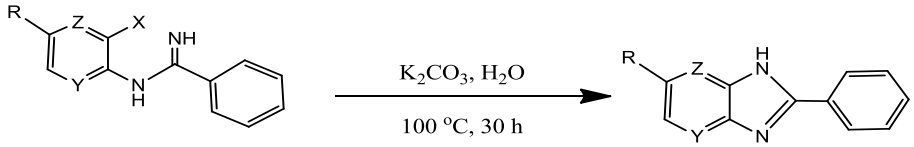


(41) (42) (43)

R1 , R2 = H or Me
Ar = C_6H_5, 2-Cl-C_6H_4, 4-Cl-C_6H_4, 3-NO_2-C_6H_4, 4-NO_2-C_6H_4, 3-OCH_3-C_6H_4, 4-OCH_3-C_6H_4, 4-CH_3-C_6H_4, 4-Br-C_6H_4, 2-thiophenyl

Scheme-1.15: Synthesis of 2-substituted benzimidazole derivatives using 1, 2-phenylenediamine derivatives and arylidene malononitrile under aqueous media.

Chunxia Chen *et al.*, 2012, developed a straightforward method for the synthesis of the benzimidazole ring system from N-(2-Halophenyl) benzamidines through a carbon-nitrogen cross-coupling reaction in the presence of 2.0 equiv. of K_2CO_3 in water at 100 °C for 30 h, the intramolecular cyclization of N-(2-iodoaryl)benzamidine provides benzimidazole derivatives in moderate to high yields (Scheme-1.16). Remarkably, the procedure occurs exclusively in water and doesn't require the use of any additional reagent/catalyst, rendering the methodology highly valuable from both environmental and economic points of view.



(44) a-i

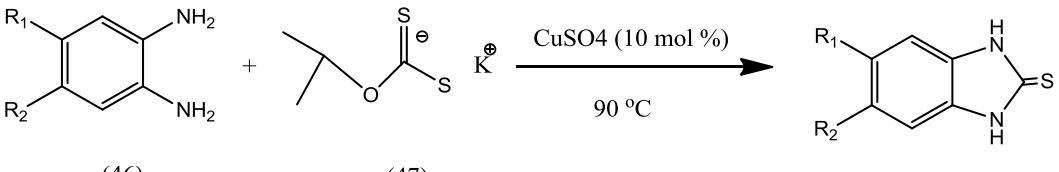
X= Br, I

(45) a-i

45a: X=I, R=H, Y=CH, Z=CH; **45b:** X=I, R=F, Y=CH, Z=CH; **45c:** X=Br, R=F, Y=CH, Z=CH; **45d:** X=Cl, R=I, Y=CH, Z=CH; **45e**: X=Br, R=I, Y=CH, Z=CH; **45f:** X=Me, R=I, Y=CH, Z=CH; **45g:** X=MeO, R=I, Y=CH, Z=CH; **45h**: X=I, R=H, Y=N, Z=CH; **45i:** X=Br, R=H, Y=N, Z=CH; **45j:** X=I, R=H, Y=CH, Z=N

Scheme-1.16: Base-mediated intramolecular C–N cross-coupling of benzamidine in water

Ranjbar-Karimi *et al.*, 2016, reported an easy, green, efficient and simple approach is for the synthesis of some benzimidazole employing the reaction of 1,2-phenylenediamines with potassium isopropyl xanthate in the presence of copper sulfate (CuSO4) as a catalyst under conventional heating (at 90°C) and ultrasonic irradiation at room temperature with high yield and short reaction time(6-7h). (Scheme-1.17)

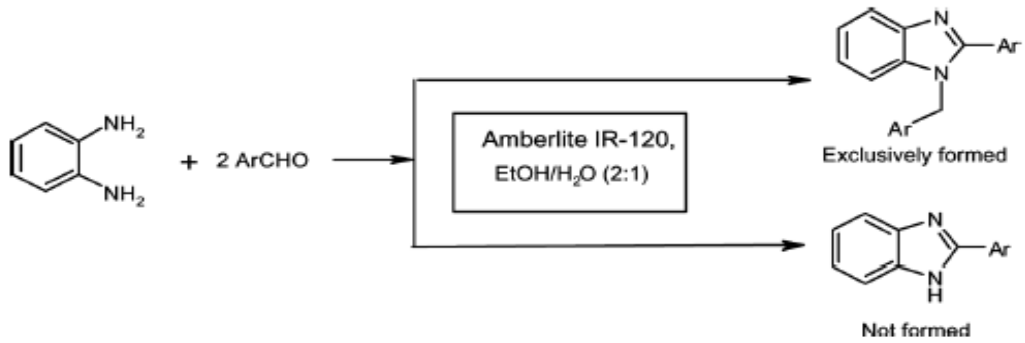


(46) (47) (48)

R_1= Me, R_2 = H

Scheme-1.17: Synthesis of benzimidazole employing the reaction of 1,2-phenylenediamines with potassium isopropyl xanthate in the presence of copper sulfate (CuSO4) as a catalyst.

S. Das Sharma *et al.*, 2009, developed a efficient, and environmentally benign method for the exclusive formation of biologically significant 2-aryl-1-arylmethyl-1H-benzimidazoles from o-phenylenediamine and various aldehydes under the heterogeneous catalysis of Amberlite IR-120 in aqueous media in excellent yields (70-95%). (Scheme-1.18). The catalyst is recyclable without loss of activity.



Scheme-1.18: 2-aryl-1-arylmethyl-1H-benzimidazoles from o-phenylenediamine and various aldehydes under the heterogeneous catalysis of Amberlite IR-120 in aqueous media.

1.6. Rational for current study

As the use of large volumes of volatile hazardous organic solvents in industrial processes poses a serious threat to the environment, development of novel, milder, and sustainable synthetic processes involving environmentally-friendly solvents and nontoxic reagents has become imperative.

One of the most sustainable alternatives to organic solvents is water, which has gained increasing popularity due to being inexpensive, non-toxic, nonflammable, widely abundant in nature, and environmentally benign.

The problem of insolubility and hydrolytic decomposition of many organic compounds in water may be solved by the use of surface-active compounds. They form a colloidal, micellar, or other organized phase and thereby, solubilize the organic reagents inside the hydrophobic interior of the organized aqueous media and allow the reaction to occur. The use of surfactants as catalysts is widespread, and has been investigated in detail for various reactions in aqueous media.

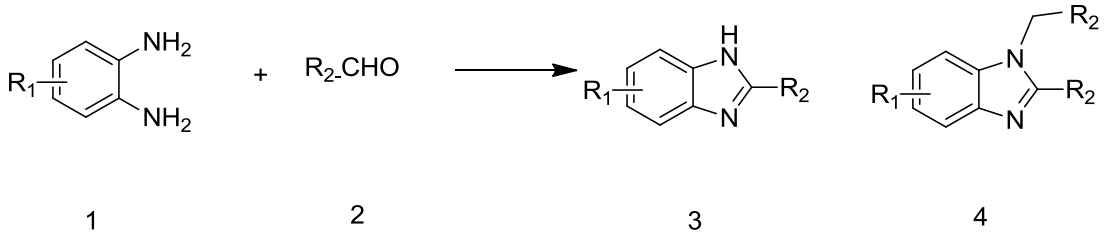
Inspired by these green synthetic approaches, we are employing several surface active agents to synthesize substituted benzimidazoles in aqueous media to find out effective alternative surfactant in this array of synthetic chemistry.

Chapter-2

Synthesis of substituted benzimidazole derivatives

2.1. Principle

Currently a number of synthetic methodologies are available for synthesizing benzimidazoles among which the condensation of o-phenylenediamine (possessing two amino groups ortho to each other) and aldehyde derivatives had been widely used for the benzimidazole synthesis. Catalytic activity of surface active agents (such as DBSA, PSSA, SDS, TBAB etc.) were investigated by several researchers for the synthesis of substituted benzimidazole derivatives in aqueous media and had gained much attention due to being inexpensive, non-toxic, nonflammable, widely abundant in nature, and environmentally benign. Reactions were carried out using o-phenylenediamine (**1**) and different aldehydes (**2**) in varying molar ratios (1:1 or 1:2) and most of the cases found 2-substituted (**3**) and 1,2-di substituted benzimidazoles (**4**) in a mixture. Usually it was found that utilizing o-phenylenediamine and aldehydes in 1:1 ratio favors 2-substituted product and 1:2 ratio favors 1, 2-di substituted products as major benzimidazole derivative (Scheme-2.1).



1 2 3 4

Scheme-2.1: Plausible transformations into substituted benzimidazoles

Inspired by green synthetic approaches in assembling benzimidazole derivatives by former investigators, in our recent synthetic work we carried out reactions between o-phenylenediamine and various aldehyde derivatives using water as reaction medium employing several cationic and anionic surface active agents such as benzalkonium chloride (BKC), sodium dodecyl sulfate (SDS), Tetra-n-butylammonium bromide (TBAB) and Tetra-n-butylammonium iodide (TBAI) as catalyst at room temperature. In this current study we

have utilized benzaldehyde, 4-OH benzaldehyde and 4-Cl benzaldehyde available at our laboratory to obtain the desired substituted benzimidazole derivatives with some modification in protocol in order to investigate the impact of molar ratios of reactants in the nature of substitutions as well as to explore the influence of surface active agents in case of selectivity in distribution of products in mixture because among the reactions of o-phenylenediamine with aldehyde, the selectivity in forming 1, 2-disubstituted benzimidazole (**4**) and 2-substituted benzimidazole (**3**) is an issue of high interest now a days.

2.2. General experimental procedures and Instrumentations

All reagents used are of analytical grade. O-phenylenediamine, 4-OH benzaldehyde and 4-Cl benzaldehyde were collected from Sigma Aldrich. Benzaldehyde from Loba Chemie was purchased from local market. Surfactants used were donated by R & D Formulation department of Incepta Pharmaceuticals Ltd., Bangladesh. Commercial aluminum backed TLC used for monitoring purpose were collected from local market. Silica gel 60 was collected from Loba Chemie, India. All organic solvents (ethyl acetate, n-hexane) collected from either central store or local market were utilized upon distillation.

All reactions were carried out in well-cleaned and well dried reaction vessels using magnetic stirrer under room temperature. Progresses of all the reactions were monitored by Thin Layer Chromatography using n-hexane and ethyl acetate as solvent. Visualization of TLC spot was accomplished initially under UV lamp and then by p-anisaldehyde solution with subsequent charring on hot plate above 120^{o}C for about 2 minutes.

Upon completion of reaction, crude mixtures were extracted by suitable organic solvent from water media using separating funnels with subsequent treatment with brine solution. Finally residual water was removed by treating the organic phage with anhydrous sodium sulfate (Na_2SO4) followed by filtration to collect the organic phage. Organic solvents were evaporated by rotary evaporator for obtaining crude mixture which was subjected to further purification by column chromatography technique using silica gel 60. The crude sample was

eluted by binary solvent system of n-hexane and ethyl acetate with gradual increase of polarity from 5:1 to 2:1 (n-hexane: ethyl acetate).

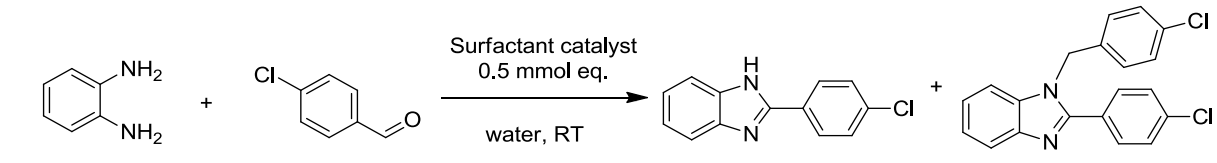
The desired transformations were confirmed by undergoing TLC with known reference samples at our laboratory and other spectroscopic techniques comprising ^{1}H-NMR.

^{1}H-NMR spectra were recorded at 400 MHz in CDCl3 on AVANCE III (Bruker, Switzerland) from Bangladesh Council of Scientific and Industrial Research (BCSIR).

2.3. Method optimization

The current synthetic procedure was preceded by initial optimization of reaction condition for the synthesis of substituted benzimidazole derivatives. For this purpose we utilized o-phenylenediamine as starting and 4-Cl-benzaldehyde as aldehyde prototype.

In order to minimize energy expenditure, here in current study we mainly focused on synthesizing benzimidazole at room temperature. Hence to establish an optimized reaction protocol, varied equivalent mmol ratios of o-phenylenediamine and 4-Cl-benzaldehyde such as 1:1 and 1:2 were used including different types of surfactants like benzalkonium chloride (BKC), sodium dodecyl sulfate (SDS), Tetra-n-butylammonium bromide (TBAB) and Tetra-n-butylammonium iodide (TBAI) in a fixed mmol equivalent proportion. All reactions are carried out at room temperature using water as reaction medium (Scheme-2.2).



o-phenylenediamine

p-chlorobenzaldehyde

2-(4-chlorophenyl)-1*H*-benzo[*d*]imidazole
Code: MS4CIB
Molecular Weight: 228.68

1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1*H*-benzo[*d*]imidazole
Code: DS4CIB
Molecular Weight: 353.24

Scheme-2.2: Reaction of O-phenylenediamine and 4-Cl-benzaldehyde under room temperature using 0.5 mmol eq. surfactant as catalyst in water media.

2.3.1. List of reagents

Reagents utilized are summarized in Table 2.1 below-

Table- 2.1: Required reagents for method optimization

Name	Purpose	MW	Purity
O-phenylenediamine	Starting	108.1	99.6%
4-Cl-benzaldehyde	Reactant	140.57	97%
Benzalkonium Chloride (BKC)	Catalyst	360	-
Sodium dodecyl sulfate (SDS)	Catalyst	288.37	-
Tetra-n-butyl ammonium bromide (TBAB)	Catalyst	322.37	-
Tetra-n-butyl ammonium iodide (TBAI)	Catalyst	369.37	-
Water	Solvent	-	Distilled

2.3.2. Procedure

Reagent admixing:

Measured amount of o-phenylenediamine (OPD) was first taken in a clean and dried 50 ml reaction vessel charged with 30 ml of distilled water. The mixture was stirred under magnetic stirrer for few minutes until all OPD get dissolved. Then measured amount of 4-chloro benzaldehyde and surfactant were added one by one into the reaction vessel with continuous stirring. At beginning there was a foamy mass witch settled down as time progresses.

Reaction progress monitoring:

The reaction progression was monitored by TLC using n-hexane: Ethyl acetate= 3:1 solvent system. Preliminary visualization of the developed TLC spots was done under UV lamp as compounds were UV active. Then the TLC plate was further soaked in p-anisaldehyde solution followed by heating on a hot plate above 120°C for about 2 minutes.

The R_f value of the product was found to be 0.314 for MS4ClB and 0.4 for DS4ClB.

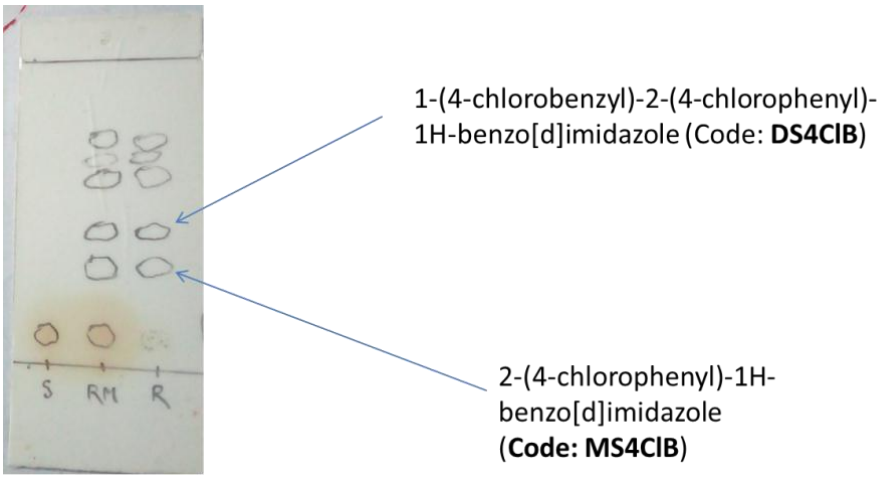


Figure: 2.1: TLC appearance during reaction of reaction of o-phenylenediamine and 4-Cl-benzaldehyde (1:1) under room temperature using 0.5 mmol eq. surfactant as catalyst in water media.

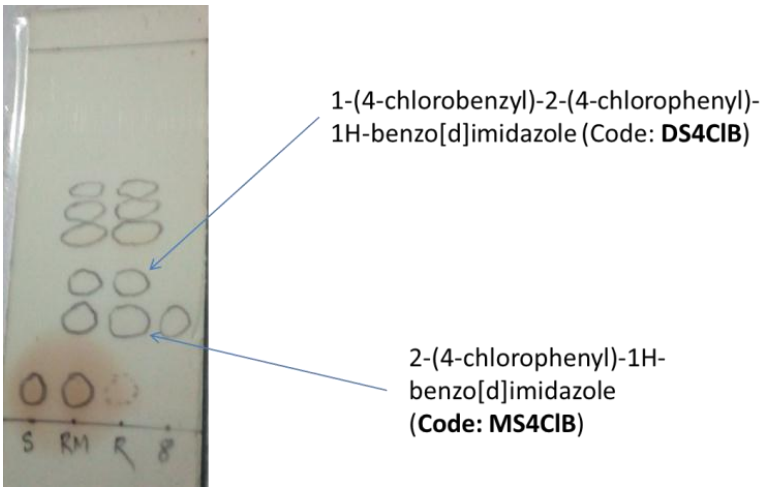


Figure: 2.2: TLC appearance during reaction of reaction of o-phenylenediamine and 4-Cl-benzaldehyde (1:2) under room temperature using 0.5 mmol eq. surfactant as catalyst in water media.

Extraction:

Upon completion of reaction, the reaction mixture was extracted with Ethyl acetate (70ml X 3). The organic layer was separated by separating funnel with subsequent washing with brine solution. Layers were separated and the organic layer was dehydrated over anhydrous Na_2SO_4 followed by filtration. The organic solvent Ethyl acetate was removed under reduced pressure at rotary evaporator.

Purification:

The crude product obtained was found to be sticky mass which was then dissolved in suitable solvent. Some silica was added to it and was swirled to make uniform slurry. Then the slurry was made dried and free flowing by evaporating under reduced pressure in rotary evaporator. The free flowing crude powder was then subjected to column chromatography for further purification and separation of the desired products using gradient elution by solvent system of n-hexane and ethyl acetate with gradual increase of polarity from 5:1 to 3:1 (n-hexane: ethyl acetate).

2.3.3. Result and discussion

All the data including respective yield values are summarized in Table-2.2. It was obvious that all surfactants were effective catalyst at room temperature providing good to moderate yields at varying ratios of o-phenylenediamine and 4-Cl benzaldehyde. We observed that at 1:1 ratio of reactants 2-substituted benzimidazole (2-(4-chlorophenyl)-1H-benzo[d]imidazole) is prominent product obtained. But at 1:2 ratios reaction condition favors 1,2-disubstituted benzimidazole (1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole). Among the four surfactants, Benzalkonium chloride shows excellent affinity to produce 2-substituted benzimidazole at both 1:1 and 1:2 ratios.

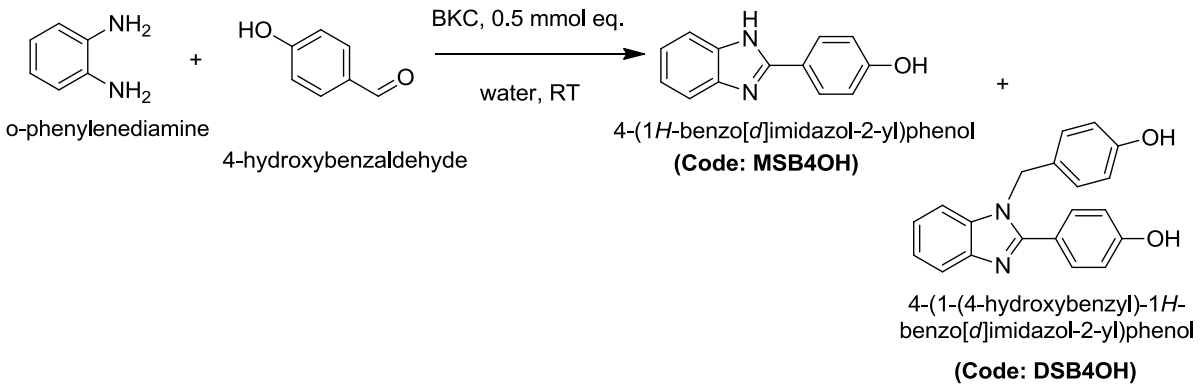
Hence, for further reactions of o-phenylenediamine with various available aldehyde derivatives, the reaction condition were optimized as – mmol equivalent ratio of o-phenylenediamine and aldehyde would be 1:1 with Benzalkonium chloride as surfactant catalyst (0.5 mmol equivalent) in water media at room temperature.

Table-2.2: Effects of surfactants on yield at varying reaction conditions.

Entry	mmol equivalent ratio of reactants	Surfactant	Quantity of surfactant (mmole equivalant)	Reaction temp.	Reaction time	Yield % of product (3)	Yield % of product (4)
1	1:1	BKC	0.5	RT	3.5 hrs.	74.79	12.17
2	1:1	SDS	0.5	RT	4 hrs.	66.57	31.19
3	1:1	TBAB	0.5	RT	4.5 hrs.	70.76	21.84
4	1:1	TBAI	0.5	RT	5 hrs.	66.81	22.78
5	1:2	BKC	0.5	RT	4 hrs.	57.11	25.95
6	1:2	SDS	0.5	RT	4.5 hrs.	32.77	66.64

2.4. Synthesis of benzimidazole derivative from OPD and 4-OH benzaldehyde

Reaction of O-phenylenediamine and 4-OH-benzaldehyde were carried out at 1:1 mmol equivalent ratio utilizing a surface active agent Benzalkonium Chloride (BKC) at 0.5 mmol equivalent ratio in water media at room temperature (Scheme-2.3).



Scheme-2.3: Reaction of O-phenylenediamine and 4-OH-benzaldehyde (1:1 mmol eq.) under room temperature using 0.5 mmol eq. BKC as catalyst in water media.

2.4.1. List of reagents

Amount of reagents utilized are summarized in Table 2.3 below-

Table- 2.3: Amount of reagents for reaction of o-phenylenediamine and 4-OH-benzaldehyde

Name	Purpose	MW	Calculated Amount	Measured amount	mmol	Equiv.
O-phenylenediamine	Starting	108.1		0.1439 gm.	1.3311	1
4-OH-benzaldehyde	Reactant	122.12	0.1625 gm.	0.1631 gm.	1.3311	1
Benzalkonium Chloride (BKC)	Catalyst	360	0.2395 gm.	0.2404 gm.	0.6655	0.5
Water	Solvent	-		20 ml		

2.4.2. Procedure

Reagent admixing:

0.1439 gm. of o-phenylenediamine (OPD) was first taken in a clean and dried 50 ml reaction vessel charged with 20 ml of distilled water. The mixture was stirred under magnetic stirrer for few minutes until all OPD get dissolved. Then 0.1631 gm. of 4-hydroxy benzaldehyde and 0.2404 gm. of catalyst BKC were added one by one into the reaction vessel with continuous stirring. At beginning there was a foamy mass witch settled down as time progresses.

Reaction progress monitoring:

The reaction progression was monitored by TLC using n-hexane: Ethyl acetate= 1:1 solvent system. Preliminary visualization of the developed TLC spots was done under UV lamp as compounds were UV active. Then the TLC plate was further soaked in p-anisaldehyde solution followed by heating on a hot plate above 120°C for about 2 minutes. The R_f value of the product was found to be 0.19 for MSB4OH and 0.11 for DSB4OH.

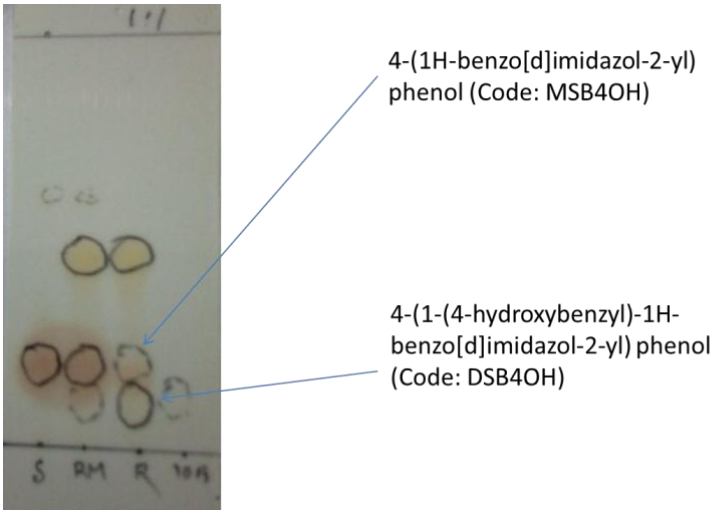


Figure: 2.3: TLC appearance during reaction of o-phenylenediamine and 4-OH-benzaldehyde

Extraction:

Upon completion of reaction, the reaction mixture was extracted with Ethyl acetate (45 ml X 3). The organic layer was separated by separating funnel with subsequent washing with brine solution. Layers were separated and the organic layer was dehydrated over anhydrous Na_2SO_4 followed by filtration. The organic solvent Ethyl acetate was removed under reduced pressure at rotary evaporator.

Purification:

The crude product obtained was found to be sticky mass which was then dissolved in suitable solvent. Some silica was added to it and was swirled to make uniform slurry. Then the slurry was made dried and free flowing by evaporating under reduced pressure in rotary evaporator. The free flowing crude powder was then subjected to column chromatography for further purification and separation of the desired products using gradient elution by solvent system of n-hexane and ethyl acetate with gradual increase of polarity from 5:1 to 2:1 (n-hexane: ethyl acetate).

2.4.3. Yield value:

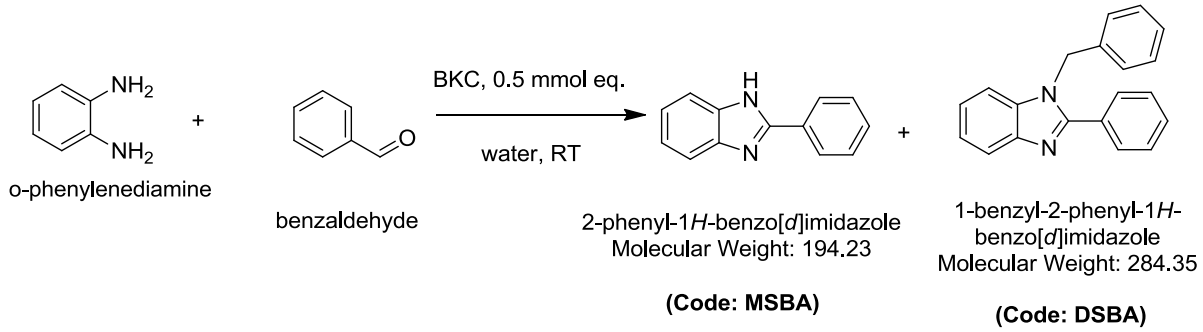
From this reaction two products were separated namely 4-(1H-benzo[d]imidazol-2-yl) phenol (Code: MSB4OH) and 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol (Code: DSB4OH). Yield value for the above products are given in Table-2.4 below-

Table-2.4: Yield value for purified products from Reaction of O-phenylenediamine and 4-OH-benzaldehyde

Code	Total weight	MW of product	mmol	mmol of OPD	Yield%
MSB4OH	145.25 mg	210.23	0.6909	1.3311	51.90
DSB4OH	55.4 mg	316.35	0.1751	1.3311	13.16

2.5. Synthesis of benzimidazole derivative from OPD and benzaldehyde

Reaction of o-phenylenediamine and benzaldehyde were carried out at 1:1 mmol equivalent ratio utilizing a surface active agent Benzalkonium Chloride (BKC) at 0.5 mmol equivalent ratio in water media at room temperature (Scheme-2.4).



Scheme-2.4: Reaction of O-phenylenediamine and benzaldehyde (1:1 mmol eq.) under room temperature using 0.5 mmol eq. BKC as catalyst in water media.

2.5.1. List of reagents

Amount of reagents utilized are summarized in Table 2.5 below-

Table- 2.5: Amount of reagents for reaction of o-phenylenediamine and benzaldehyde

Name	Purpose	MW	Density	Calculated Amount	Measured amount	mmol	Equiv.
o-phenylenediamine	Starting	108.1			0.1063 gm.	0.9838	1
Benzaldehyde	Reactant	106.12	1.044 gm./ml	0.1044 gm. (100µl)	100 µl	0.9838	1
Benzalkonium Chloride (BKC)	Catalyst	360		0.1771 gm.	0.1774 gm.	0.4919	0.5
Water	Solvent	-			20 ml		

2.5.2. Procedure

Reagent admixing:

0.1063 gm. of o-phenylenediamine (OPD) was first taken in a clean and dried 50 ml reaction vessel charged with 20 ml of distilled water. The mixture was stirred under magnetic stirrer for few minutes until all OPD get dissolved. Then 100µl of benzaldehyde and 0.1774 gm. of catalyst BKC were added one by one into the reaction vessel with continuous stirring. At beginning there was a foamy mass witch settled down as time progresses.

Reaction progress monitoring:

The reaction progression was monitored by TLC using n-hexane: Ethyl acetate= 1:1 solvent system. Preliminary visualization of the developed TLC spots was done under UV lamp as compounds were UV active. Then the TLC plate was further soaked in p-anisaldehyde solution followed by heating on a hot plate above 120°C for about 2 minutes. The Rf value of the product was found to be 0.42 for MSBA and 0.49 for DSBA.

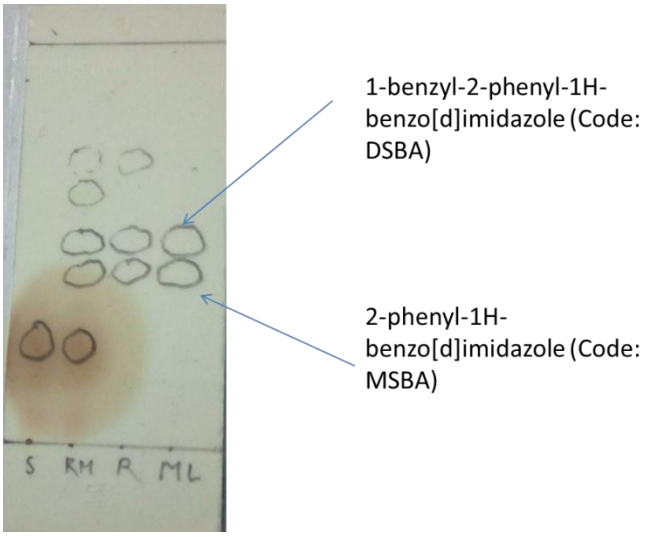


Figure: 2.4: TLC appearance during reaction of o-phenylenediamine and benzaldehyde

Extraction:

Upon completion of reaction, the reaction mixture was extracted with Ethyl acetate (45 ml X 3). The organic layer was separated by separating funnel with subsequent washing with brine solution. Layers were separated and the organic layer was dehydrated over anhydrous Na_2SO_4 followed by filtration. The organic solvent Ethyl acetate was removed under reduced pressure at rotary evaporator.

Purification:

The crude product obtained was found to be sticky mass which was then dissolved in suitable solvent. Some silica was added to it and was swirled to make uniform slurry. Then the slurry was made dried and free flowing by evaporating under reduced pressure in rotary evaporator. The free flowing crude powder was then subjected to column chromatography for further purification and separation of the desired products using gradient elution by solvent system of n-hexane and ethyl acetate with gradual increase of polarity from 5:1 to 2:1 (n-hexane: ethyl acetate).

2.5.3. Yield value:

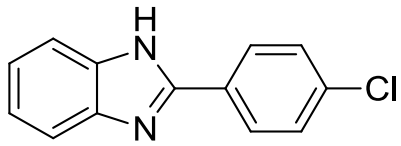
From this reaction two products were separated namely 2-phenyl-1H-benzo[d]imidazole (Code: MSBA) and 1-benzyl-2-phenyl-1H-benzo[d]imidazole (Code: DSBA). Yield value for the above products are given in Table-2.6 below-

Table-2.6: Yield value for purified products from Reaction of o-phenylenediamine and benzaldehyde

Code	**Total weight**	**MW of product**	**mmol**	**mmol of OPD**	**Yield%**
MSBA	85.8 mg	194.23	0.4417	0.9838	44.17
DSBA	40.4 mg	284.35	0.1421	0.9838	14.44

2.6. Identification of synthesized benzimidazole derivatives

Product Code: MS4ClB: 2-(4-chlorophenyl)-1H-benzo[d]imidazole



Appearance: Off-white powder

Molecular weight (calculated): 228.68

R_f value: 0.314 (solvent system: n-hexane: Ethyl acetate: 3:1)

^{1}H-NMR (400 MHz, TMS, CDCl3, δ/ppm): 8.002 (d, 2H, Ar H), 7.6 (d, 2H, Ar H), 7.3 (m, 2H, Ar H), 7.2 m (m, 2H, Ar H)

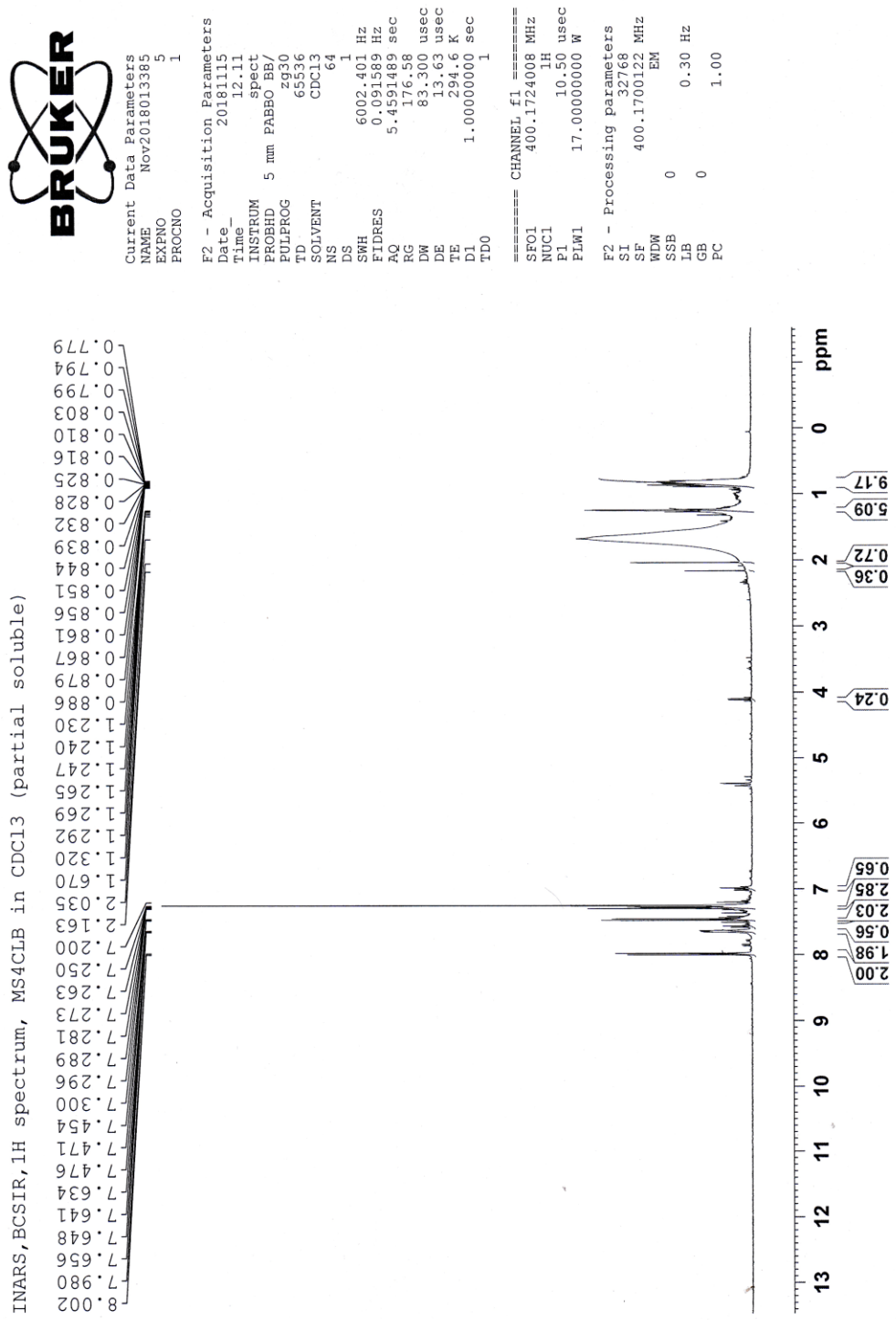
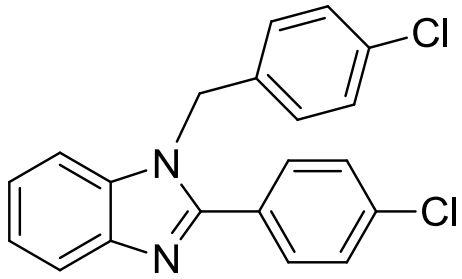


Figure 2.5: ^{1}H-NMR spectroscopy of 2-(4-chlorophenyl)-1H-benzo[d]imidazole

Product Code: DS4ClB: 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole



Appearance: Off-white powder

Molecular weight (calculated): 353.24

R_f value: 0.4 (solvent system: n-hexane: Ethyl acetate: 3:1)

^{1}H-NMR (400 MHz, TMS, CDCl3, δ/ppm): 5.38 (2H, s, CH2) 7.18- 7.86 (12H, m, Ar–H)

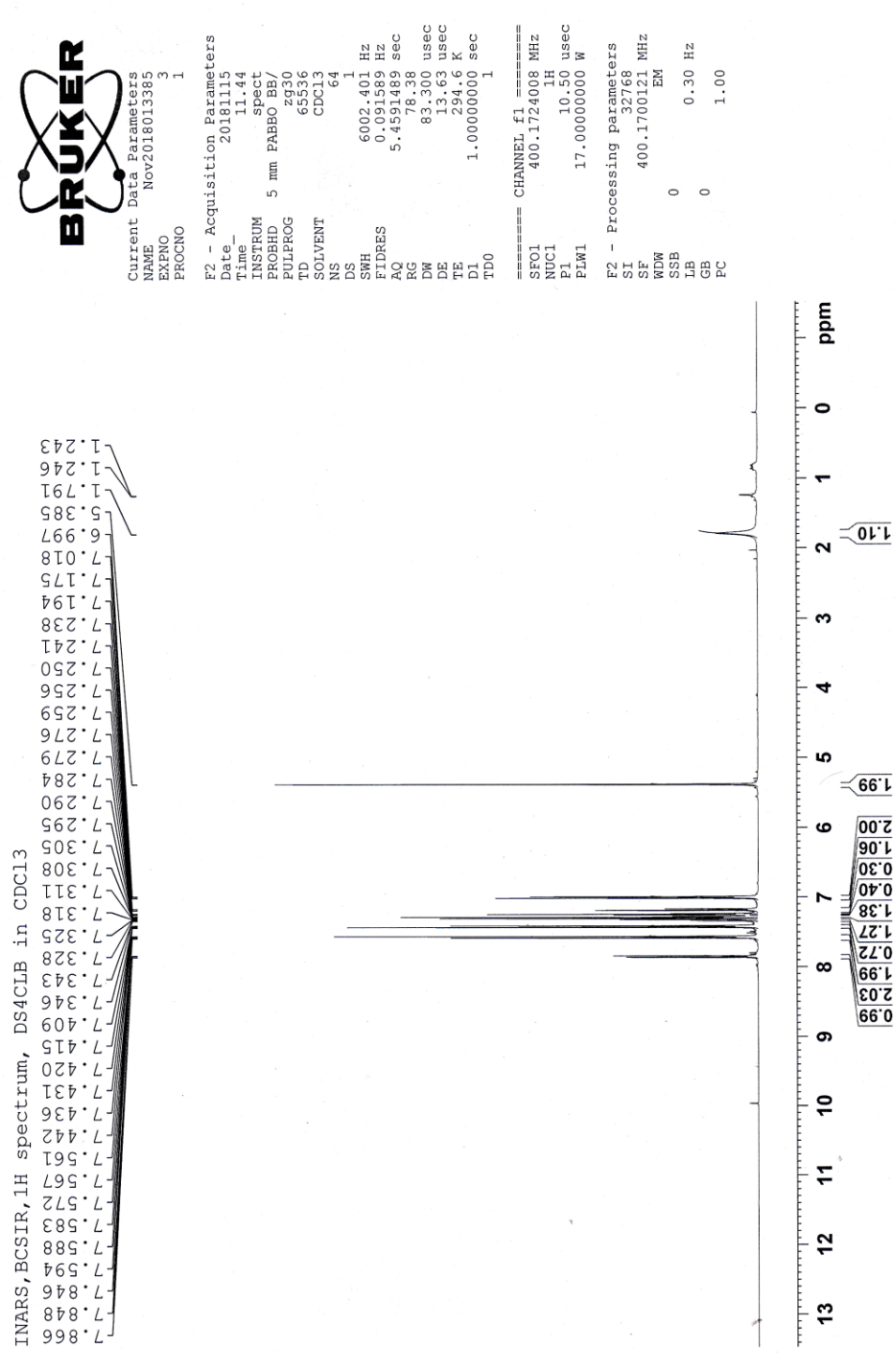
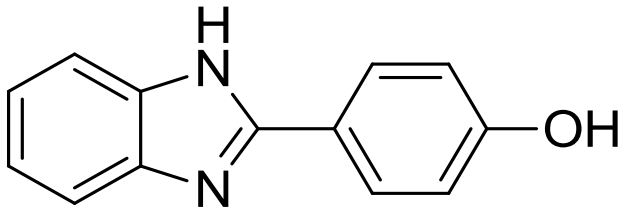


Figure 2.6: ^{1}H-NMR spectroscopy of 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole

Product Code- MSB4OH: 4-(1H-benzo[d]imidazol-2-yl) phenol



Appearance: slightly yellowish powder

Molecular weight (calculated): 210.23

R_f: 0.19 (solvent system – n-hexane: Ethyl acetate: 1:1)

^{1}H-NMR (400 MHz, TMS, CDCl3, δ/ppm): 9.8 (s, H, NH-) 6.5-7.8 (m, 8H,-Ar)

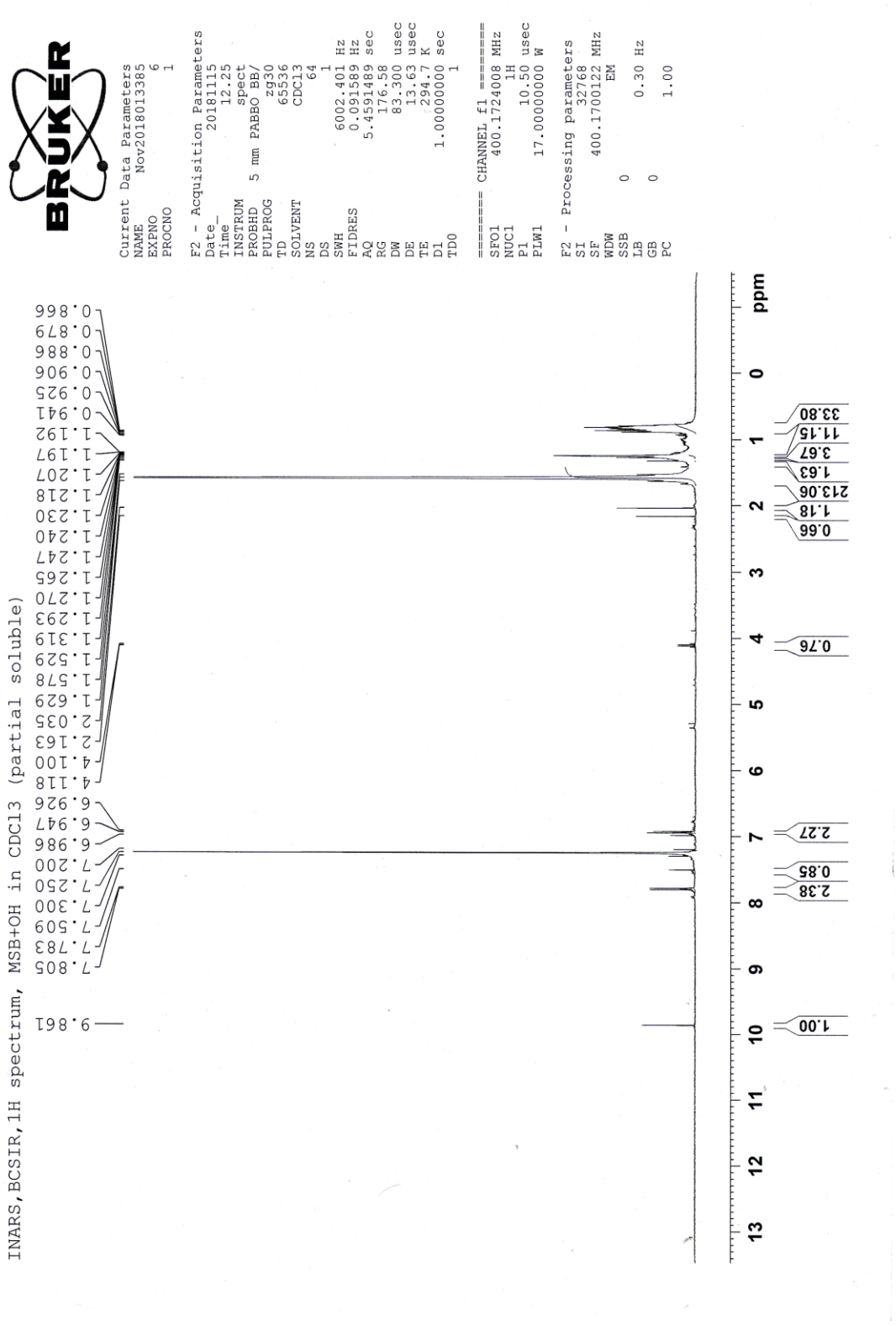
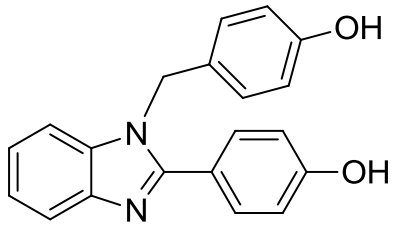


Figure 2.7: ^{1}H-NMR spectroscopy of 4-(1H-benzo[d]imidazol-2-yl) phenol

Product Code: DSB4OH: 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol



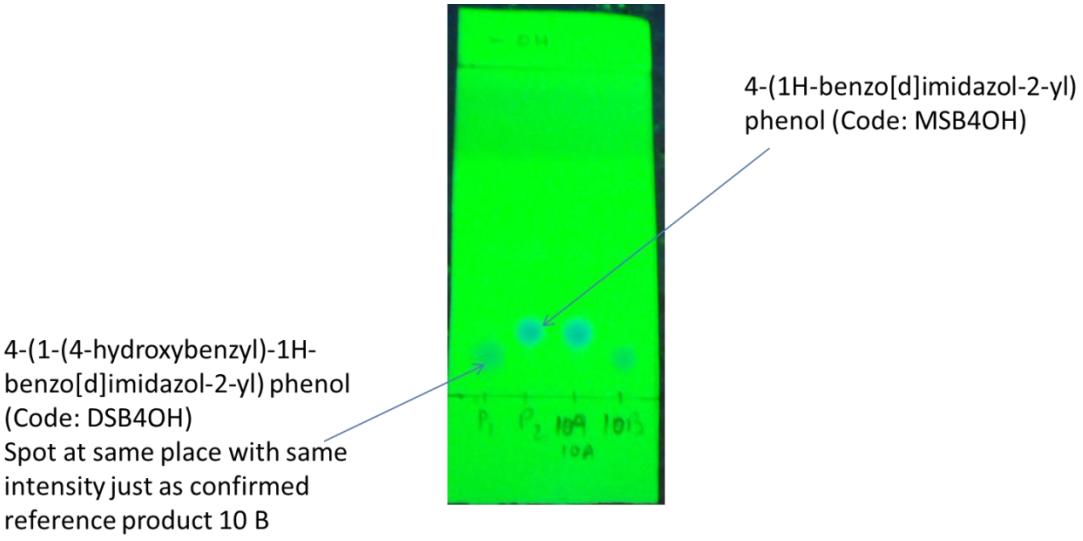
Appearance: Brown solid

Molecular weight (calculated): 316.35

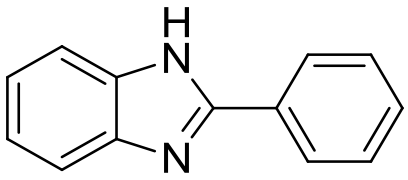
R_f value: 0.11 (solvent system – n-hexane: Ethyl acetate: 1:1)

^{1}H-NMR: data could not be obtained due to solubility problem.

Product is identified by TLC appearance comparing with reference product established at our laboratory.



Product Code: MSBA: 2-phenyl-1H-benzo[d]imidazole



Appearance: Cream color powder

Molecular weight (calculated): 194.23

R_f value: 0.42 (Solvent system: n-hexane: Ethyl acetate=1:1).

^{1}H-NMR (400 MHz, TMS, CDCl3, δ/ppm): 8.06 (m, 2H, Ar-H), 7.25-7.4 (m, 5H, Ar-H), 7.05(2H, Ar-H), 5.46 (s, 2H).

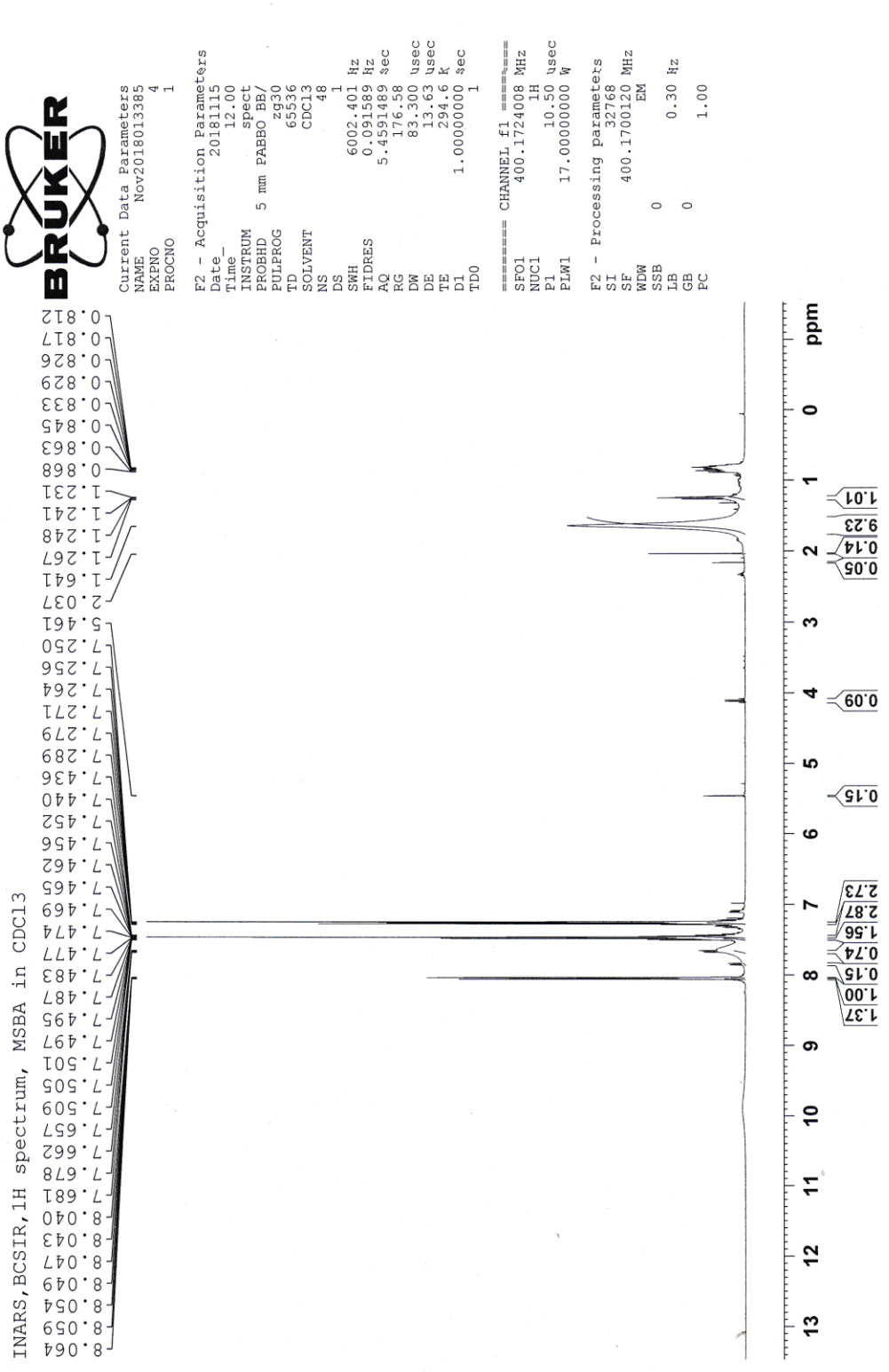
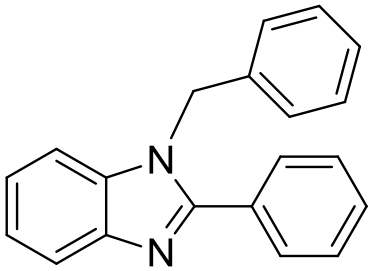


Figure 2.8: ^{1}H-NMR spectroscopy of 2-phenyl-1H-benzo[d]imidazole

Code: DSBA: 1-benzyl-2-phenyl-1H-benzo[d]imidazole



Appearance: Off white powder

Molecular weight (calculated): 284.35

R_f value: 0.49 (Solvent system: n-hexane: Ethyl acetate=1:1)

^{1}H-NMR (400 MHz, TMS, CDCl3, δ/ppm): δ 5.4 (s, 2H), 7.09 (d, 2 H, J = 7.6 Hz),

7.25-7.28 (m, 5H), 7.67-7.69 (m, 4H), 7.86 (m, 3H)

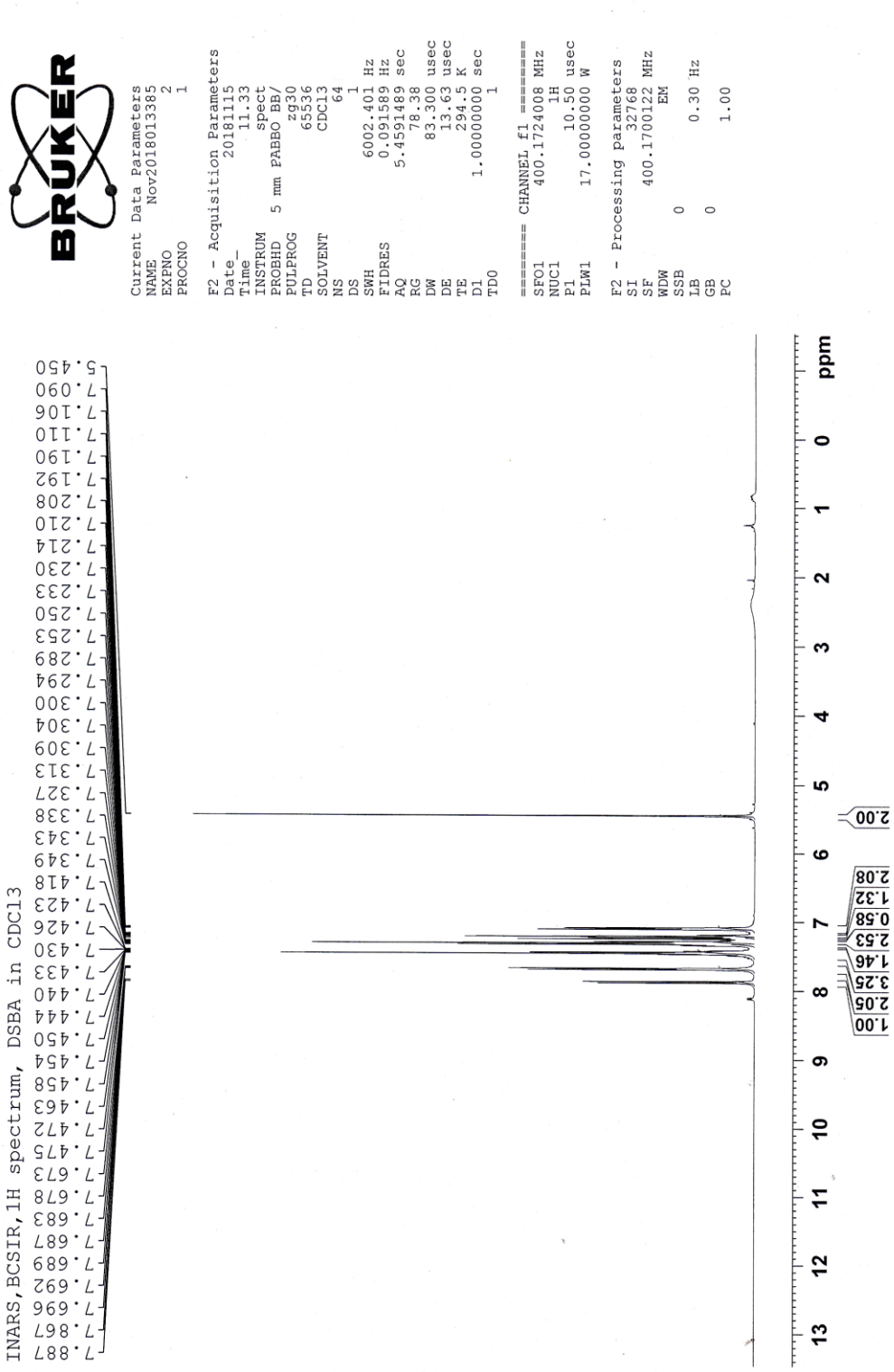


Figure 2.9: ^{1}H-NMR spectroscopy of 1-benzyl-2-phenyl-1H-benzo[d]imidazole

Chapter-3

Assumption of biologic activity through molecular docking

3.1. Assumption of biologic activity through molecular docking

Benzimidazole derivatives such as 2-substituted and 1,2 disubstituted molecules are diversified in pharmacological activities ranging from antimicrobial, anti-inflammatory, antidiabetic to more sophisticated chemotherapeutic activity. Extensive biologic activity screening was done on biological species like microbes or animals by several researchers, but as per our knowledge analysis of activities by computer aided molecular docking.

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Lengauer T. *et al*., 1996). Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

The associations between biologically relevant molecules such as proteins, peptides, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore, docking is useful for predicting both the strength and type of signal produced.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behavior plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes (Kitchen DB *et al*., 2004).

Here, in this current work, the synthesized six substituted benzimidazole derivatives were screened through molecular docking on Acetylcholine esterase enzyme to recognize the binding site and affinities for better inhibition. Pharmacologically acetylcholine esterase enzyme inhibitors are widely used in several indications and clinical situations including Alzheimer's disease, particularly apathy.

3.2. Methods and materials

Software and databases that were utilized during molecular docking were listed in Table-3.1 below-

Table-3.1: Software and databases for docking.

Software/Database	Purpose
ChemDraw Ultra 12.0	Building 2D structure of molecules
Chem3D Pro 12.0	Building 3D structure of molecules, energy minimization/optimization of those structures and saving them in PDB format
Protein Data Bank	Downloading protein structure
PyMol	Removing water and ligand from protein structure, molecular visualization and saving drug-enzyme complex in PDB format
PyRx	Docking, obtaining binding energy and getting the "out" file
Discovery Studio	Visualizing ligand interaction, getting both 2D and 3D plot of ligand interaction and obtaining surface structures of binding pocket

Standard drug:

Standard drug utilized was pralidoxime. Pralidoxime (2-pyridine aldoxime methyl chloride) or 2-PAM, usually as the chloride or iodide salts, belongs to a family of compounds called oximes that bind to organophosphate-inactivated acetylcholinesterase (Jokanović M. *et al*., 2006). It is used to treat organophosphate poisoning (Jokanović M *et al*., 2009) in conjunction with atropine and diazepam.

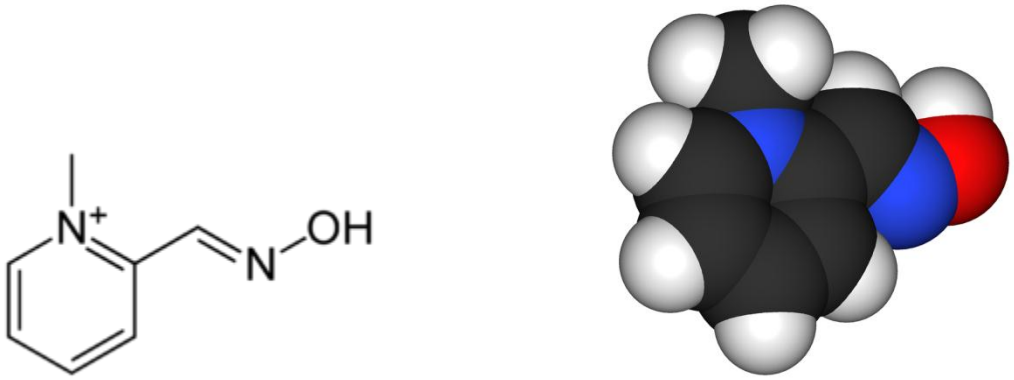


Figure-3.1: Structure of pralidoxime (on left); Space-filling model of pralidoxime (on right)

Target protein:

Molecular docking analysis of six synthesized benzimidazole derivatives was carried out on acetylcholine esterase enzyme for studying the characteristics and efficacy of binding interactions.

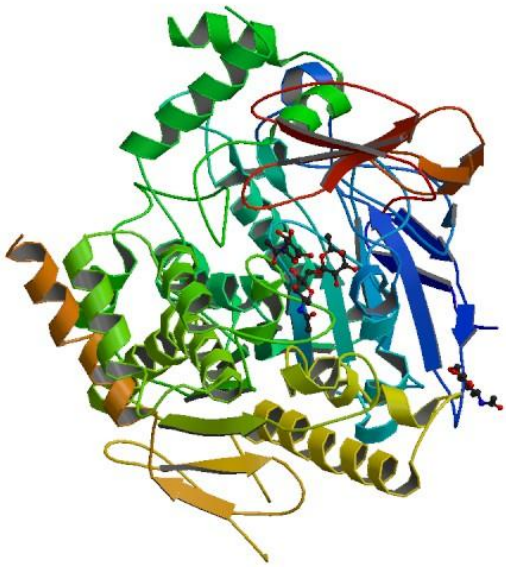


Figure- 3.2: Acetylcholinesterase structure

3.3. Screening of Acetylcholine binding affinity of pralidoxime

Pralidoxime which has strong affinity to bind to active site of acetylcholine esterase enzyme through phosphate linkage shows very strong binding affinity with maximum value of -5.7 with corresponding rmsd value near 0. (Table 3.2)

Table 3.2: Binding affinity of pralidoxime to acetylcholine esterase

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Acetylcholinesterase_5hfa_min_pralidoxime_standard	-5.7	0	0
Acetylcholinesterase_5hfa_min_pralidoxime_standard	-5.5	2.201	1.117
Acetylcholinesterase_5hfa_min_pralidoxime_standard	-5.4	3.414	2.257
Acetylcholinesterase_5hfa_min_pralidoxime_standard	-5.3	36.538	35.373
Acetylcholinesterase_5hfa_min_pralidoxime_standard	-5.3	2.346	1.718
Acetylcholinesterase_5hfa_min_pralidoxime_standard	-5.2	12.32	11.13
Acetylcholinesterase_5hfa_min_pralidoxime_standard	-5.2	29.028	28.824
Acetylcholinesterase_5hfa_min_pralidoxime_standard	-5.1	4.31	3.353
Acetylcholinesterase_5hfa_min_pralidoxime_standard	-5.1	37.325	36.34

3.4. Screening of Acetylcholine binding affinity of 2-(4-chlorophenyl)-1H-benzo[d]imidazole (product code-MS4ClB)

3.4.1. Binding affinity

Table-3.3: Binding affinity of product 2-(4-chlorophenyl)-1H-benzo[d]imidazole

Ligand	Binding Affinity	rmsd/ub	msd/lb
Acetylcholinesterase_5hfa_min_Analogoue MS4ClB _MM2_Min	-9	0	0
Acetylcholinesterase_5hfa_min_Analogoue MS4ClB _MM2_Min	-8.8	6.316	0.883
Acetylcholinesterase_5hfa_min_Analogoue MS4ClB _MM2_Min	-8.5	2.215	1
Acetylcholinesterase_5hfa_min_Analogoue MS4ClB _MM2_Min	-8.4	7.41	4.674
Acetylcholinesterase_5hfa_min_Analogoue MS4ClB _MM2_Min	-8.1	9.868	5.036
Acetylcholinesterase_5hfa_min_Analogoue MS4ClB _MM2_Min	-7.1	11.02	7.264
Acetylcholinesterase_5hfa_min_Analogoue MS4ClB _MM2_Min	-7.1	11.442	8.412
Acetylcholinesterase_5hfa_min_Analogoue MS4ClB _MM2_Min	-6.7	79.963	76.765
Acetylcholinesterase_5hfa_min_Analogoue1_MM2_Min	-6.6	32.209	30.551

3.4.2. Bond interactions

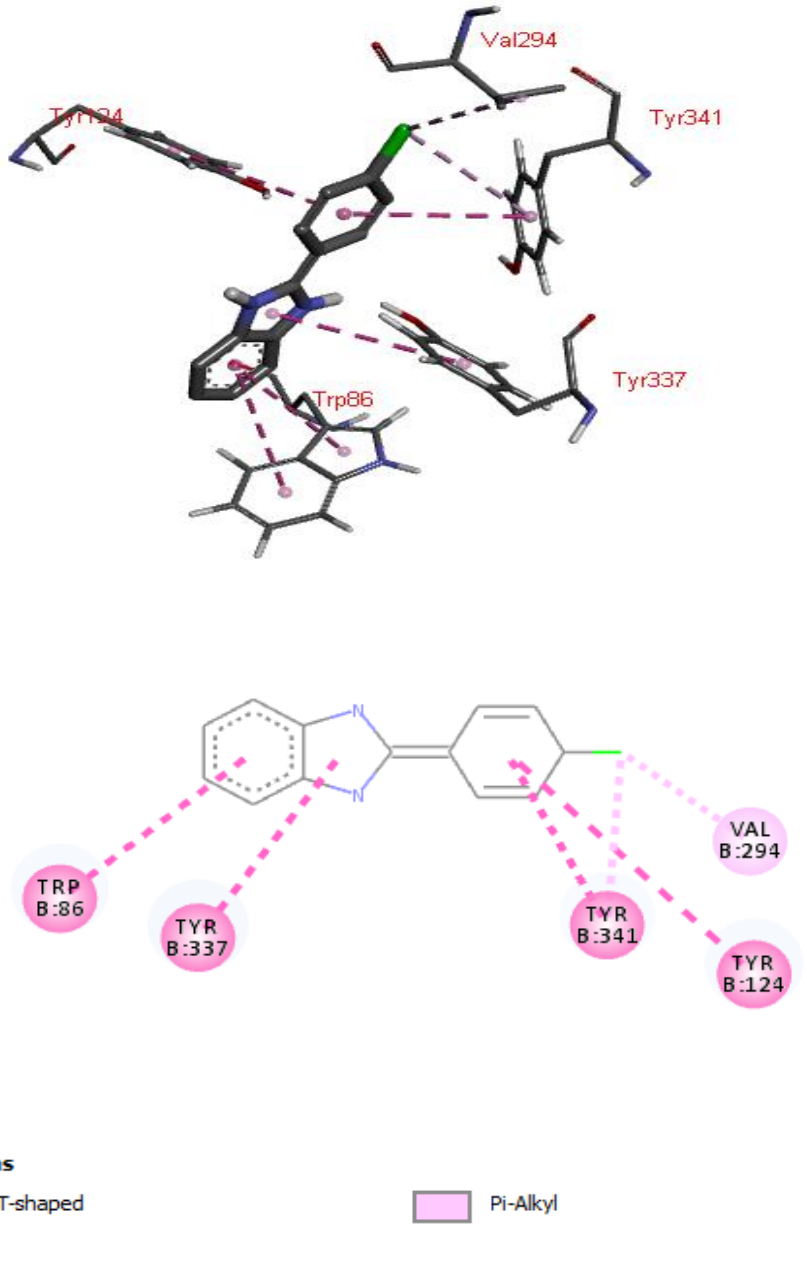


Figure-3.3: Interaction of 2-(4-chlorophenyl)-1H-benzo[d]imidazole with binding site of acetylcholine esterase enzyme.

3.4.3. Bond orientation at active site

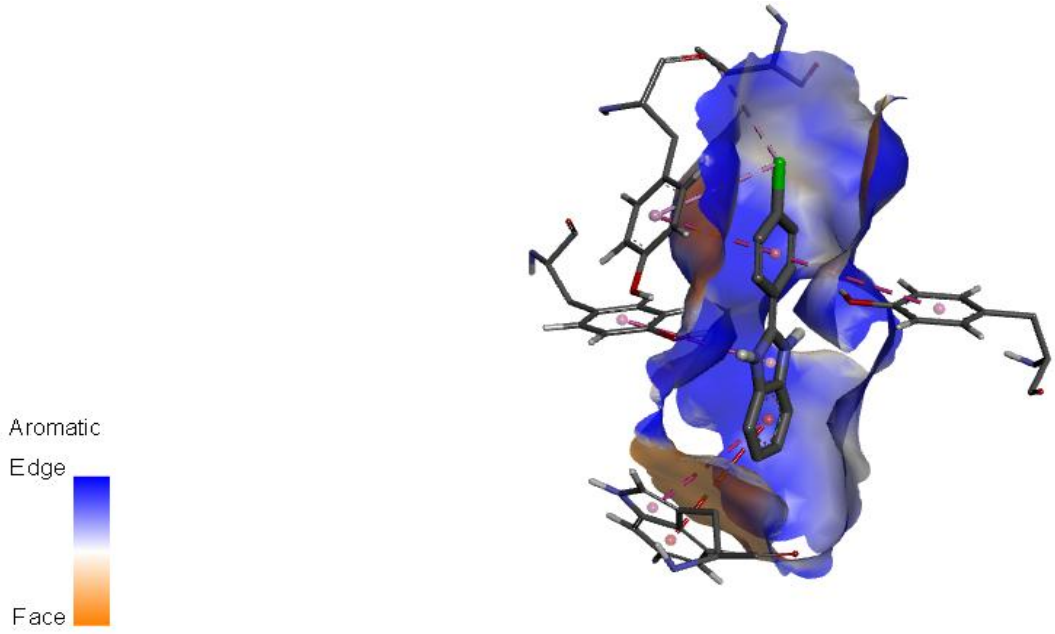


Figure-3.4: Bond orientation of 2-(4-chlorophenyl)-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

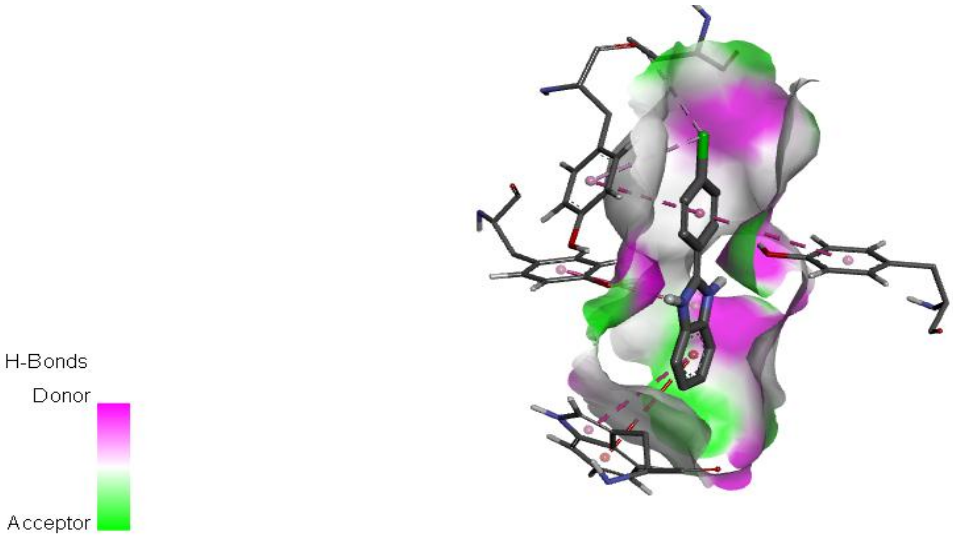


Figure-3.5: H-Bonding capability of 2-(4-chlorophenyl)-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

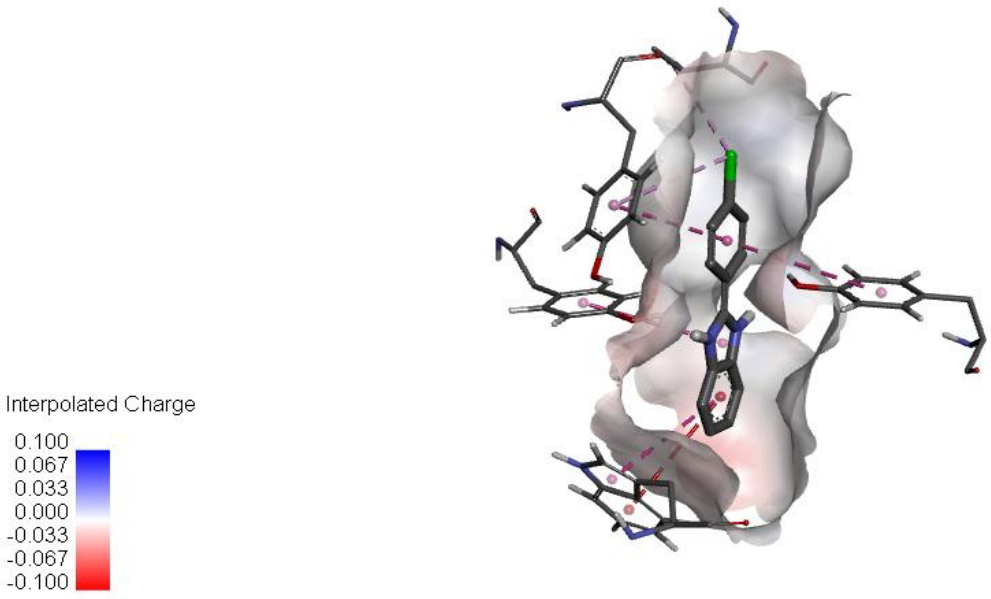


Figure-3.6: Interpolated charge of 2-(4-chlorophenyl)-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

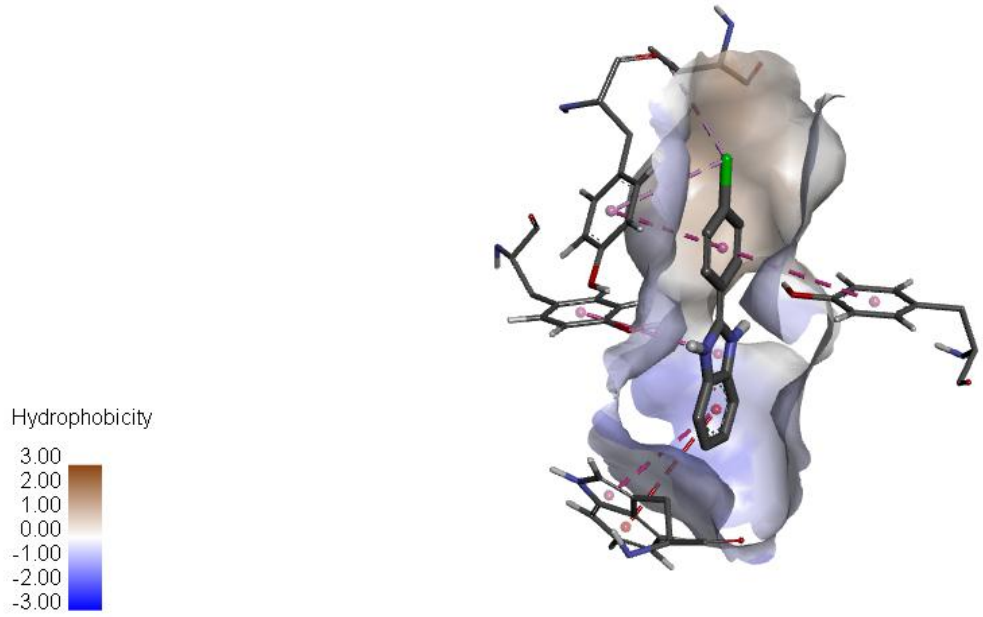


Figure-3.7: Hydrophobicity of 2-(4-chlorophenyl)-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

3.4.4. Discussion

Binding affinity of 2-(4-chlorophenyl)-1H-benzo[d]imidazole to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -9 compared to -5.7 of pralidoxime (Table-3.3). The product MS4ClB tends to interact with five amino acid residues present at the binding site namely val294, Tyr341, Tyr337, Tyr124 and Trp86 (Figure-3.3). This interaction is due to pi to pi bond interaction with tryptophan and tyrosine residues, alkyl and alkyl to pi interaction with valine residue at the binding site. This interaction is not sufficiently strong for prominent inhibitory effect on the enzyme. This binding interaction is also affected by the molecular orientation at the binding site. Docking study reveals that the molecule is oriented to the edge of the binding site where the aromatic system of the interacting amino acids faces in slightly different angle rendering the interaction much weaker (Figure-3.4). The binding site of the enzyme contains both H-bond donor and acceptor groups. The chloride substitution on the side chain renders the molecule to interact with the H-bonding accepting group at binding site. But bond distance are much longer to create a strong bonding (Figure-3.5). The molecules also possess bulkier chlorophenyl side chain which can interact with hydrophobic interaction at hydrophobic region of the binding site (Figure-3.6 and Figure-3.7).

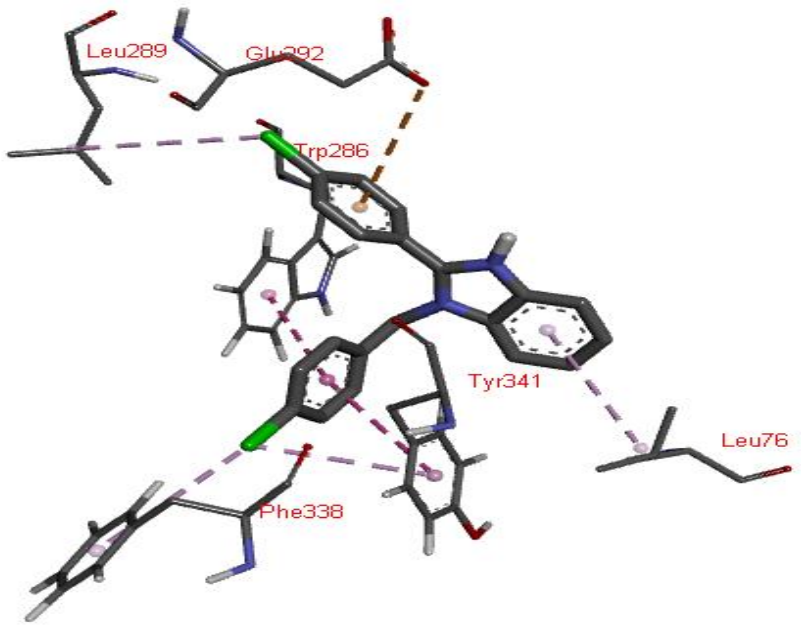
3.5. Screening of Acetylcholine binding affinity of 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole (product code-DS4ClB)

3.5.1. Binding affinity

Table-3.4: Binding affinity of product 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Acetylcholinesterase_5hfa_min_ DS4ClB _MM2_Min	-8.9	0	0
Acetylcholinesterase_5hfa_min_ DS4ClB _MM2_Min	-8.4	5.804	2.102
Acetylcholinesterase_5hfa_min_ DS4ClB _MM2_Min	-8	21.762	18.745
Acetylcholinesterase_5hfa_min_ DS4ClB _MM2_Min	-8	43.084	40.637
Acetylcholinesterase_5hfa_min_ DS4ClB _MM2_Min	-7.9	5.414	1.497
Acetylcholinesterase_5hfa_min_ DS4ClB _MM2_Min	-7.8	4.822	2.692
Acetylcholinesterase_5hfa_min_ DS4ClB _MM2_Min	-7.8	38.091	35.602
Acetylcholinesterase_5hfa_min_ DS4ClB _MM2_Min	-7.5	36.776	33.344
Acetylcholinesterase_5hfa_min_ DS4ClB _MM2_Min	-7.3	59.94	57.12

3.5.2. Bond interactions



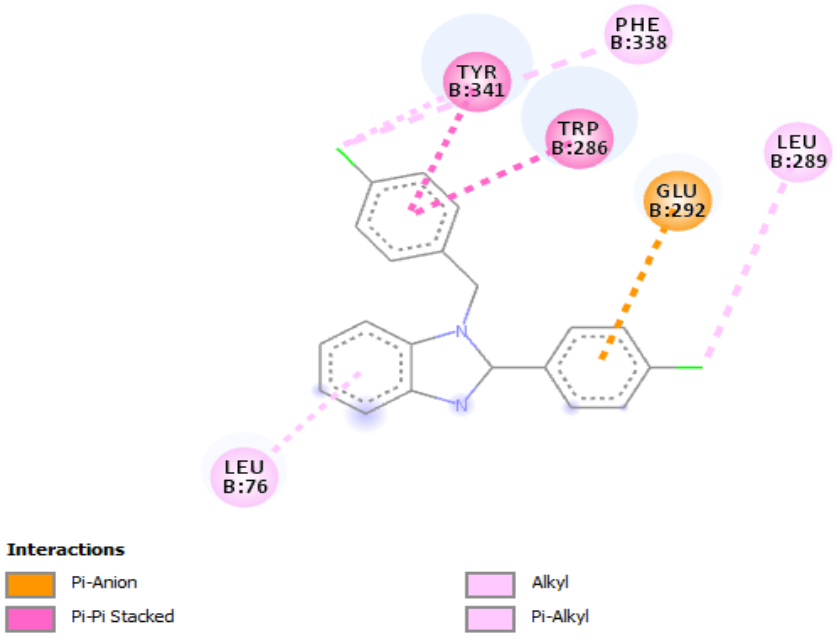


Figure-3.8: Interaction of product 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole with binding site of acetylcholine esterase enzyme.

3.5.3. Bond orientation at active site

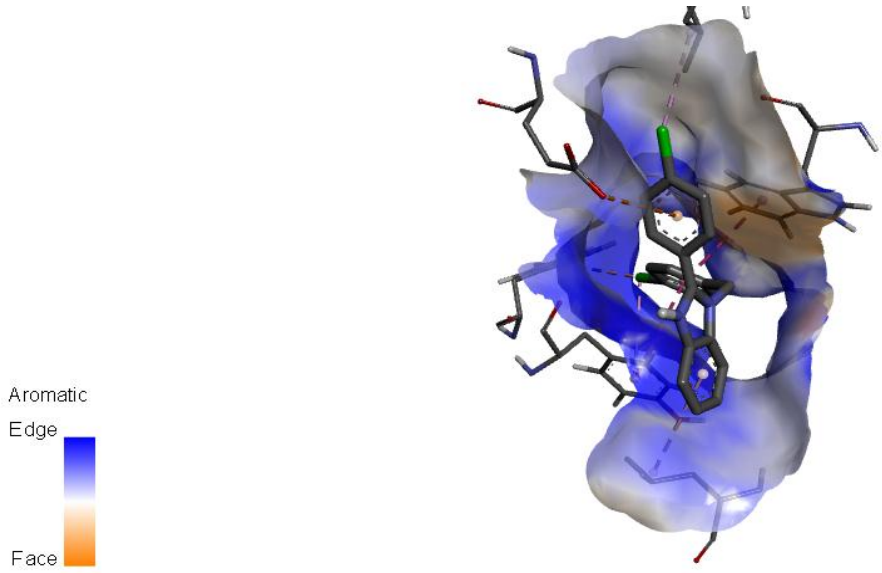


Figure-3.9: Bond orientation of product 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

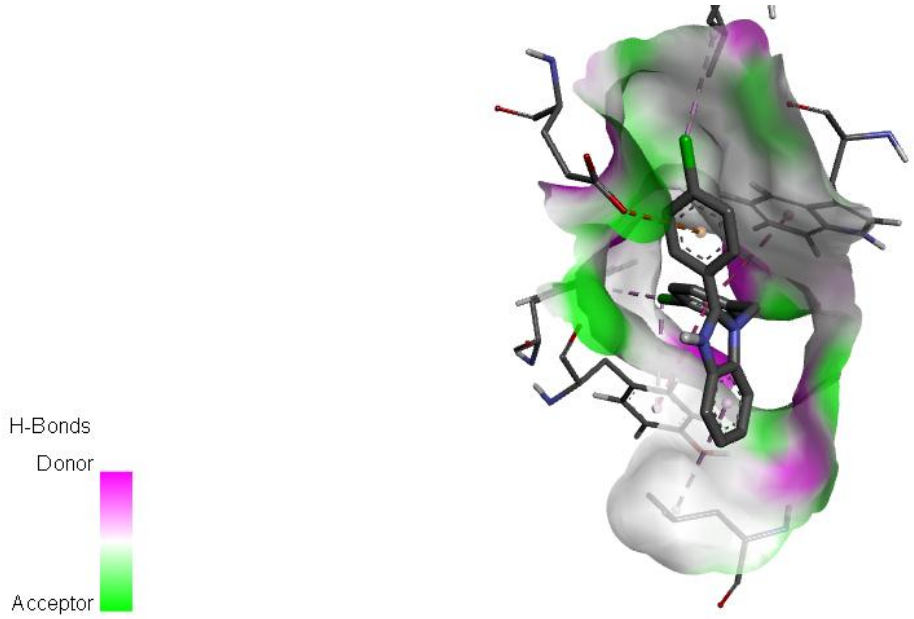


Figure-3.10: H-Bonding capability of 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

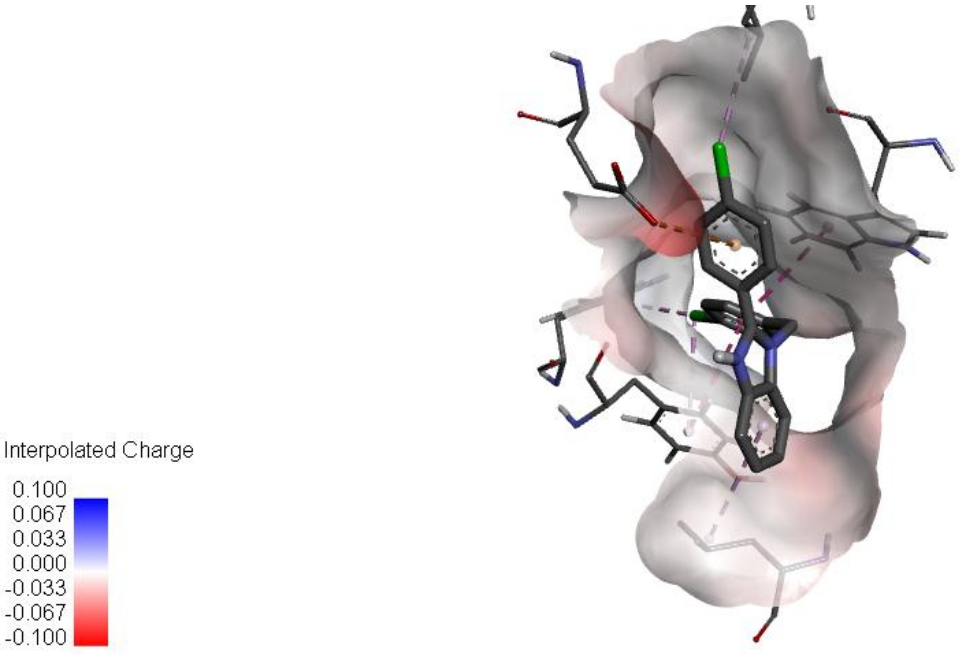


Figure-3.11: Interpolated charge of 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

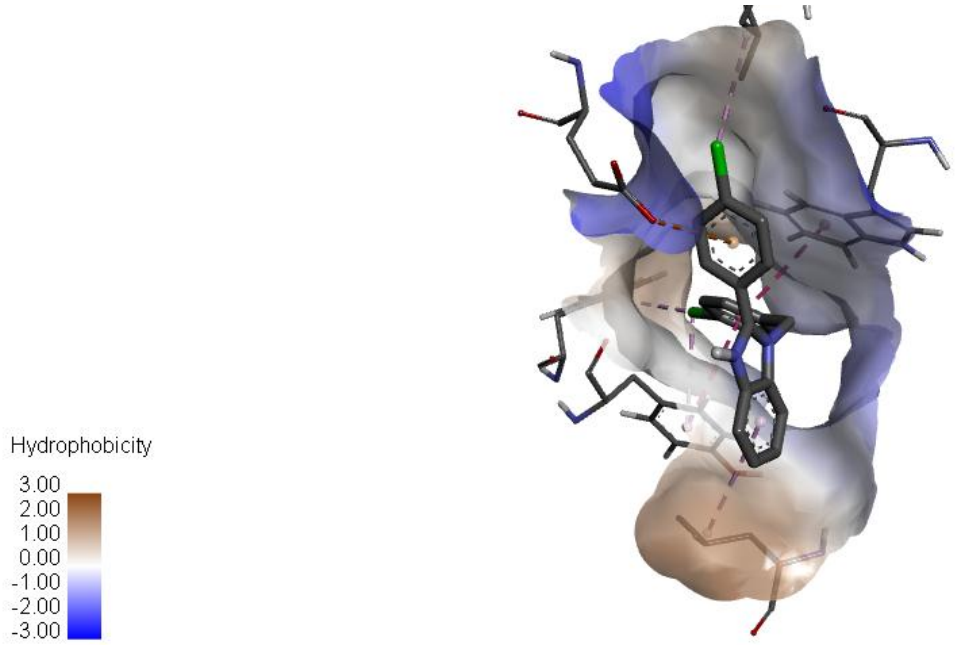


Figure-3.12: Hydrophobicity of 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

3.5.4. Discussion

Binding affinity of 2-(4-chlorophenyl)-1H-benzo[d]imidazole to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -8.9 compared to -5.7 of pralidoxime (Table-3.4). Docking of this molecule at binding site reveals the possible pi-anion interaction with Glu 292 residue, pi-pi stacked interaction with Tyr341 and Trp286 through benzyl side chain of the substitution (Figure-3.8). Docking study reveals that the benzyl side chain substituted at 1-possition is oriented to amino acid residue face (Figure-3.9) as well as to H-bonding accepting site (Figure-3.10) which enables this molecule to exhibit greater affinity than its mono substituted congener. But bond distance are much longer to create a strong bonding. The molecule also possesses bulkier chlorophenyl side chain which can interact with hydrophobic interaction at hydrophobic region of the binding site (Figure-3.11 and Figure-3.12).

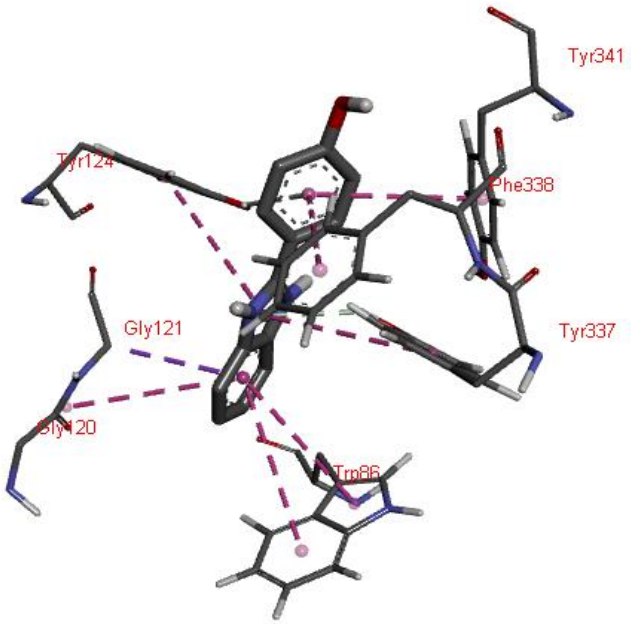
3.6. Screening of Acetylcholine binding affinity of 4-(1H-benzo[d]imidazol-2-yl) phenol (product code-MSB4OH)

3.6.1. Binding affinity

Table-3.5: Binding affinity of product 4-(1H-benzo[d]imidazol-2-yl) phenol

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Acetylcholinesterase_5hfa_min_ MSB4OH _MM2_Min	-8.3	0	0
Acetylcholinesterase_5hfa_min_ MSB4OH _MM2_Min	-7.9	2.346	1.641
Acetylcholinesterase_5hfa_min_ MSB4OH _MM2_Min	-7.8	6.21	2.869
Acetylcholinesterase_5hfa_min_ MSB4OH _MM2_Min	-6.4	29.759	28.17
Acetylcholinesterase_5hfa_min_ MSB4OH _MM2_Min	-6.3	56.267	54.561
Acetylcholinesterase_5hfa_min_ MSB4OH _MM2_Min	-6.3	44.681	42.137
Acetylcholinesterase_5hfa_min_ MSB4OH _MM2_Min	-6.2	29.364	28.062
Acetylcholinesterase_5hfa_min_ MSB4OH _MM2_Min	-6.2	19.023	18.019
Acetylcholinesterase_5hfa_min_ MSB4OH _MM2_Min	-6.2	19.825	18.245

3.6.2. Bond interactions



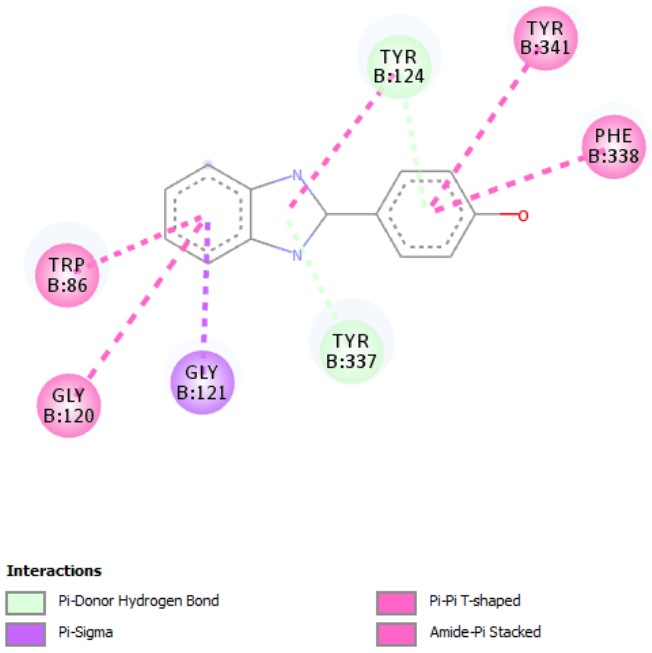


Figure-3.13: Interaction of product 4-(1H-benzo[d]imidazol-2-yl)phenol with binding site of acetylcholine esterase enzyme.

3.6.3. Bond orientation at active site

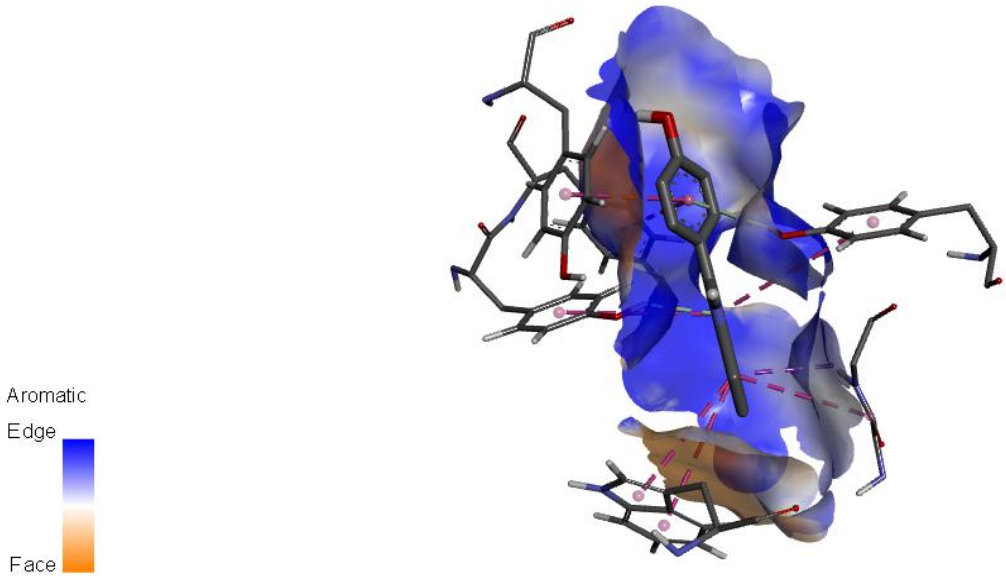


Figure-3.14: Bond orientation of product 4-(1H-benzo[d]imidazol-2-yl)phenol at binding grove of acetylcholine esterase enzyme.

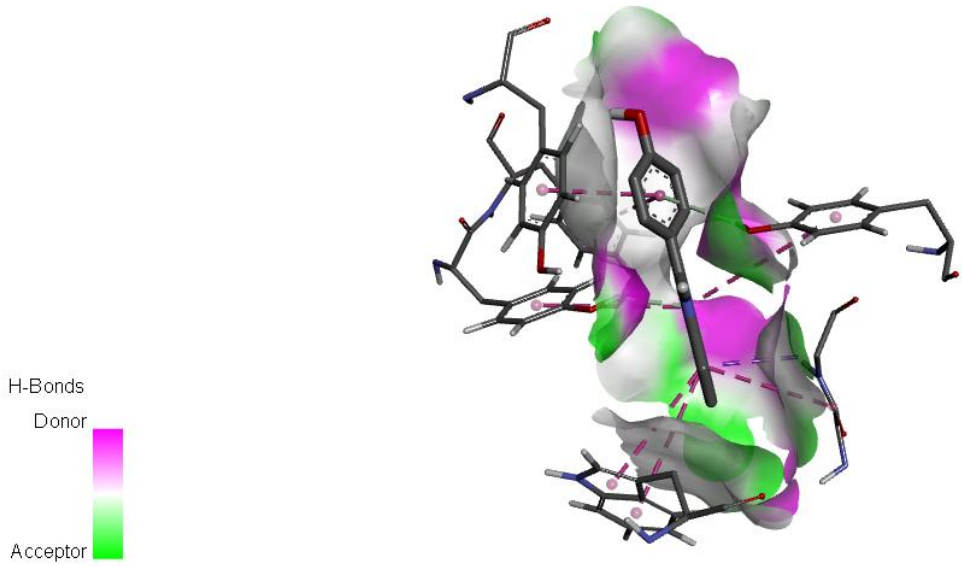


Figure-3.15: H-Bonding capability of 4-(1H-benzo[d]imidazol-2-yl)phenol at binding grove of acetylcholine esterase enzyme.

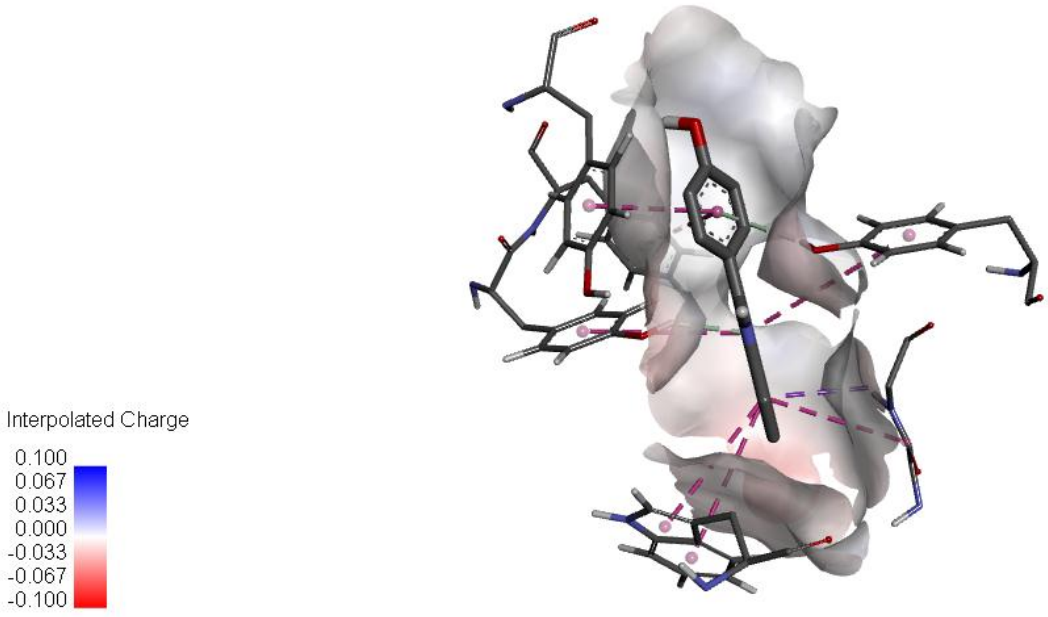


Figure-3.16: Interpolated charge of 4-(1H-benzo[d]imidazol-2-yl)phenol at binding grove of acetylcholine esterase enzyme.

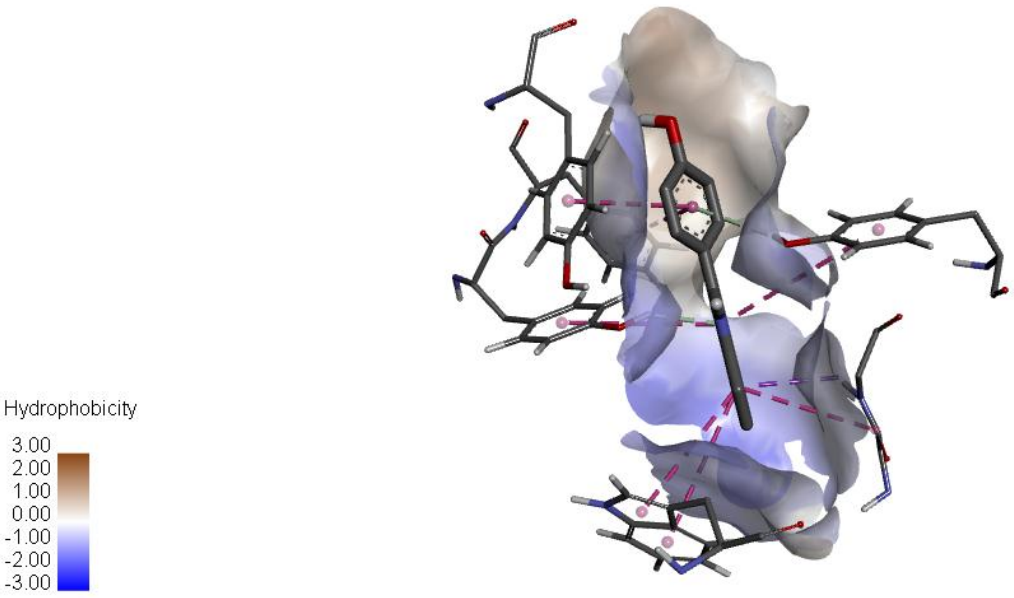


Figure-3.17: Hydrophobicity of 4-(1H-benzo[d]imidazol-2-yl)phenol at binding grove of acetylcholine esterase enzyme.

3.6.4. Discussion

Binding affinity of 4-(1H-benzo[d]imidazol-2-yl) phenol to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -8.3 compared to -5.7 of pralidoxime (Table-3.5). Molecular Docking reveals that molecule MSB4OH is oriented at the edge surfaces of most of the interacting amino acid residues (Figure- 3.14) and is oriented far from the H-bond accepting site of the enzyme. The molecule interacts with pi-donor H bond with Tyr 124 and tyr337 (Figure-3.15**)**. The molecule possesses hydroxyl group at side chain rendering weak interaction with hydrophobic region of the binding site (Figure-3.16 and Figure-3.17).

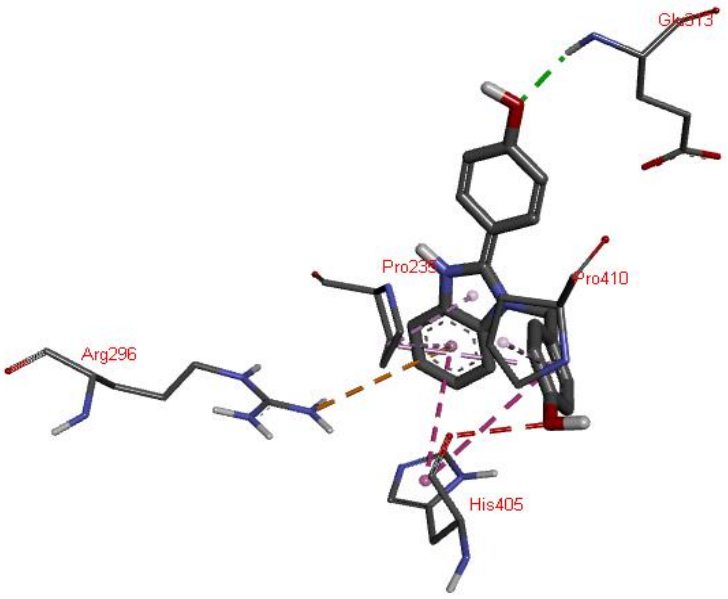
3.7. Screening of Acetylcholine binding affinity of 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol (product code-DSB4OH)

3.7.1. Binding affinity

Table-3.6: Binding affinity of product 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Acetylcholinesterase_5hfa_min_ DSB4OH _MM2_Min	-8.3	0	0
Acetylcholinesterase_5hfa_min_ DSB4OH _MM2_Min	-8	31.764	29.374
Acetylcholinesterase_5hfa_min_ DSB4OH _MM2_Min	-8	31.584	29.365
Acetylcholinesterase_5hfa_min_ DSB4OH _MM2_Min	-7.9	4.724	2.36
Acetylcholinesterase_5hfa_min_ DSB4OH _MM2_Min	-7.8	31.704	29.423
Acetylcholinesterase_5hfa_min_ DSB4OH _MM2_Min	-7.8	30.918	28.436
Acetylcholinesterase_5hfa_min_ DSB4OH _MM2_Min	-7.4	68.851	66.364
Acetylcholinesterase_5hfa_min_ DSB4OH _MM2_Min	-7.4	31.973	29.408
Acetylcholinesterase_5hfa_min_ DSB4OH _MM2_Min	-7.1	49.831	46.803

3.7.2. Bond interactions



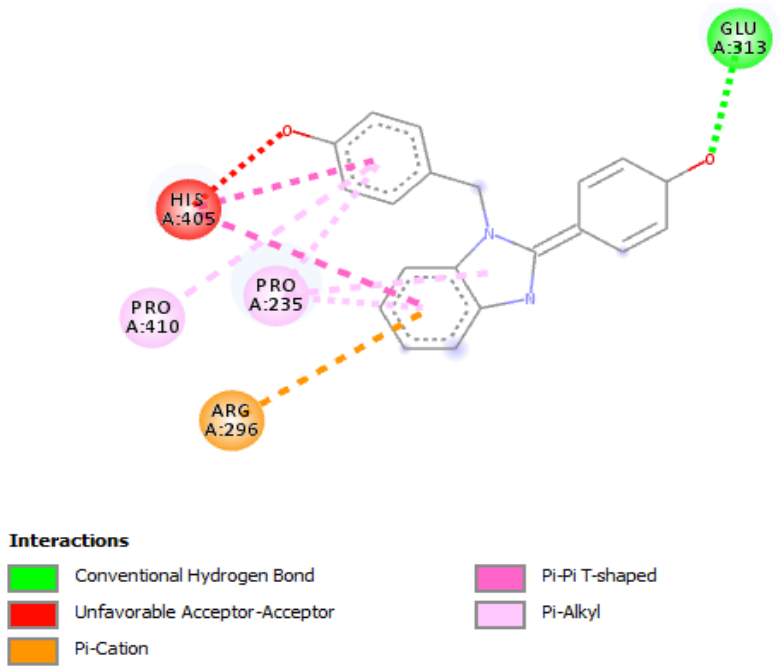


Figure-3.18: Interaction of product 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol with binding site of acetylcholine esterase enzyme.

3.7.3. Bond orientation at active site

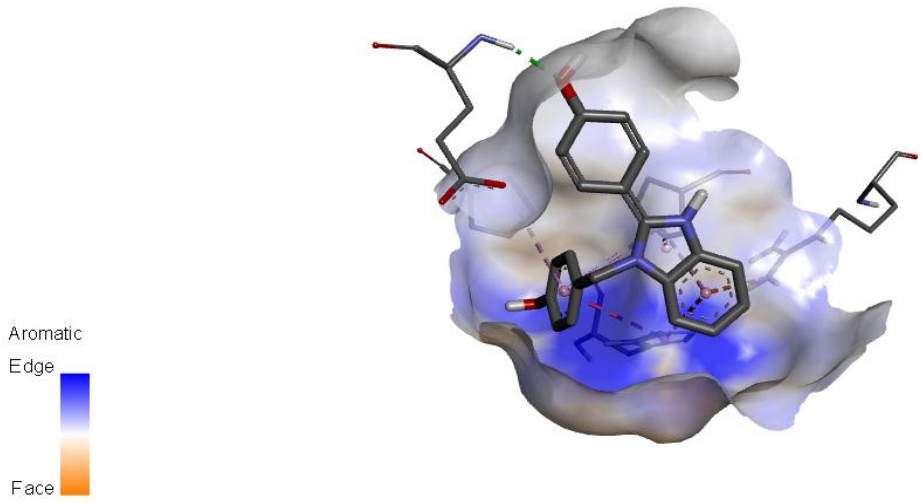


Figure-3.19: Bond orientation of product 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol at binding grove of acetylcholine esterase enzyme.

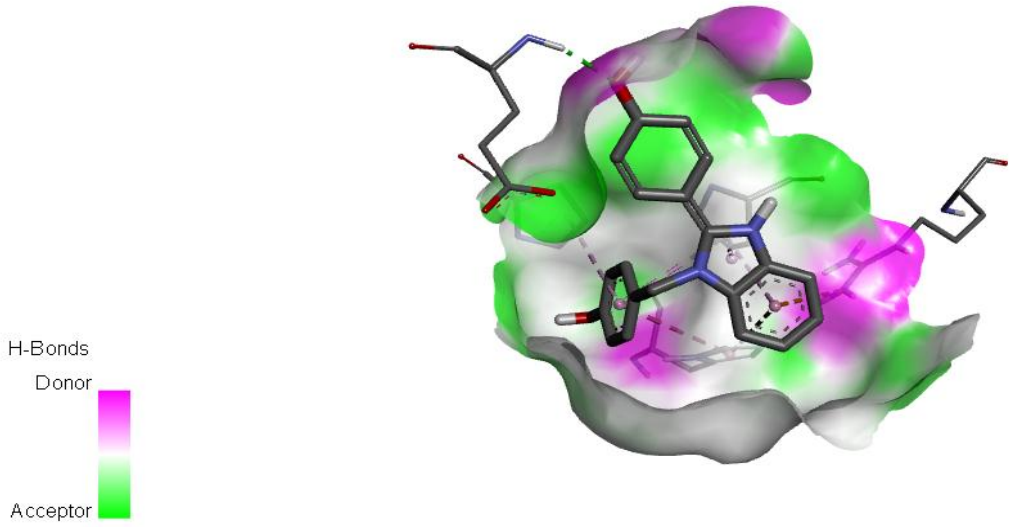


Figure-3.20: H-Bonding capability of 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol at binding grove of acetylcholine esterase enzyme.

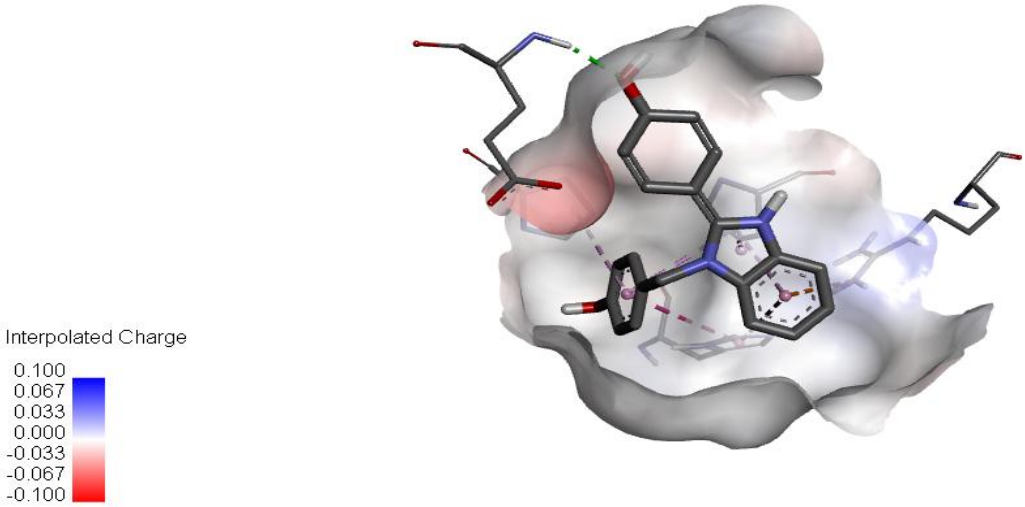


Figure-3.21: Interpolated charge of 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol at binding grove of acetylcholine esterase enzyme.

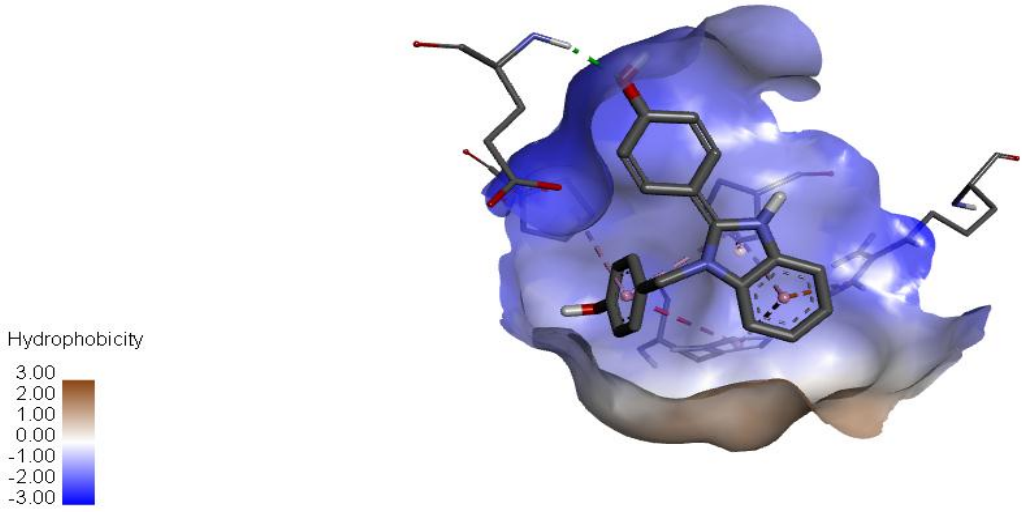


Figure-3.22: Hydrophobicity of 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol at binding grove of acetylcholine esterase enzyme.

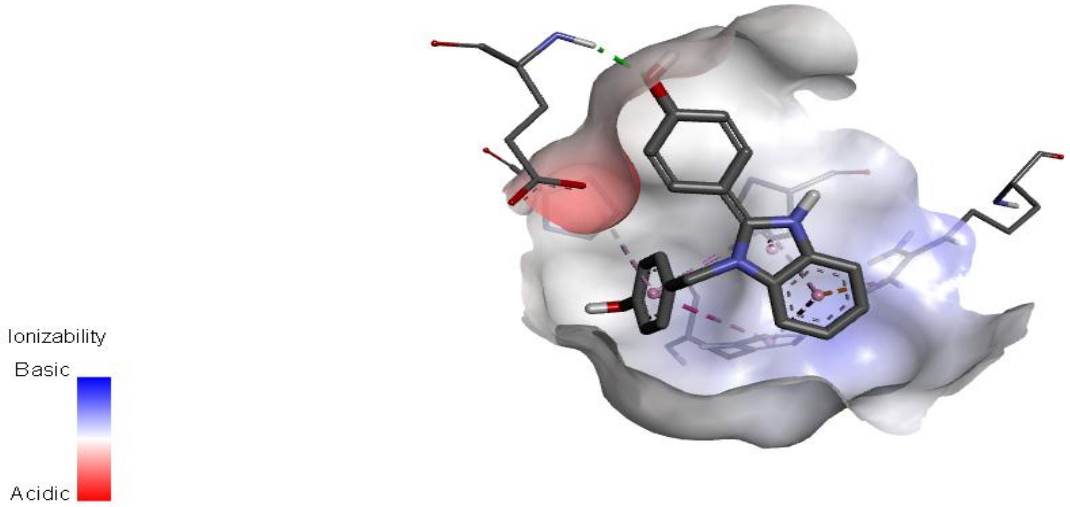


Figure-3.23: Ionizability of 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol at binding grove of acetylcholine esterase enzyme.

3.7.4. Discussion

Binding affinity of 4-(1-(4-hydroxybenzyl)-1H-benzo[d]imidazol-2-yl) phenol to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -8.3 compared to -5.7 of pralidoxime (Table-3.6). Molecular Docking reveals that molecule DSB4OH is oriented at the edge surfaces of most of the interacting amino acid residues (Figure- 3.19) and is oriented to the H-bond accepting site of the enzyme which promotes conventional H-bonding with Glu313 residue at binding pocket (Figure-3.20). The molecule possesses hydroxyl groups at side chain rendering weak interaction with hydrophobic region of the binding site (Figure-3.21 and Figure-3.22).

3.8. Screening of Acetylcholine binding affinity of 2-phenyl-1H-benzo[d]imidazole (product code-MSBA)

3.8.1. Binding affinity

Table-3.7: Binding affinity of product 2-phenyl-1H-benzo[d]imidazole

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Acetylcholinesterase_5hfa_min_ MSBA _MM2_Min	-10.8	0	0
Acetylcholinesterase_5hfa_min_ MSBA _MM2_Min	-8.4	58.398	56.564
Acetylcholinesterase_5hfa_min_ MSBA _MM2_Min	-8.3	61.352	59.175
Acetylcholinesterase_5hfa_min_ MSBA _MM2_Min	-8.1	58.51	56.026
Acetylcholinesterase_5hfa_min_ MSBA _MM2_Min	-8.1	58.25	56.206
Acetylcholinesterase_5hfa_min_ MSBA _MM2_Min	-8	58.508	56.104

3.8.2. Bond interactions

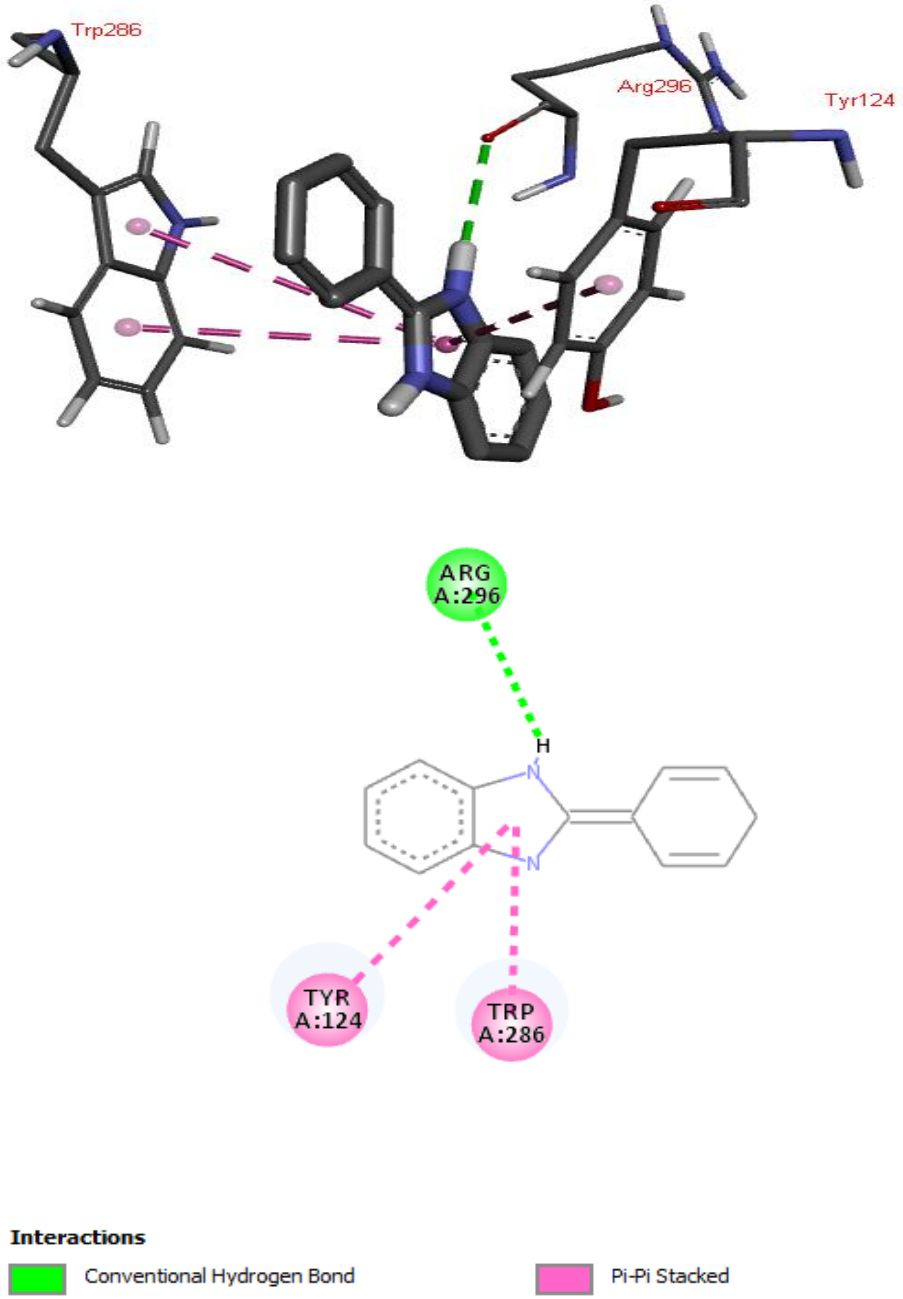


Figure-3.24: Interaction of product 2-phenyl-1H-benzo[d]imidazole with binding site of acetylcholine esterase enzyme.

3.8.3. Bond orientation at active site

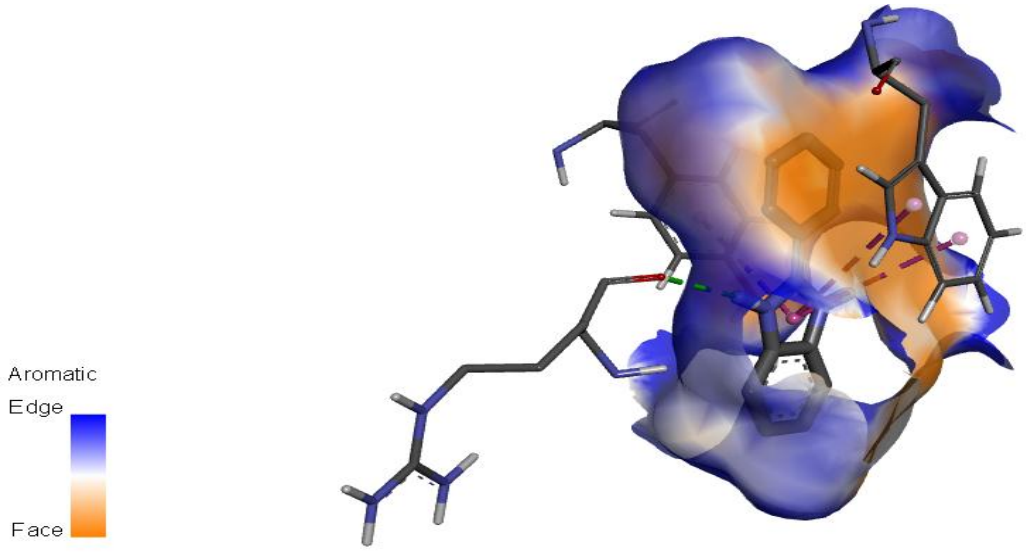


Figure-3.25: Bond orientation of product 2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

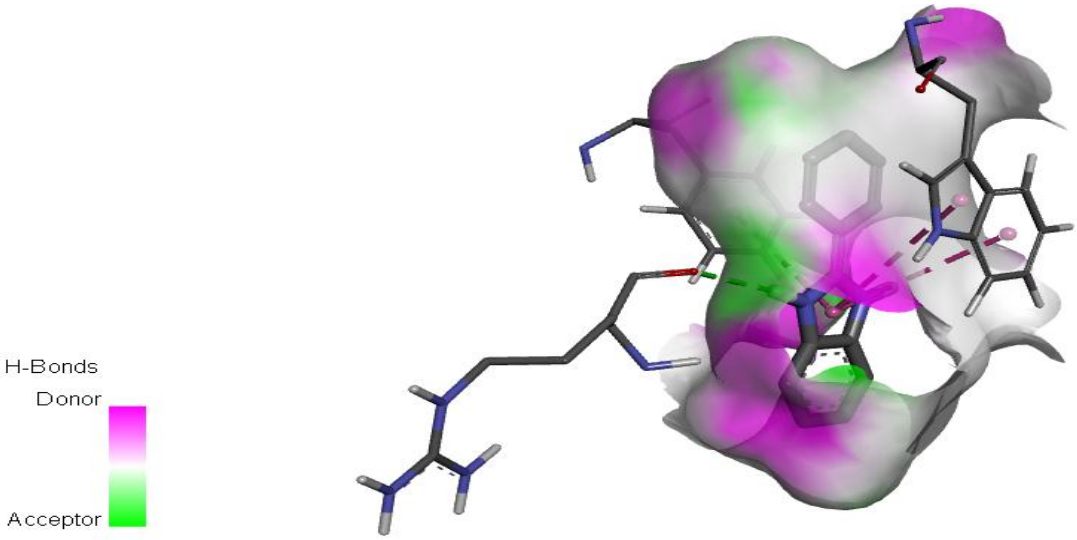


Figure-3.26: H-Bonding capability of 2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

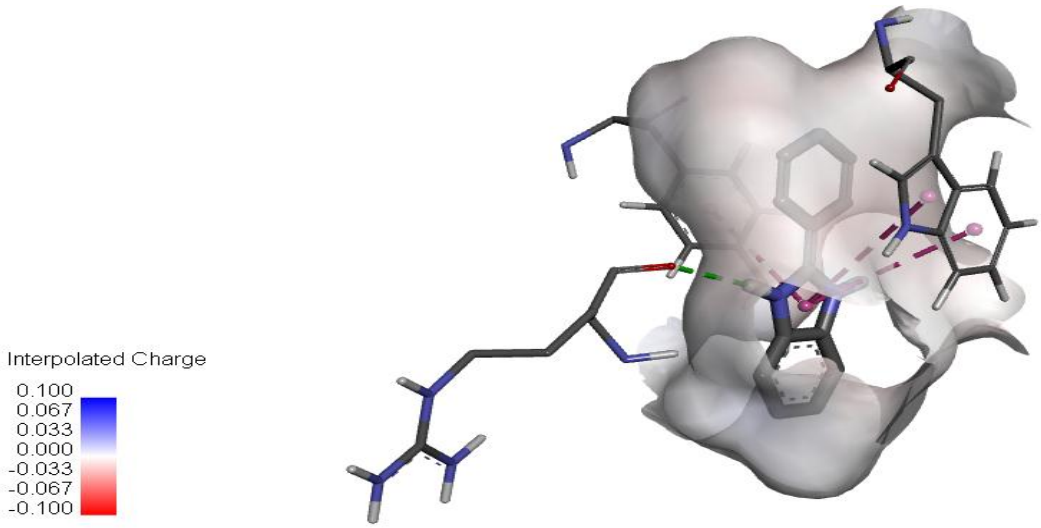


Figure-3.27: Interpolated charge of 2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

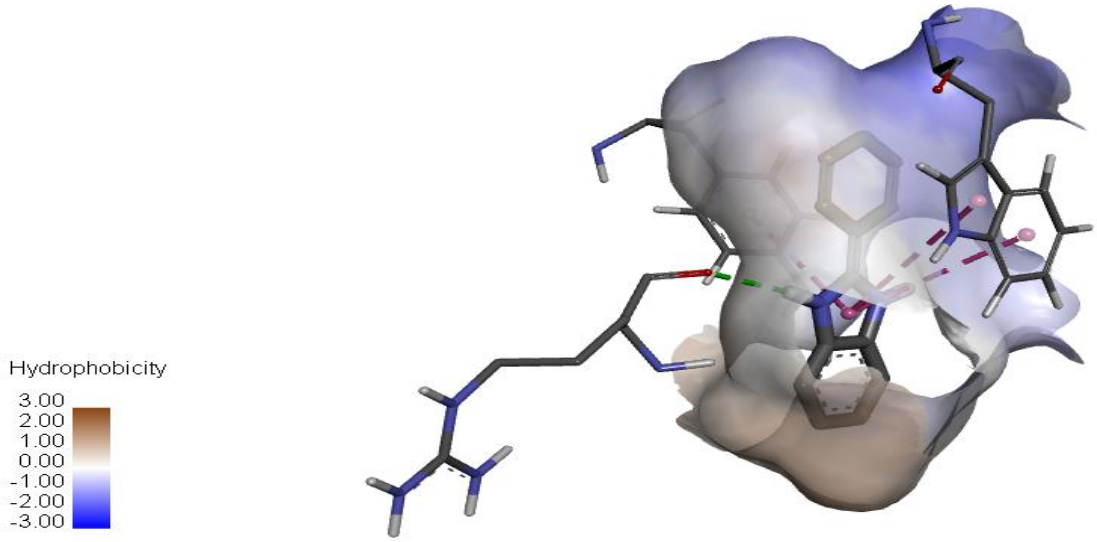


Figure-3.28: Hydrophobicity of 2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

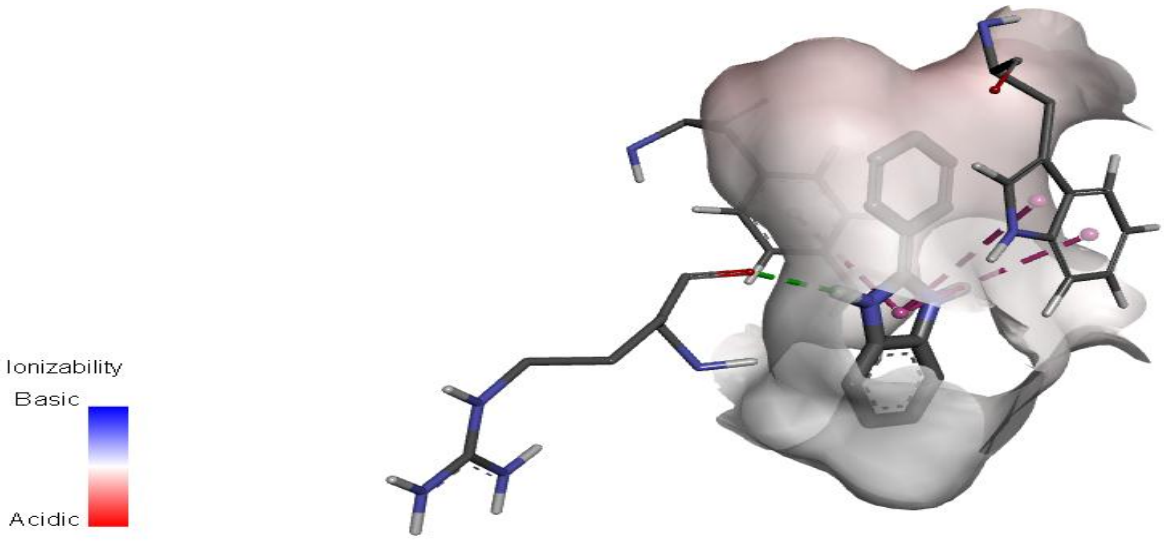


Figure-3.29: Ionizability of 2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

3.8.4. Discussion

Binding affinity of 2-phenyl-1H-benzo[d]imidazole to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -10.8 compared to -5.7 of pralidoxime (Table-3.7). Molecular Docking reveals that this smaller molecule MSBA is oriented at the face surfaces of most of the interacting amino acid residues (Figure-3.25) and is oriented to the H-bond donor site of the enzyme which promotes conventional H-bonding with Arg296 residue at binding pocket (Figure-3.26). Benzene ring of benzimidazole can protrude to hydrophobic region to some extent (Figure-3.27 to 3.29).

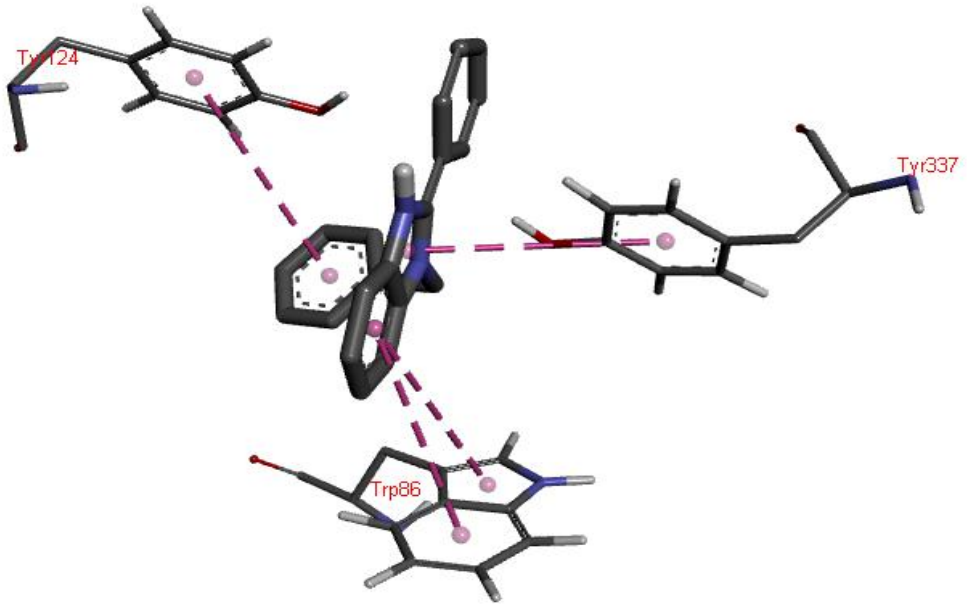
3.9. Screening of Acetylcholine binding affinity of 1-benzyl-2-phenyl-1H-benzo[d]imidazole (product code-DSBA)

3.9.1. Binding affinity

Table-3.8: Binding affinity of product 1-benzyl-2-phenyl-1H-benzo[d]imidazole

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Acetylcholinesterase_5hfa_min_ DSBA_MM2_Min	-9	0	0
Acetylcholinesterase_5hfa_min_ DSBA_MM2_Min	-9	10.177	6.509
Acetylcholinesterase_5hfa_min_ DSBA_MM2_Min	-8.4	12.552	8.746
Acetylcholinesterase_5hfa_min_ DSBA_MM2_Min	-7.9	20.286	19.068
Acetylcholinesterase_5hfa_min_ DSBA_MM2_Min	-7.7	46.232	43.458
Acetylcholinesterase_5hfa_min_ DSBA_MM2_Min	-7.6	20.873	19.465
Acetylcholinesterase_5hfa_min_ DSBA_MM2_Min	-7.6	32.222	30.084
Acetylcholinesterase_5hfa_min_ DSBA_MM2_Min	-7.3	12.378	8.551
Acetylcholinesterase_5hfa_min_ DSBA_MM2_Min	-7.3	32.391	30.211

3.9.2. Bond interactions



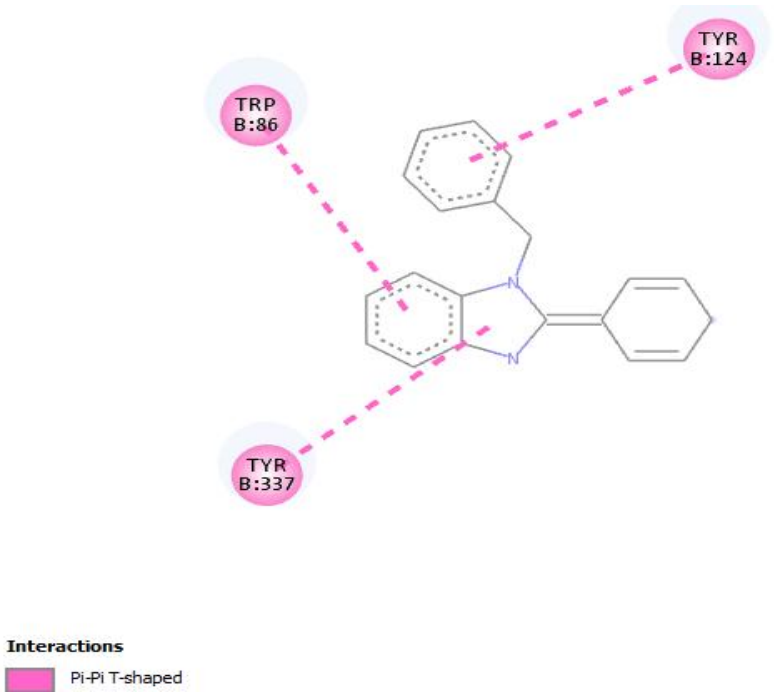


Figure-3.30: Interaction of product 1-benzyl-2-phenyl-1H-benzo[d]imidazole with binding site of acetylcholine esterase enzyme.

3.9.3. Bond orientation at active site

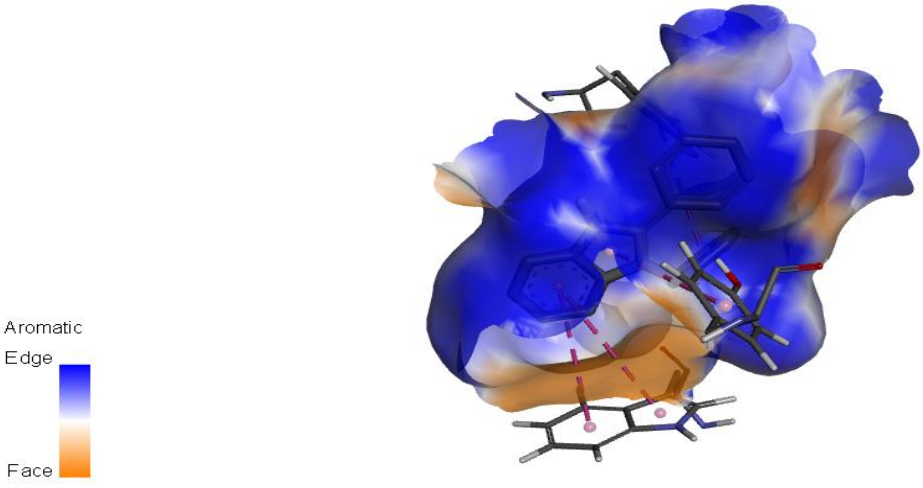


Figure-3.31: Bond orientation of product 1-benzyl-2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

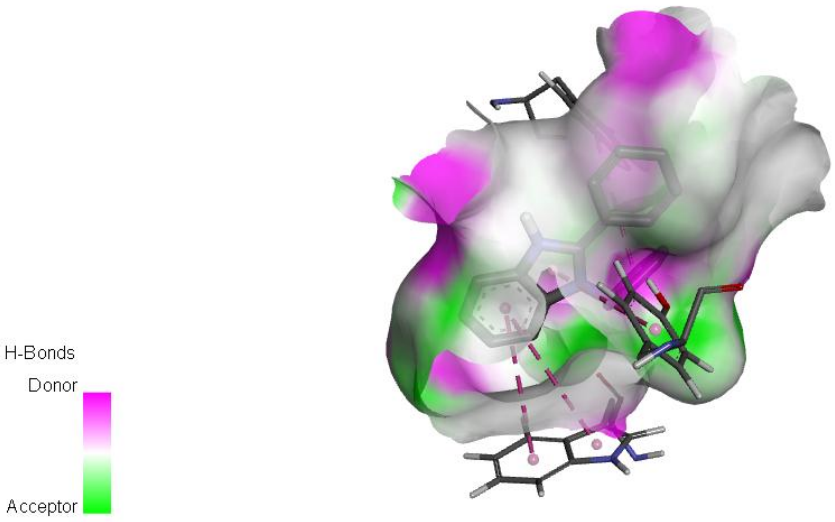


Figure-3.32: H-Bonding capability of 1-benzyl-2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

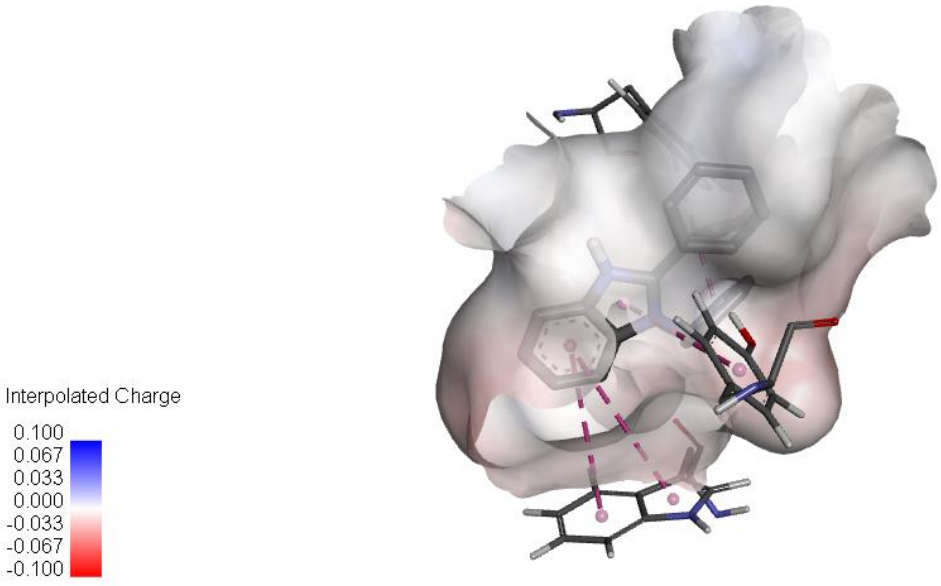


Figure-3.33: Interpolated charge of 1-benzyl-2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

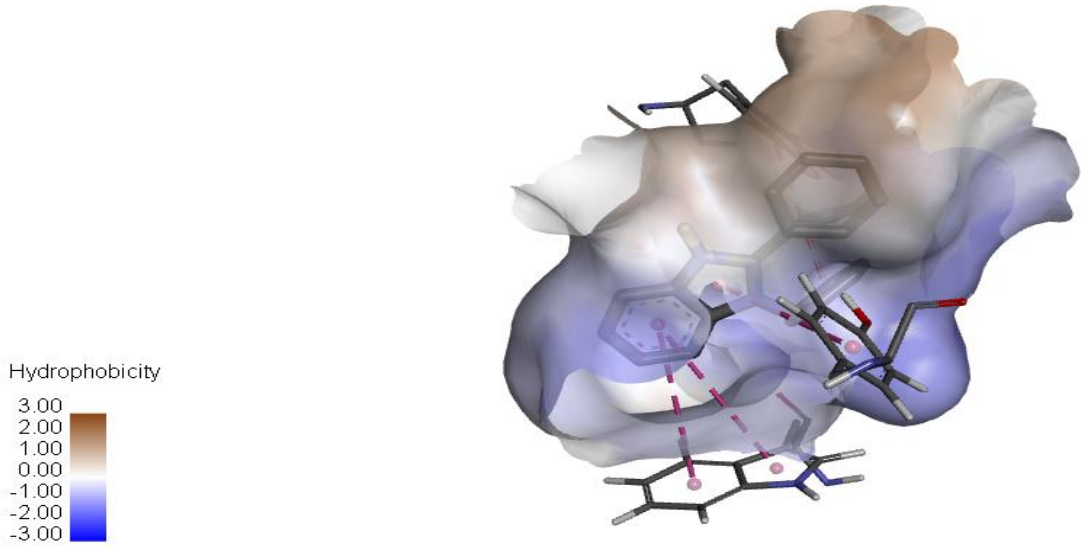


Figure-3.34: Hydrophobicity of 1-benzyl-2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

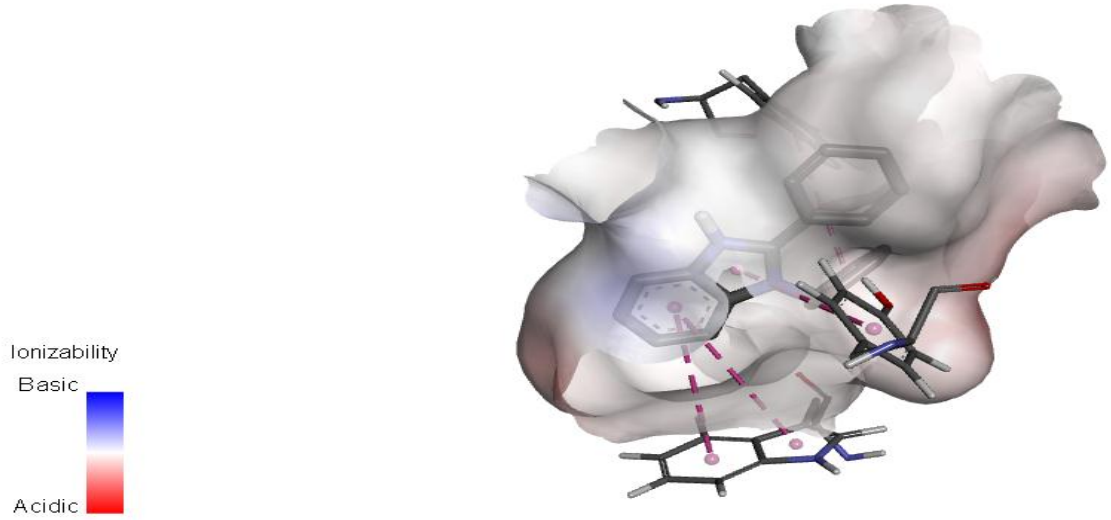


Figure-3.35: Ionizability of 1-benzyl-2-phenyl-1H-benzo[d]imidazole at binding grove of acetylcholine esterase enzyme.

3.9.4. Discussion

Binding affinity of 1-benzyl-2-phenyl-1H-benzo[d]imidazole to acetylcholine esterase is much stronger than that of pralidoxime with maximum binding affinity of -9 compared to -5.7 of pralidoxime (Table-3.8). Molecular Docking reveals that this smaller molecule DSBA is oriented at the edge surfaces of most of the interacting amino acid residues (Figure- 3.30 and 3.31) and is not oriented to the H-bond donor site of the enzyme. Hence this molecule forms pi-pi weak interaction with amino acid residue like Trp86 and Tyr124 and Tyr337 (Figure- 3.32 and 3.33). This molecule possesses weak interpolated charge and much oriented to hydrophobic interaction in weak ionizing environment (Figure- 3.33 and 3.35).

Chapter-4

Conclusion

Conclusion

From a green chemistry perspective it is imperative to find sustainable alternatives to organic solvents. Water is this case has gained increasing popularity due to being inexpensive, non-toxic, nonflammable, widely abundant in nature, and environmentally benign. To solve the problem of solubility and hydrolytic decomposition of organic molecule surface-active compounds have gained enormous potentiality.

Benzalkonium chloride is normally a pharmaceutically used excipient having a antimicrobial properties. This cationic surfactant is found to be effective in synthesizing benzimidazole derivatives. Among the four surfactants used in this study, benzalkonium chloride shows excellent affinity to produce 2-substituted benzimidazole as major product at both 1:1 and 1:2 ratios of o-phenylenediamine and aldehyde. Thus benzalkonium chloride is effective and environmentally benign alternative for synthetic purpose with easy workup procedure.

Molecular docking reveals good binding affinities for the candidates synthesized in this study and can be promising drug entity with some structural optimization. There is lacking of formation of strong classical H-bonding among the candidates with the amino acid residues at the binding site of Acetylcholine esterase enzyme. Hydrogen atom at position 1 of benzimidazole ring system of 2-phenyl-1H-benzo[d]imidazole (product code-MSBA) was found to possess conventional H-bonding with arginine residue at binding site but bond distance was much higher. Hydrogen bonding as well as hydrophobicity can be improved by substituting different groups on the benzene ring system on both benzimidazole heterocycles and on the side chain to render the candidates as good irreversible Acetylcholine esterase enzyme inhibitor of clinical importance.

Chapter-5

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