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Research paper

2-Aryl benzimidazoles: Synthesis, *In vitro* α-amylase inhibitory activity, and molecular docking study

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ABSTRACT

Despite of many diverse biological activities exhibited by benzimidazole scaffold, it is rarely explored for the α-amylase inhibitory activity. For that purpose, 2-aryl benzimidazole derivatives **1**–**45** were synthesized and screened for *in vitro* α-amylase inhibitory activity. Structures of all synthetic compounds were deduced by various spectroscopic techniques. All compounds revealed inhibition potential with IC_{50} values of 1.48 ± 0.38–2.99 ± 0.14 μM, when compared to the standard acarbose ($IC_{50} = 1.46 \pm 0.26$ μM). Limited SAR suggested that the variation in the inhibitory activities of the compounds are the result of different substitutions on aryl ring. In order to rationalize the binding interactions of most active compounds with the active site of α-amylase enzyme, *in silico* study was conducted.

1. Introduction

α-Amylase (E.C.3.2.1.1) is a metalloenzyme having Ca^{+2} ion in its active site. It catalyzes the hydrolysis of starch into glucose and maltose. This enzyme has attracted considerable attention due to its ability of hydrolyzing α-1,4-glycosidic linkage of starch and the activities that can be carried out owing to the hydrolysis. High quantity of carbohydrate uptake is associated with severe health issues such as diabetes, obesity, and oral diseases [1–4]. Diabetes mellitus (DM) is a type of metabolic disorder categorized by both fasting and post-prandial hyperglycemia and causes due to deficiency in insulin action (type 2 diabetes) or insulin secretion (type 1 diabetes), or both, thus promoting disturbance in the metabolism of carbohydrate, fat, and protein. Other pathological conditions that are associated with the long term complications of diabetes mellitus include neuropathy, retinopathy, micro-angiopathy, and cardiovascular diseases. The different therapeutic strategies used for the treatment of diabetes mellitus are stimulating the endogenous secretion of insulin, increasing the action of insulin at the target tissues, reduction of the demand for insulin, and the inhibition of degradation of oligo and disaccharides. The clinically used drugs to control diabetes are insulin, biguanide, thiazolidinediones, sulfonylureas, aldose reductase inhibitor, glucosidase inhibitors, carbamoylmethyl benzoic acid, and insulin-like growth factor. Acarbose, miglitol, and voglibose are the drugs which basically function by inhibiting α-amylase and α-glycosidase enzymes that are responsible for hydrolyzing the disaccharides and

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oligosaccharides into monosaccharides and thus prevents the absorption of glucose [5–8]. Although these medications are being clinically used for the management of type-II diabetes mellitus but also associated with many adverse effects such as diarrhea, abdominal discomfort, meteorism, and flatulence which lead to discontinuation of therapy. Therefore, for the sake of efficacy, they oftenly used with other antidiabetic agents as combination therapy. So, it is upmost necessity to find new therapies for the management of type-II diabetes mellitus with no or low risk of side effects [9–12].

Nitrogen containing heterocycles have attracted considerable attention due to their wide occurrence and pharmacological importance. Benzimidazole is an aromatic heterocyclic organic compound having benzene ring fused with an imidazole ring. Imidazole ring is the part of many natural products including purine, histamine, histidine, and nucleic acid. Due to its polar and ionisable ability, it proves to be a characteristics pharmacokinetic for lead molecules by enhancing their solubility [13–15]. Benzimidazole is one of the privileged structures in medicinal chemistry due to its wide range of activities including analgesic, antidiabetic, antiinflammatory, antiulcer, antifungal, antimicrobial, anticancer, antiprotozoal, antiviral activities *etc* [16–19].

Our research group has been working on design and synthesis of heterocyclic compounds including imidazole and benzimidazole derivatives in search of potential lead compounds since many years and had found promising results [11,20–33]. We have reported the 5-bromo-2-aryl benzimidazole derivatives as potent inhibitors for α-glucosidase enzyme [12]. Few benzimidazole chalcone derivatives are reported as α-amylase inhibitors [34] but there is still need to explore more compounds for this activity in order to identify lead candidates for more advances research in future. Thus, we decided to screen a library of substituted 2-aryl benzimidazoles for α-amylase inhibitory activity.

2. Results and discussion

2.1. Chemistry

2-Aryl benzimidazoles were prepared according to our previously reported procedure [12]. Five derivatives of *o*-phenylene diamine including unsubstituted benzene-1,2-diamine, 4,5-dimethylbenzene-1,2-diamine, 4-fluorobenzene-1,2-diamine, 4-chlorobenzene-1,2-diamine, and 4-nitrobenzene-1,2-diamine were reacted with a variety of benzaldehyde derivatives in *N,N*-dimethylformamide (DMF) as solvent. Sodium metabisulfite ($Na_2S_2O_5$) was used as catalyst (Scheme 1, Table 1). Compounds were structurally characterized by various spectroscopic techniques such as EI-MS, HREI-MS, ^{1}H NMR, and IR. ^{13}C NMR was performed for representative compounds. To the best of our knowledge, except compound **3** [35], all derivatives are new.

2.2. In vitro α-amylase inhibitory activity

Synthetic compounds **1**–**45** were evaluated for *in vitro* α-amylase inhibitory activity. All derivatives showed their inhibitory activity in the range of $IC_{50} = 1.48 \pm 0.38$–2.99 ± 0.14 μM as compared to standard acarbose ($IC_{50} = 1.46 \pm 0.26$ μM). Compounds **3**, **10**–**12**, **16**, **19**, **23**, **27**, **31**, **32**, **34**, **35**, **38**, **42**, and **43** were found to be excellent inhibitors based on their IC_{50} values (Table 1).

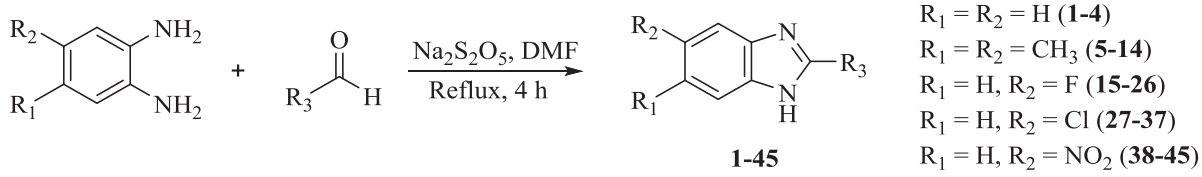
2.3. Structure-activity relati|onship (SAR)

On the basis of different substitutions R_2 and R_1 at positions-5 and -6 of benzimidazole scaffold, 2-aryl benzimidazole derivatives **1**–**45** are classified into five categories **A**-**E**. In order to simplify the SAR, it was assumed that variations in the inhibitory activities of compounds in a particular category is attributed by different aryl substitutions R_3 at position-2 of benzimidazole scaffold (Fig. 1, Table 1). So, a limited SAR was established by analyzing the effect of R_3 on inhibitory potential.

Compounds **1**–**4** of category-**A** do not have any substitutions as R_1 and R_2. Among this category, compound **3** having bromo and dimethoxy substitutions showed excellent activity comparable to the standard acarbose. Its excellent activity is might be due to the presence of three groups with positive mesomeric effect which increases the electron density on the aryl ring to better interact with the active site of enzyme. Activity comparison of compound **3** with compound **2** shown that the absence of methoxy group *para* to benzimidazole ring and presence of hydroxy *ortho* to benzimidazole ring decreased the inhibitory activity. Apparently, it revealed that the hydroxy group is not participating in the inhibition potential. Activity of compound **2** can be compared with compound **4** having bromo and chloro *ortho* and *para* to hydroxy, respectively, which showed that absence of methoxy further decreased the activity, however, the role of chloro is not clearly judged at this stage. Compound **1** having chloro and methoxy *ortho* to each other, showed moderate inhibitory activity might be due to weak involvement of chloro group to the inhibitory activity (Table 1, Category-**A**).

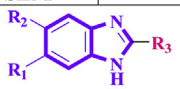
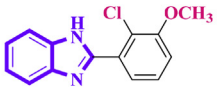
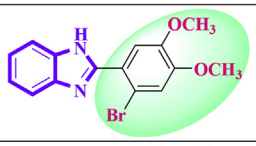
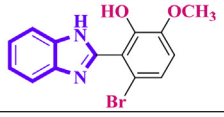
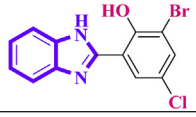
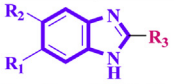
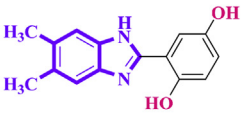
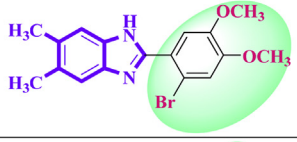
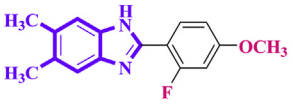
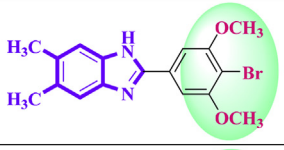
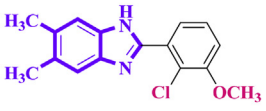
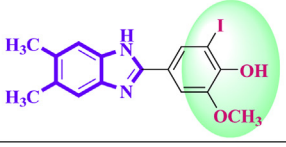
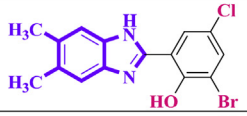
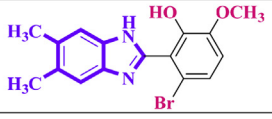
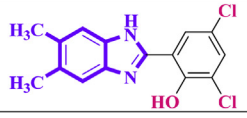
Compounds **5**–**14** of category-**B** possess methyl substitutions as R_1 and R_2. Again in this category, compound **10** with bromo and dimethoxy substitutions was found to be the most active compound. Its positional isomer **11** showed slightly diminished activity. Activity of compound **11** may be compared with compound **9** which has bromo, hydroxy, and methoxy substitutions, demonstrated moderate activity which again reveal the less involvement of hydroxy as observed in category-**A**. It was further confirmed by the weakest inhibitory activity among this category by the di-hydroxy substituted compound **5**. Similarly, compound **12** having combination of methoxy, hydroxy, and iodo also resulted a slightly less inhibitory activity as compared to compound **10**. Compound **8** with tri-methoxy substitutions was found to be moderately active it confirms that the combination of methoxy and bromo is worth important for the inhibitory activity. Compounds with combinations of hydroxy with halogens such as **13** and **14** were found to be moderately active. Similarly, combinations of methoxy with fluoro or chloro in compounds **6** and **7** demonstrated moderate inhibitory activity (Table 1, Category-**B**).

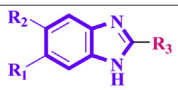
Compounds **15**–**26** of category-**C** have fluoro group as R_2. Almost similar activity pattern was observed as in the case of



Scheme 1. Synthesis of benzimidazole derivatives 1–45.

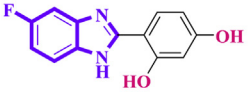
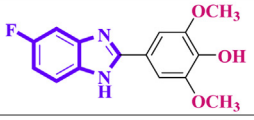
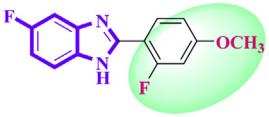
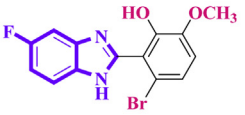
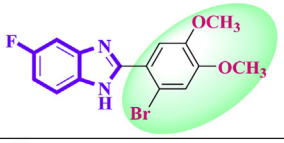
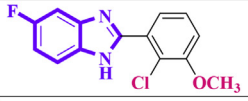
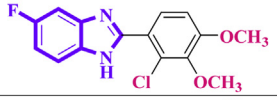
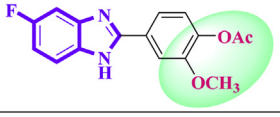
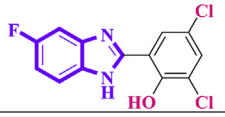
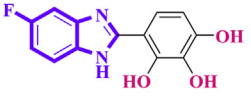
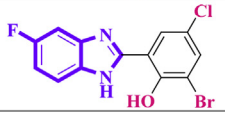
Table 1
In vitro α-amylase inhibitory activity of 2-aryl benzimidazole derivatives **1**–**45**.

Comp.	Structures	IC_{50} ± SEM[a]	Comp.	Structures	IC_{50} ± SEM[a]
	 $R_1 = R_2 = H$ **(1-4)** **Category "A"**				
1		2.74 ± 0.07	**3**		1.51 ± 1.82
2		2.48 ± 0.11	**4**		2.85 ± 0.08
	 $R_1 = R_2 = CH_3$ **(5-14)** **Category "B"**				
5		2.99 ± 0.14	**10**		1.48 ± 0.38
6		2.78 ± 0.02	**11**		1.59 ± 0.67
7		2.84 ± 0.05	**12**		1.77 ± 0.23
8		2.3 ± 0.14	**13**		2.92 ± 0.06
9		2.26 ± 0.02	**14**		2.98 ± 0.05



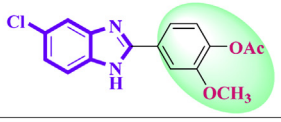
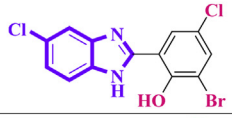
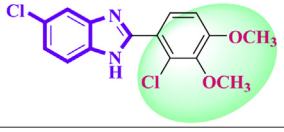
R_1 = H, R_2 = F (15-26)

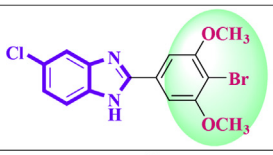
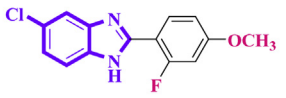
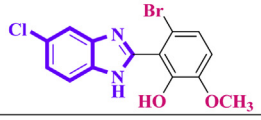
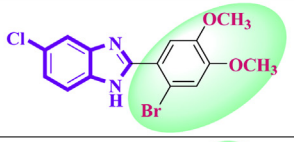
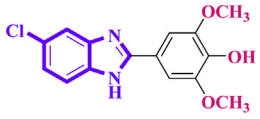
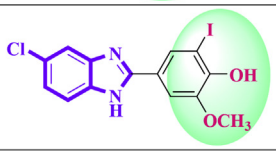
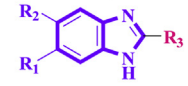
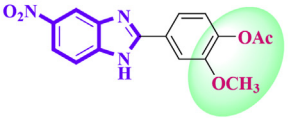
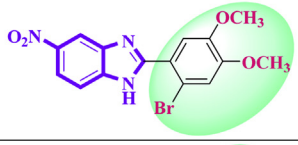
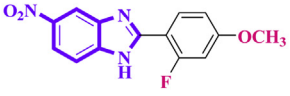
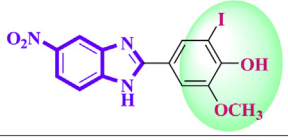
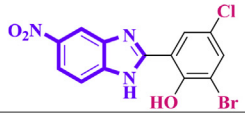
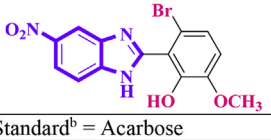
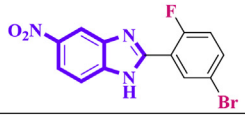
Category "C"

15		2.98 ± 0.21	**21**		2.49 ± 0.05
16		1.59 ± 0.16	**22**		2.17 ± 0.08
17		2.60 ± 0.03	**23**		1.57 ± 1.07
18		2.62 ± 0.08	**24**		2.56 ± 0.11
19		1.68 ± 0.62	**25**		2.87 ± 0.06
20		2.54 ± 0.10	**26**		2.86 ± 0.08

R_1 = H, R_2 = Cl (27-37)

Category "D"

27		1.68 ± 0.23	**33**		2.86 ± 0.17
28		2.78 ± 0.05	**34**		1.51 ± 0.38

29		2.62 ± 0.07	**35**		1.62 ± 0.17
30		2.74 ± 0.05	**36**		2.21 ± 0.11
31		1.73 ± 0.16	**37**		2.52 ± 0.09
32		1.61 ± 0.2	-	-	-
 R_1 = H, R_2 = NO_2 (38-45) **Category "E"**					
38		1.73 ± 0.06	**42**		1.87 ± 0.32
39		2.75 ± 0.02	**43**		1.72 ± 0.12
40		2.78 ± 0.06	**44**		2.87 ± 0.05
41		2.29 ± 0.14	**45**		2.31 ± 0.07
Standard[b] = Acarbose		1.46 ± 0.26	-	-	-

SEM[a] (Standard Error Mean); Standard[b] (Standard Inhibitor for α-Amylase Enzyme)

SEM[a] (Standard Error Mean); Standard[b] (Standard Inhibitor for α-Amylase Enzyme).

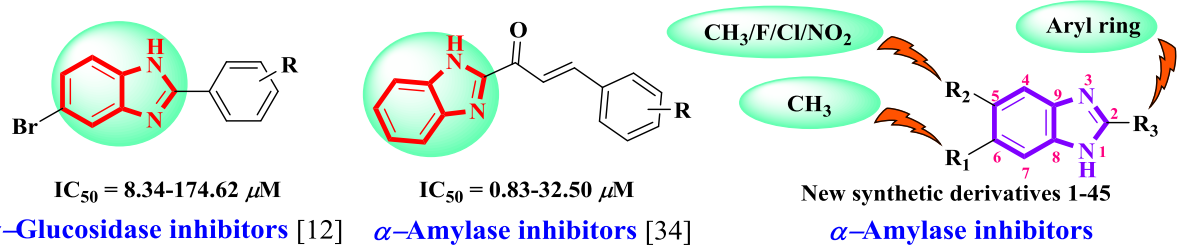


Fig. 1. Benzimidazole based already identified inhibitors of α-glucosidase and α-amylase enzymes and newly synthesized derivatives as α-amylase inhibitors 1–45.

category-**B**. It is worth-noting that bromo and di-methoxy substituted analog **23** was again found to be the most potent. Compounds including **21**, **22**, and **24** with the combinations of methoxy with hydroxy, bromo, and chloro, were again found to be moderately active. Similarly, compounds **25** and **26** with the combination of hydroxy with chloro and bromo were demonstrated

moderate inhibitory potential. Furthermore, compound **16** with methoxy and fluoro groups was found to be the second most active molecule of this category. Its structurally related molecules **17** and **18** with combination of methoxy with bromo and chloro demonstrated moderate inhibitory potential. Another derivative **19** with methoxy and acetoxy groups *ortho* to each other was found to be the third most active compound of this category (Table 1, Category-**C**).

Compounds **27**–**37** of category-**D** with chloro substituent as R_2 showed activity in the range of $IC_{50} = 1.51 \pm 0.38$–2.86 ± 0.17 μM. In this category, compound **34** with chloro and di-methoxy substituents was found to be the most active analog. Two positional isomers **31** and **35** with di-methoxy and bromo substitutions showed close but excellent inhibitory activities. Furthermore, compound **32** with hydroxy *ortho* to methoxy and iodo, also showed comparable activity to standard acarbose. Compound **36** with hydroxy *ortho* to methoxy and *meta* to bromo, revealed low activity than compound **36**. Amongst the compounds **27**–**30** which have methoxy group in combination with acetoxy, chloro, bromo, and fluoro, compound **27** showed comparable inhibitory potential to standard acarbose (Table 1, Category-**D**).

Compounds **38**–**45** belong to category-**E** having nitro group as R_2 showed inhibitory activity with IC_{50} values of 1.72 ± 0.12–2.87 ± 0.05 μM. In this category, compound demonstrated almost same activity pattern as observed in previous categories. Compound **43** with hydroxy *ortho* to methoxy and iodo, was found to be the most active analog. Its structurally related analog **41** with hydroxy *ortho* to methoxy but *meta* to bromo, exhibited moderate inhibitory activity. Compound **38** with methoxy and acetoxy *ortho* to each other was found to be the second most active analog in this category. Other compounds **39** and **40** with the combinations of methoxy with fluoro and chloro showed moderate inhibitory activity. Compound **42** with bromo and di-methoxy substituents was found to be the third most active in this category. Compounds **44** with fluoro and bromo *para* to each other and **45** with combination of bromo, chloro, and hydroxy, showed moderate inhibitory potential (Table 1, Category-**E**).

However, to support the *in vitro* α-amylase inhibition studies and to get the real picture of the involvement of different structural motifs of the compounds in binding with the active site of α-amylase enzyme, *in silico* molecular modeling was also performed.

2.4. Molecular docking

In silico molecular docking study was performed to study the binding mechanism of 2-aryl benzimidazole derivatives in the active site of α-amylase. The docking results showed the best accommodation of all the compounds in the active site of the target enzyme (Table 2).

Compound **3** (docking score = −5.468) the most potent compound in the category-**A**, formed a H-bond between the nitrogen atom of imidazole moiety and carboxylate oxygen of Asp300 (2.5 Å) and the benzene ring of compound showed π-π interaction with Leu162. Whereas, other residues Trp59, Tyr62, Tyr151, Leu162, and Ile235 of active site showed hydrophobic interactions with compound **3** (Fig. 2a). The compound has electron rich centers, may allow it to interact with the polar area of receptor active site, while the non-polar parts of ligand with non-polar area of the receptor which may responsible for potent inhibitory activity of the compound **3**. However, the removal of one OCH_3 group and insertion of Cl instead of Br in compound **1** showed slightly inferior activity. Similarly, the removal of both OCH_3 groups and the addition of Cl, Br, or OH groups in compound **4** resulted almost similar behavior to compound **1**. However, the presence of one OCH_3 and OH group in compound **2** resulted comparable activity as shown by compounds

Table 2
Docking scores and report of predicted interactions of docked conformations.

Interaction report						
Comp.	Docking score	Ligand	Receptor		Interaction	Distance
1	−4.845	N 11	CE1	HIS201	H-acceptor	3.54
		5-ring	CD1	LEU162	π-H	3.92
2	−5.033	BR 25	OD1	ASP197	H-donor	3.52
		6-ring	CD2	LEU162	π-H	3.99
3	−5.468	N 13	OD1	ASP300	H-donor	2.5
		6-ring	CD1	LEU162	π-H	3.6
4	−4.944	N 14	OD1	ASP300	H-donor	3.26
		BR 25	OD2	ASP197	H-donor	3.53
5	−4.78	O 23	OG1	THR163	H-acceptor	3.22
6	−5.572	O 31	NE2	HIS299	H-acceptor	3.23
7	−5.311	N 11	OD1	ASP300	H-donor	3.07
		6-ring	CD2	LEU162	π-H	4.37
8	−5.051	O 29	NH2	ARG195	H-acceptor	3.20
		5-ring	CB	TRP59	π-H	4.17
9	−5.072	BR 23	OD1	ASP197	H-donor	3.56
		6-ring	CD2	LEU162	π-H	4.35
10	−5.933	C 16	OE1	GLU233	H-donor	2.5
		BR 29	OD1	ASP197	H-donor	3.4
		N 10	CD1	LEU162	H-bond	2.9
		N 10	CD2	LEU162	H-bond	2.9
11	−5.908	N 11	OD1	ASP300	H-donor	3.50
		BR 25	OE1	GLU233	H-donor	3.44
12	−5.558	I 23	OD2	ASP356	H-donor	3.98
		6-ring	6-ring	TRP59	π-π	3.68
13	−5.161	BR 23	OD2	ASP356	H-donor	3.55
14	−5.091	CL 24	OD2	ASP356	H-donor	3.46
15	−5.197	O 23	OD1	ASP197	H-donor	3.24
		O 26	OD2	ASP300	H-donor	3.13
16	−5.195	O 25	NE2	HIS299	H-acceptor	3.13
17	−4.994	BR 24	OD2	ASP300	H-donor	3.20
		5-ring	CD2	LEU162	π-H	4.03
18	−4.766	C 16	OE1	GLU233	H-donor	3.44
		6-ring	CB	TRP59	π-H	4.71
19	−5.734	O 26	NE2	HIS299	H-acceptor	3.10
		6-ring	6-ring	TRP59	π-π	4.00
20	−5.192	O 25	OD1	ASP197	H-donor	2.97
		6-ring	5-ring	TRP59	π-π	3.85
21	−5.561	O 23	OE1	GLU233	H-donor	2.95
		6-ring	CD2	LEU162	π-H	4.25
22	−5.327	F 25	NE2	HIS299	H-acceptor	2.99
		6-ring	CB	TRP59	π-H	4.40
23	−5.564	C 1	OE1	GLU233	H-donor	2.6
		N 12	OD1	ASP300	H-donor	2.4
		C 25	6-ring	TRP59	H-π	2.9
		6-ring	CB	TRP59	π-H	2.7
24	−5.423	C 16	OE1	GLU233	H-donor	3.40
		6-ring	CD2	LEU162	π-H	4.35
25	−4.88	CL 24	OE2	GLU233	H-donor	3.26
26	−4.73	N 10	NE2	HIS299	H-acceptor	3.03
27	−5.941	C 17	OD1	ASP300	H-donor	3.35
		O 26	NE2	HIS299	H-acceptor	2.98
28	−4.997	CL 21	OE1	GLU233	H-donor	3.25
29	−5.517	N 12	OD1	ASP300	H-donor	2.91
30	−5.523	O 25	NE2	HIS299	H-acceptor	3.08
31	−6.026	C 30	OE1	GLU233	H-donor	3.51
		C 25	5-ring	HIS299	H-π	4.75
32	−5.378	N 12	OD1	ASP300	H-donor	3.14
		I 24	OD2	ASP197	H-donor	3.75
33	−4.556	BR 24	OE2	GLU233	H-donor	3.32
34	−5.403	C 30	O	TRP59	H-donor	2.4
		Cl 4	CD1	ILEU235	H-donor	2.7
		Cl 4	CZ	PHE256	H-donor	3.0
35	−5.52	N 10	OG1	THR163	H-acceptor	3.18
		6-ring	CD2	LEU162	π-H	4.56
36	−4.963	O 22	OD2	ASP300	H-donor	2.92
37	−5.351	N 12	OD1	ASP300	H-donor	2.98
		O 23	OE1	GLU233	H-donor	3.02
38	−6.152	O 25	NE2	HIS299	H-acceptor	3.02
		6-ring	6-ring	TRP59	π-π	3.62
39	−5.398	O 26	NE2	HIS299	H-acceptor	2.95
40	−5.049	C 1	OD2	ASP356	H-donor	3.58
		CL 23	OD1	ASP300	H-donor	3.10
41	−5.057	C 16	OE1	GLU233	H-donor	3.39

(*continued on next page*)

Table 2 (*continued*)

Interaction report						
Comp.	Docking score	Ligand	Receptor		Interaction	Distance
		6-ring	CD1	LEU162	π-H	4.54
42	−5.826	BR 22	OD1	ASP197	H-donor	3.32
		6-ring	CD2	LEU162	π-H	4.43
43	−5.883	N 12	OD1	ASP300	H-donor	3.09
		I 24	OD2	ASP197	H-donor	3.74
44	−5.303	N 12	OD1	ASP300	H-donor	3.67
		O 22	OD2	ASP300	H-donor	3.10
45	−5.083	BR 24	OE1	GLU233	H-donor	3.55
		6-ring	CD2	LEU165	π-H	4.16

1 and **4** but lower than compound **3**. The docking poses of these low inhibitory activity compounds shows that these compounds have less interactions than the most active compound **3**.

In category-**B**, compound **10** (docking score = −5.933) showed good binding interactions with the active side residues. The Leu162 was observed making two H-bonds (2.9 Å & 2.9 Å) with imidazole moiety and the carboxylate oxygen of Glu233 showed one H-bond (2.5 Å) with benzene moiety. Asp197 showed an interaction (3.4 Å) towards Br atom of compound. Try59 and Leu162 were also observed to make arene-hydrogen interactions with compound (Fig. 2b). The potent inhibitory activity might be due to the presence of the electron donating groups. Nevertheless, the other compounds of this category containing OH and OCH_3 groups (**5**, **6**, **7**, **8**, **9**, **11**, and **12**) at different positions did not improve the potency as in the compound **10**. As the positions and number of functional groups at aromatic rings have great role in the activity of the compounds, so the compounds containing OH, OCH_3 (**5**, **6**, **7**, **8**, **9**, **11**, and **12**) and halogen groups (**13** and **14**) at different positions did not improve the potency as compared to compound **10**.

In case of compound **23** (docking score = −5.564) from category-**C**, the fluorobenzene moiety formed H-bond (2.6 Å) with Glu233, whereas carboxylate group of Asp300 also forms H-bond (2.4 Å) with the imidazole nitrogen and Try59 showed arene-hydrogen interaction with the methoxy moiety of compound (Fig. 2c). The inhibitory activity of the compound may be due to the presence of the electron withdrawing (fluorine atom) and donating groups (methoxy groups) which create an electron flow making the compound more active, polarizable, and potent. The remaining compounds of this category also exhibit the same results with less potent compounds of category-**B** and the docking results revealed their less inhibition and low potency.

The compound **34** (docking score = −5.403) of category-**D**, the Phe256 and Ileu235 made hydrogen bonds (3.0 Å & 2.7 Å) with Cl atom and Try59 made single hydrogen bond (2.4 Å) with methoxy (Fig. 2d). The compound inhibitory activity might be due to uniform electron flow through compound having both electron and electron withdrawing groups. In docking studies, the remaining compounds showed low potency as compared to compound **3**. Introduction of acetic acid in compounds (**27** and **38**) showed slightly improve activity. The remaining compounds containing methoxy and halogens at various positions, all showed low potency against α-amylase.

The whole series of compounds containing the same core structure 2-phenyl-1*H*-benzoimidazole showed almost same relative activities with little differences. The most active compounds in each category have *para* and *meta* methoxy and *ortho* Cl or Br atom at phenyl ring which may be responsible for the inhibitory activity of these compounds. As in each category the addition of Cl, F, CH_3, and NO_2 to the benzene ring of core structure have no considerable effect on the activity of these four most active compounds (**3**, **10**, **23**, and **34**) and showed almost the same activity. All the compounds showed good docking results which correlate with the biological results.

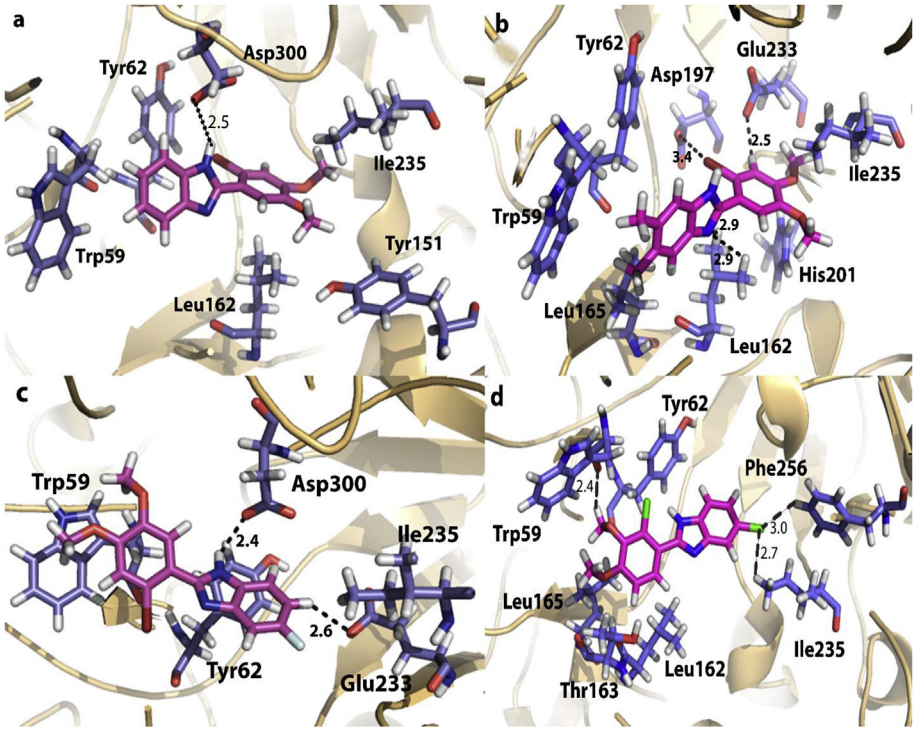


Fig. 2. Putative binding interaction of most active compounds 3, 10, 23, and 34 (a, b, c, and d) (pink) inside the active site of α-amylase (blue), hydrogen bonds are shown in black dashed line. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Conclusion

New synthetic 2-aryl benzimidazole derivatives **1**–**45** were evaluated for *in vitro* α-amylase inhibitory activity and showed good to moderate inhibitory activity ($IC_{50} = 1.48 \pm 0.38–2.99 \pm 0.14$ μM) as compared to standard acarbose ($IC_{50} = 1.46 \pm 0.26$ μM). Limited SAR suggested that the compounds showed mixed type of behavior with different combinations of groups but it is clear from the results that the derivatives with combination of bromo and di-methoxy were found to be significantly active. In addition to that *in silico* studies suggested that compounds with *para* and *meta* methoxy group as well as *ortho* chloro or bromo atom at phenyl ring were the most active analogs in each category. Current study has identified a whole series of lead molecules which can be used in further advance research in order to obtain a powerful inhibitor for α-amylase enzyme for the development of insulin-independent antidiabetic agents.

4. Experimental

4.1. Materials and methods

Analytical grade reagents and solvents were purchased from Sigma-Aldrich, USA and used as received. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). TLC chromatograms were visualized under ultraviolet light at 254 and 366 nm. Mass spectra were recorded under electron impact (EI) on MAT 312 and MAT 113D mass spectrometer. The ^1H and ^{13}C NMR were recorded on Avance Bruker AM spectrometers, operating at 300 and 400 MHz instrument. The chemical shift values are presented in ppm (δ), relative to tetramethylsilane (TMS) as an internal standard and the coupling constant (J) are in Hz. Multiplicities are reported as singlet (s), doublet (d), triplet (t), doublet of doublets (dd), doublet of triplets (dt), quartet (q) or multiplet (m). Melting points of the compounds were determined on Stuart® SMP10 melting point apparatus. IR spectra (KBr discs) were run on FTS 3000 MX, Bio-RAD Merlin (Excalibur Model) spectrophotometer.

4.2. General procedure for the synthesis of 2-aryl benzimidazoles

o-Phenylene diamine derivative (1 mmol) and substituted benzaldehyde (1 mmol) were taken in *N*,*N*-dimethylformamide (10 mL) into a round-bottomed flask 100 mL. A pinch of sodium metabisulfite ($Na_2S_2O_5$) was added into it and refluxed solution for 4 h. Progress of reaction was checked by thin layer chromatography (TLC). After completion, reaction mixture was poured onto crushed ice (100 mL). Precipitates were appeared immediately which were filtered. The obtained solid crude products were crystallized from ethanol. Compounds were structurally characterized by various spectroscopic techniques.

4.2.1. 2-(2′-Chloro-3′-methoxyphenyl)-1H-benzo[d]imidazole (1)

Pale brown solid, 0.131 g (50.6% yield); M.P. 268–271 °C; R_f: 0.42 (ethyl acetate/hexane, 1:1); ^1H NMR (300 MHz, DMSO-d6): δ_H 7.63–7.60 (m, 2H, H-7, H-4), 7.49–7.42 (m, 2H, H-6′, H-5′), 7.32 (dd, 1H, $J_{4',5'} = 7.5$ Hz, $J_{4',6'} = 2.1$ Hz, H-4′), 7.24–7.21 (m, 2H, H-6, H-5), 3.93 (s, 3H, 3′-OCH_3); IR (KBr, cm^{-1}): 3414 (N–H), 3054, 2934, 2840 (C–H), 1665 (C=N), 1573, 1433 (C=C), 1537 (N–H), 1466 (CH), 1320 (C–N), 1270, 1060 (C–O), 983 (C–Cl); EI-MS: *m/z* (rel. abund. %), 258 [M^+] (100), 260 [M^++2] (61), 243 (6), 215 (39), 193 (5), 180 (9), 129 (7), 90 (6); HREI-MS: *m/z* Calcd for $C_{14}H_{11}ClN_2O$ [M^+] 258.0560; Found 258.0560.

4.2.2. 2′-(1H-Benzo[d]imidazole-2-yl)-3′-bromo-6′-methoxyphenol (2)

Yellow solid, 0.133 g (41.8% yield); M.P. 178–181 °C; R_f: 0.55 (ethyl acetate/hexane, 1:1); ^1H NMR (400 MHz, DMSO-d6): δ_H 11.77–11.70 (br d, 1H, 1′-OH), 7.62–7.60 (m, 2H, H-7, H-4), 7.24–7.22 (m, 2H, H-6, H-5), 7.18 (d, 1H, $J_{4',5'} = 8.8$ Hz, H-4′), 7.07 (d, 1H, $J_{5',4'} = 8.8$ Hz, H-5′), 3.84 (s, 3H, 6′-OCH_3); ^{13}C NMR (75 MHz, DMSO-d6): δ_C 148.3 (C-2), 148.1 (C-6′), 147.6 (C-1′), 137.6 (C-9), 137.6 (C-8), 122.5 (C-5′), 122.5 (C-4′), 122.1 (C-6) 122.1 (C-5), 118.6 (C-2′), 114.2 (C-7) 114.2 (C-4), 112.3 (C-3′), 56.1 (C-7′); IR (KBr, cm^{-1}): 3336 (O–H), 3100, 2925 (C–H), 1585 (C=C), 1450 (C–H), 1348 (C–N), 1245 (C–O) 879 (C–Br); EI-MS: *m/z* (rel. abund. %), 318 [M^+] (89), 320 [M^++2] (100), 303 (14), 291 (31), 275 (28), 240 (14), 209 (7), 195 (9), 167 (22), 44 (3); HREI-MS: *m/z* Calcd for $C_{14}H_{11}BrN_2O_2$ [M^+] 318.0004; Found 318.0015.

4.2.3. 2-(2′-Bromo-4′,5′-dimethoxyphenyl)-1H-benzo[d]imidazole (3)

Pale-brown, 0.205 g (61.6% yield); M.P. 186–188 °C; R_f: 0.34 (ethyl acetate/hexane, 1:1); ^1H NMR (400 MHz, DMSO-d6): δ_H 12.55 (s, 1H, -NH), 7.67 (d, 1H, $J_{4,5} = 7.6$ Hz, H-4), 7.53 (d, 1H, $J_{7,6} = 7.2$ Hz, H-7), 7.33 (s, 1H, H-3′), 7.30 (s, 1H, H-6′), 7.24–7.18 (m, H-6, H-5), 3.85 (s, 3H, 5′-OCH_3), 3.81 (s, 3H, 4′-OCH_3); IR (KBr, cm^{-1}): 3300 (N–H), 3053, 2959, 2840 (C–H), 1598, 1501 (C=C), 1441 (C–H), 1332 (C–N), 1210 (C–O), 866 (C–Br); EI-MS: *m/z* (rel. abund. %), 332 [M^+] (100), 334 [M^++2] (99), 319 (21), 303 (26), 288 (14), 254 (4), 210 (6), 195 (22), 167 (15), 105 (5), 43 (5); HREI-MS: *m/z* Calcd for $C_{15}H_{13}N_2O_2Br$ [M^+] 332.0160; Found 332.0144.

4.2.4. 6′-(1H-Benzo[d]imidazole-2-yl)-2-bromo-4-chlorophenol (4)

Pale yellow solid, 0.291 g (89.9% yield); M.P. 266–268 °C; R_f: 0.67 (ethyl acetate/hexane, 1:1); ^1H NMR (400 MHz, DMSO-d6): δ_H 8.20 (d, 1H, $J_{5',3'} = 2.4$ Hz, H-5′), 7.81 (d, 1H, $J_{3',5'} = 2.4$ Hz, H-3′) 7.71–7.69 (m, broad, 2H, H-7, H-4), 7.34–7.33 (m, 2H, H-6, H-5); IR (KBr, cm^{-1}): 3391 (O–H), 3067 (C–H), 1662 (C=N), 1584, 1453 (C=C), 1521 (N–H), 1317 (C–N), 1178 (C–O), 1007 (C–Cl), 910 (C–Br); EI-MS: *m/z* (rel. abund. %), 322 [M^+] (74), 324 [M^++2] (100), 326 [M^++4] (25), 243 (7), 215 (35), 180 (8), 162 (3), 108 (5), 90 (8); HREI-MS: *m/z* Calcd for $C_{13}H_8N_2OBrCl$ [M^+] 321.9509; Found 321.9508.

4.2.5. 2′-(5,6-Dimethyl-1H-benzo[d]imidazole-2-yl)benzene-1′,4′-diol (5)

Brown solid, 0.214 g (84.2% yield); M.P. 315-217 °C; R_f: 0.67 (ethyl acetate/hexane, 1:1); ^1H NMR (400 MHz, DMSO-d6): δ_H 12.80 (br s, 1H, -NH), 9.11 (s, 1H, 4′-OH), 7.42 (s, 2H, H-7, H-4), 7.38 (s, 1H, H-3′), 6.85 (s, 2H, H-6′, H-5′), 2.33 (s, 6H, 6-CH_3, 5-CH_3); IR (KBr, cm^{-1}): 3481 (O–H), 3260 (N–H), 2920, 2856 (C–H), 1626, 1503 (C=C), 1316 (C–N), 1098 (C–O), 1145 (C–F), 885 (C–Br); EI-MS: *m/z* (rel. abund. %), 254 [M^+] (100), 255 [M^++1] (15), 239 (9), 197 (16), 120 (3), 91 (4); HREI-MS: *m/z* Calcd for $C_{15}H_{14}N_2O_2$ [M^+] 254.1055; Found 254.1065.

4.2.6. 2-(2′-Fluoro-4′-methoxyphenyl)-5,6-dimethyl-1H-benzo[d]imidazole (6)

White solid, 0.082 g (30.3% yield); M.P. 143–147 °C; R_f: 0.48 (ethyl acetate/hexane, 1:1); ^1H NMR (300 MHz, DMSO-d6): δ_H 8.10 (t, 1H, $J_{6',5'} = 9.0$ Hz, H-6′), 7.48 (s, 2H, H-7, H-4), 7.19 (dd, 1H, $J_{3',2'F} = 13.5$ Hz, $J_{3',5'} = 2.4$ Hz, H-3′), 7.09 (dd, 1H, $J_{5',6'} = 9.0$ Hz, $J_{5',3'} = 2.4$ Hz, H-5′), 3.88 (s, 3H, 4′-OCH_3), 2.36 (s, 6H, 6-CH_3, 5-CH_3); IR (KBr, cm^{-1}): 3412 (N–H), 3034, 2919, 2847 (C–H), 1625, 1499 (C=C), 1466 (C–H), 1242, 1019 (C–O), 1145 (C–F), 885 (C–Br); EI-MS: *m/z* (rel. abund. %), 270 [M^+] (100), 271 [M^++1] (49), 255 (90), 240 (4), 227 (13), 207 (8), 135 (10), 91 (5); HREI-MS: *m/z* Calcd for $C_{16}H_{15}FN_2O$ [M^+] 270.1168; Found 270.1171.

*4.2.7. 2-(2′-Chloro-3′-methoxyphenyl)-5,6-dimethyl-1H-benzo[d] imidazole **(7)***

White solid, 0.152 g (53.0% yield); M.P. 210–212 °C; R_f: 0.40 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 7.49–7.43 (m, 2H, H-6′, H-5′), 7.40 (s, 2H, H-7, H-4), 7.33 (dd, 1H, $J_{4',5'} = 7.5$ Hz, $J_{4',6'} = 2.1$ Hz, H-4′), 3.92 (s, 3H, 3′-OCH_3), 2.33 (s, 6H, 6-CH_3, 5-CH_3); IR (KBr, cm^{-1}): 3390 (N–H), 3040, 2922, 2859 (C–H), 1666 (C=N), 1574 (C=C), 1458 (C–H), 1352 (C–N), 1268, 1056 (C–O); EI-MS: *m/z* (rel. abund. %), 286 [M^+] (100), 288 [M^++2] (41), 273 (9), 271 (25), 243 (8), 228 (4), 207 (3), 143 (5), 135 (5), 118 (4), 91 (4); HREI-MS: *m/z* Calcd for $C_{16}H_{15}N_2OCl$ [M^+] 286.0873; Found 286.0869.

*4.2.8. 5,6-Dimethyl-2-(2′,3′,4′-trimethoxyphenyl)-1H-benzo[d] imidazole **(8)***

White solid, 0.153 g (49.0% yield); M.P. 188–190 °C; R_f: 0.3 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 11.93 (br s, 1H, -NH), 7.92 (d, 1H, $J_{6',5'} = 8.8$ Hz, H-6′), 7.35 (s, 2H, H-7, H-4), 6.98 (d, 1H, $J_{5',6'} = 9.2$ Hz, H-5′), 3.88 (s, 3H, 2′-OCH_3), 3.86 (s, 3H, 4′-OCH_3), 3.82 (s, 3H, 3′-OCH_3), 2.30 (s, 6H, 6-CH_3, 5-CH_3); ^{13}C NMR (75 MHz, DMSO-d6): δ_C 154.6 (C-4′), 151.2 (C-2), 147.6 (C-2′), 141.7 (C-3′), 130.0 (C-9) 130.0 (C-8), 130.0 (C-6), 130.0 (C-5), 124.3 (C-6′), 116.1 (C-1′), 115.06 (C-5′), 108.4 (C-7), 108.4 (C-4), 61.2 (C-7′), 60.5 (C-9′), 55.9 (C-8′), 19.9 (C-11) 19.9 (C-10); IR (KBr, cm^{-1}): 3314 (N–H), 3102, 2943 (C–H), 1597, 1479 (C=C), 1457 (C–H), 1288, 1083 (C–O); EI-MS: *m/z* (rel. abund. %), 312 [M^+] (94), 313 [M^++2] (21), 297 (100), 281 (19), 266 (23), 254 (26), 156 (11), 183 (14), 91 (7), 64 (9); HREI-MS: *m/z* Calcd for $C_{18}H_{20}N_2O_3$ [M^+] 312.1474; Found 312.1470.

*4.2.9. 3′-Bromo-2′-(5,6-dimethyl-1H-benzo[d]imidazole-2-yl)-6′-methoxyphenol **(9)***

Brown solid, 0.263 g (75.7% yield); M.P. 236–239 °C; R_f: 0.52 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 11.88 (br s, 1H, 1′-OH), 7.39 (s, 2H, H-7, H-4), 7.16 (d, 1H, $J_{4',5'} = 8.8$ Hz, H-4′), 7.04 (d, 1H, $J_{5',4'} = 8.8$ Hz, H-5′), 3.83 (s, 3H, 6′-OCH_3), 2.32 (s, 6H, 6-CH_3, 5-CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 158.7 (C-4), 157.6 (C-2), 153.0 (C-8), 144.4 (C-6), 138.3 (C-1′), 129.4 (C-3′), 128.4 (C-5′), 126.5 (C-7), 124.5 (C-4′), 122.9 (C-2′), 122.9 (C-6′), 120.8 (C-5), 114.3 (C-9), 55.8 (C-7′), 19.7 (C-11), 19.7 (C-10); IR (KBr, cm^{-1}): 3359 (O–H), 2931, 2846 (C–H), 1583 (C=C), 1461 (C–H), 1348 (C–N), 1251 (C–O), 1056 (C–Br); EI-MS: *m/z* (rel. abund. %), 346 [M^+] (100), 348 [M^++2] (95), 328 (28), 317 (38), 305 (43), 268 (21), 250 (15), 225 (9), 195 (46), 90 (13); HREI-MS: *m/z* Calcd for $C_{16}H_{15}BrN_2O_2$ [M^+] 346.0317; Found 346.0295.

*4.2.10. 2-(2′-Bromo-4′,5′-dimethoxyphenyl)-5,6-dimethyl-1H-benzo[d]imidazole **(10)***

Pale brown solid, 0.184 g (51.0% yield); M.P. 234–237 °C; R_f: 0.33 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 7.40 (s, 2H, H-7, H-4), 7.34 (s, 1H, H-3′), 7.31 (s, 1H, H-6′), 3.86 (s, 3H, 5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 2.33 (s, 6H, 6-CH_3, 5-CH_3); IR (KBr, cm^{-1}): 3419 (N–H), 3010, 2936, 2845 (C–H), 1600, 1501 (C=C), 1439 (C–H), 1334 (C–N), 1252, 1212 (C–O); EI-MS: *m/z* (rel. abund. %), 360 [M^+] (82), 362 [M^++2] (100), 345 (27), 331 (26), 316 (24), 119 (14), 223 (31), 195 (14), 180 (21), 172 (15), 111 (21), 91 (24); HREI-MS: *m/z* Calcd for $C_{17}H_{17}N_2O_2Br$ [M^+] 360.0473; Found 360.0480.

*4.2.11. 2-(4′-Bromo-3′,5′-dimethoxyphenyl)-5,6-dimethyl-1H-benzo[d]imidazole **(11)***

Yellow solid, 0.310 g (85.8% yield); M.P. 293–295 °C; R_f: 0.48 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 12.70 (s, 1H, -NH), 7.50 (s, 2H, H-6′, H-2′), 7.43 (s, 1H, H-7), 7.31 (s, 1H, H-4), 3.95 (s, 6H, 5′-OCH_3, 3′-OCH_3), 2.33 (s, 3H, 6-CH_3), 2.31 (s, 3H, 5-CH_3); IR (KBr, cm^{-1}): 3415 (N–H), 3090, 2937, 2853 (C–H), 1631 (C=N), 1586 (C=C), 1464 (C–H), 1312 (C–N), 1243, 1121 (C–O) 852 (C–Br); EI-MS: *m/z* (rel. abund. %), 360 [M^+] (100), 362 [M^++2] (95), 345 (3), 331 (8), 251 (29), 221 (32), 195 (15), 181 (6), 118 (6), 91 (5); HREI-MS: *m/z* Calcd for $C_{17}H_{17}BrN_2O_2$ [M^+] 360.0468; Found 360.0465.

*4.2.12. 4′-(5,6-Dimethyl-1H-benzo[d]imidazole-2-yl)-2′-iodo-6′-methoxyphenol **(12)***

Pale yellow solid, 0.338 g (85.7% yield); M.P. 243–246 °C; R_f: 0.56 (ethyl acetate/hexane, 3:7); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 10.25 (br s, 1H, 1′-OH), 8.09 (d, 1H, $J_{3',5'} = 1.6$ Hz, H-3′), 7.74 (d, 1H, $J_{5',3'} = 1.2$ Hz, H-5′), 7.39 (s, 2H, H-7, H-4), 3.92 (s, 3H, 6′-OCH_3), 2.33 (s, 6H, 6-CH_3, 5-CH_3); IR (KBr, cm^{-1}): 3390 (O–H), 3085, 2926, 2854 (C–H), 1630, 1598 (C=C), 1469 (C–H), 1353 (C–N), 1288, 1039 (C–O), 615 (C–I); EI-MS: *m/z* (rel. abund. %), 394 [M^+] (100), 395 [M^++1] (60), 379 (15), 351 (6), 267 (14), 252 (9), 237 (16), 209 (4), 197 (14), 119 (5), 91 (3), 44 (3); HREI-MS: *m/z* Calcd for $C_{16}H_{15}N_2O_2I$ [M^+] 394.0178; Found 394.0177.

*4.2.13. 2′-Bromo-4′-chloro-6′-(5,6-dimethyl-1H-benzo[d] imidazole-2-yl)phenol **(13)***

Yellow solid, 0.340 g (96.7% yield); M.P. 278–280 °C; R_f: 0.70 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 8.15 (d, 1H, $J_{5',3'} = 2.4$ Hz, H-5′), 7.76 (d, 1H, $J_{3',5'} = 2.4$ Hz, H-3′), 7.45 (s, 2H, H-7, H-4), 2.34 (s, 6H, 6-CH_3, 5-CH_3); IR (KBr, cm^{-1}): 3350 (O–H), 3071, 2921, 2855 (C–H), 1617,1560 (C=C), 1520 (N–H), 1479 (C–H), 1310 (C–N), 1139 (C–O), 964 (C–Cl), 862 (C–Br); EI-MS: *m/z* (rel. abund. %), 350 [M^+] (75), 352 [M^++2] (100), 354 [M^++4] (26), 337 (10), 271 (5), 208 (3), 176 (3), 114 (4), 91 (3); HREI-MS: *m/z* Calcd for $C_{15}H_{12}N_2OBrCl$ [M^+] 349.9822; Found 349.9827.

*4.2.14. 2′,4′-Dichloro-6′-(5,6-dimethyl-1H-benzo[d]imidazole-2-yl) phenol **(14)***

Yellow solid,.Yellow solid, 0.301 g (98.0% yield); M.P. 305–308 °C; Rf: 0.69 (ethyl acetate/hexane, 1:1); 1H NMR (400 MHz, DMSO-d6): δ_H 13.71 (br s, 1H, -NH), 8.11 (d, 1H, J5′,3′ = 2.0 Hz, H-5′), 7.65 (d, 1H, $J_{3',5'} = 2.4$ Hz, H-3′), 7.46 (s, 2H, H-7, H-4), 2.34 (s, 6H, 6-CH_3, 5-CH_3); IR (KBr, cm^{-1}): 3383 (O–H), 3165, 3071, 2970 (C–H), 1618, 1485 (C=C), 1309 (C–N), 1267, 1025 (C–O), 885 (C–Cl); EI-MS: *m/z* (rel. abund. %), 306 [M^+] (100), 308 [M^++2] (66), 310 [M^++4] (8), 291(14), 271 (4), 243 (6), 153 (3), 118 (2), 91 (3); HREI-MS: *m/z* Calcd for $C_{15}H_{12}N_2OCl_2$ [M+] 306.0327; Found 306.0334.

*4.2.15. 4′-(5-Fluoro-1H-benzo[d]imidazole-2-yl)benzene-1′,3′-diol **(15)***

Brown solid, 0.160 g (65.6% yield); M.P. 260–263 °C; R_f: 0.70 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 13.06 (br s, 1H, -NH), 10.14 (s, 1H, 1′-OH), 7.84 (d, 1H, $J_{5',6'} = 8.4$ Hz, H-5′), 7.62–7.59 (m, 1H, H-4), 7.44 (dd, 1H, $J_{7,6} = 8.8$ Hz, $J_{7,5F} = 1.2$ Hz, H-7), 7.15 (td, 1H, $J_{6,7} = 8.8$ Hz, $J_{6,4} = 2.0$ Hz, H-6), 6.47 (dd, 1H, $J_{6',5'} = 8.8$ Hz, $J_{6',2'} = 1.6$ Hz, H-6′), 6.43 (s, 1H, H-2′); IR (KBr, cm^{-1}): 3347 (O–H), 1617, 1492 (C=C), 1145 (C–F), 1110 (C–O); EI-MS: *m/z* (rel. abund. %), 244 [M^+] (100), 245 [M^++1] (20), 215 (9), 187 (44), 174 (4), 161 (4), 136 (2), 108 (6), 83 (3); HREI-MS: *m/z* Calcd for $C_{13}H_9N_2O_2F$ [M^+] 244.0648; Found 244.0651.

*4.2.16. 5-Fluoro-2-(2′-fluoro-4′-methoxyphenyl)-1H-benzo[d] imidazole **(16)***

Brown solid, 0.130 g (50.0% yield); M.P. 160–162 °C; R_f: 0.53 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 8.15 (t, 1H, $J_{6',5'} = 8.7$ Hz, H-6′), 7.60–7.55 (m, 1H, H-4), 7.38 (d, 1H, $J_{7,6} = 9.0$ Hz, H-7), 7.08–7.01 (m, 2H, H-6, H-3′), 6.99 (dd, 1H, $J_{5',6'} = 8.7$ Hz, $J_{5',3'} = 2.1$ Hz, H-5′), 3.85 (s, 3H, 4′-OCH_3); IR (KBr,

cm^{-1}): 3535 (N–H), 3199, 3005, 2949, 2845 (C–H), 1668 (C=N), 1630, 1587 (C=C), 1446 (C–H), 1359 (C–N), 1297 (C–F), 1093 (C–O); EI-MS: *m/z* (rel. abund. %), 260 [M^+] (100), 261 [M^++1] (34), 245 (83), 217 (34), 130 (7), 197 (25), 108 (7); HREI-MS: *m/z* Calcd for $C_{14}H_{10}F_2N_2O$ [M^+] 260.0756; Found 260.0777.

4.2.17. 2-(5′-Bromo-2′-methoxyphenyl)-5-fluoro-1H-benzo[d] imidazole **(17)**

Brown solid, 0.248 g (77.2% yield); M.P. 191–193 °C; R_f: 0.50 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 8.38 (d, 1H, $J_{6',4'} = 2.4$ Hz, H-6′), 7.67–7.60 (m, 2H, H-7, H-4), 7.42 (dd, 1H, $J_{4',3'} = 9.6$ Hz, $J_{4',6'} = 2.4$ Hz, H-4′), 7.25 (d, 1H, $J_{3',4'} = 9.0$ Hz, H-3′), 7.11 (td, 1H, $J_{6,7} = 9.0$ Hz, $J_{6,4} = 2.7$ Hz, H-6), 4.02 (s, 3H, 2′-OCH_3); IR (KBr, cm^{-1}): 3326 (N–H), 3160, 2942, 2844 (C–H), 1631 (C=N), 1629, 1519, 1470 (C=C), 1519 (N–H), 1433 (C–H), 1348 (C–N), 1259 (C–F), 1086, 1020 (C–O), 895 (C–Br); EI-MS: *m/z* (rel. abund. %), 320 [M^+] (100), 322 [M^++2] (94), 302 (4), 292 (30), 211 (48), 198 (18), 137 (77), 111 (13), 99 (13); HREI-MS: *m/z* Calcd for $C_{14}H_{10}BrFN_2O$ [M^+] 319.9961; Found 319.9928.

4.2.18. 2-(2′-Chloro-3′-methoxyphenyl)-5-fluoro-1H-benzo[d] imidazole **(18)**

Brown solid, 0.179 g (64.7% yield); M.P. 215–217 °C; R_f: 0.45 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 7.63–7.59 (m, 1H, H-4), 7.49–7.39 (m, 3H, H-7, H-6′, H-5′), 7.32 (dd, 1H, $J_{4',5'} = 7.5$ Hz, $J_{4',6'} = 2.4$ Hz, H-4′), 7.12 (td, 1H, $J_{6,7} = 9.6$ Hz, $J_{6,4} = 2.4$ Hz, H-6), 3.92 (s, 3H, 3′-OCH_3); IR (KBr, cm^{-1}): 3427 (N–H), 2933, 2885 (C–H), 1629 (C=N), 1600, 1572, 1435 (C=C), 1463 (C–H), 1318 (C–N), 1263 (C–F), 1144, 1059 (C–O), 989 (C–Cl); EI-MS: *m/z* (rel. abund. %), 276 [M^+] (100), 278 [M^++2] (76), 261 (6), 211 (7), 198 (14), 171 (3), 138 (6), 108 (7), 99 (5); HREI-MS: *m/z* Calcd for $C_{14}H_{10}ClFN_2O$ [M^+] 276.0466; Found 276.0453.

4.2.19. 4′-(5-Fluoro-1H-benzo[d]imidazole-2-yl)-2′-methoxyphenyl acetate **(19)**

Brown solid, 0.164 g (55.0% yield); M.P. 124–127 °C; R_f: 0.36 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 7.89 (d, 1H, $J_{3',5'} = 1.6$ Hz, H-3′), 7.75 (dd, 1H, $J_{5',6'} = 8.4$ Hz, $J_{5',3'} = 1.6$ Hz, H-5′), 7.63–7.60 (m, 1H, H-4), 7.44 (dd, 1H, $J_{7,6} = 9.2$ Hz, $J_{7,5F} = 2.0$ Hz, H-7), 7.29 (d, 1H, $J_{6',5'} = 8.0$ Hz, H-6′), 7.12 (td, 1H, $J_{6,7} = 9.2$ Hz, $J_{6,4} = 2.4$ Hz, H-6), 3.89 (s, 3H, 2′-OCH_3), 2.29 (s, 3H, 1′-$OCOCH_3$); IR (KBr, cm^{-1}): 3415 (N–H), 3079, 2932, 2854 (C–H), 1761 (C=O), 1633 (C=N), 1603, 1504 (C=C), 1475 (C–H), 1375 (C–N), 1207 (C–F), 1139 (C–O); EI-MS: *m/z* (rel. abund. %), 300 [M^+] (10), 258 (100), 243 (13), 228 (12), 215 (18), 200 (11), 187 (9), 43 (7); HREI-MS: *m/z* Calcd for $C_{16}H_{13}FN_2O_3$ [8 M^+] 300.0910; Found 300.0903.

4.2.20. 4′-(5-Fluoro-1H-benzo[d]imidazole-2-yl)benzene-1′,2′,3′-triol **(20)**

Brown solid, 0.237 g (91.0% yield); M.P. 287–288 °C; R_f: 0.64 (ethyl acetate/hexane, 3:7); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 13.00 (br s, 2H, -NH, 3′-OH), 9.61 (br d, 1H, 2′-OH), 8.58 (br s, 1H, 1′-OH), 7.62–7.58 (m, 1H, H-4), 7.43 (d, 1H, $J_{7,6} = 8.0$ Hz, H-7), 7.35 (d, 1H, $J_{5',6'} = 8.8$ Hz, H-5′), 7.14 (dt, 1H, $J_{6,5F} = 9.6$ Hz, $J_{6,4} = 2.0$ Hz, H-6), 6.49 (d, 1H, $J_{6',5'} = 8.4$ Hz, H-6′); IR (KBr, cm^{-1}): ~3400 (broad O–H), 3240 (N–H), 3066 (C–H), 1624, 1494 (C=C), 1461 (C–H), 1307 (C–N), 1143 (C–F), 1110 (C–O); EI-MS: *m/z* (rel. abund. %), 260 [M^+] (100), 261 [M^++1] (29), 231 (24), 203 (4), 186 (12), 187 (13), 161 (24), 44 (53); HREI-MS: *m/z* Calcd for $C_{15}H_9FN_2O_3$ [M^+] 260.0597; Found 260.0599.

4.2.21. 4′-(5-Fluoro-1H-benzo[d]imidazole-2-yl)-2′,6′-dimethoxyphenol **(21)**

Brown solid, 0.161 g (55.9% yield); M.P. 311–313 °C; R_f: 0.36 (ethyl acetate/hexane, 3:7); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 9.29 (br s, 1H, 1′-OH), 7.68–7.64 (m, 1H, H-4), 7.51–7.49 (m, 3H, H-7, H-5′, H-3′), 7.21 (td, 1H, $J_{6,7} = 10.0$ Hz, $J_{6,4} = 2.4$ Hz, H-6), 3.88 (s, 6H, 2′-OCH_3, 6′-OCH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 160.3 (C-5), 158.0 (C-2), 152.0 (C-6′) 152.0 (C-2′), 148.2 (C-9), 139.2 (C-1′), 136.3 (C-8), 116.0 (C-4″), 115.0 (C-7), 111.8 (C-6), 111.5 (C-4), 104.8 (C-5′) 104.8 (C-3′), 56.2 (C-8′) 56.2 (C-7′); IR (KBr, cm^{-1}): 3516 (O–H), 3375 (N–H), 3100, 2937 (C–H), 1612, 1510 (C=C), 1477 (C–H), 1340 (C–N), 1232 (C–F), 1145, 1112 (C–O); EI-MS: *m/z* (rel. abund. %), 288 [M^+] (100), 289 [M^++1] (19), 273 (10), 257 (16), 245 (18), 213 (7), 108 (3), 83 (7); HREI-MS: *m/z* Calcd for $C_{15}H_{13}FN_2O_3$ [M^+] 288.0910; Found 288.0908.

4.2.22. 3′-Bromo-2′-(5-fluoro-1H-benzo[d]imidazole-2-yl)-6′-methoxyphenol **(22)**

Brown solid, 0.219 g (65.0% yield); M.P. 210–213 °C; R_f: 0.50 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 11.56 (br s, 1′-OH), 7.61–7.57 (m, 1H, H-4), 7.40 (dd, 1H, $J_{7,6} = 7.6$ Hz, H-7), 7.17 (d, 1H, $J_{4',5'} = 8.8$ Hz, H-4′), 7.10–7.05 (m, 2H, H-6, H-5′), 3.84 (s, 3H, 6′-OCH_3); ^{13}C NMR (75 MHz, DMSO-d6): δ_C 160.0 (C-5), 156.9 (C-2), 149.6 (C-6′), 147.8 (C-1′), 147.5 (C-9) 122.5 (C-8), 118.7 (C-5′), 118.0 (C-4′), 117.6 (C-2′), 114.3 (C-7), 112.5 (C-3′), 110.2 (C-6), 109.9 (C-4), 56.1 (C7′); IR (KBr, cm^{-1}): 3336 (O–H), 3100, 2931 (C–H), 1591 (C=C), 1450 (C–H), 1348 (C–N), 1247 (C–F), 1136 (C–O); EI-MS: *m/z* (rel. abund. %), 336 [M^+] (99), 338 [M^++2] (100), 320 (22), 318 (20), 307 (40), 293 (42), 258 (25), 240 (15), 227 (7), 185 (38), 110 (6); HREI-MS: *m/z* Calcd for $C_{14}H_{10}BrFN_2O_2$ [M^+] 335.9910; Found 335.9904.

4.2.23. 2-(2′-Bromo-4′,5′-dimethoxyphenyl)-5-fluoro-1H-benzo[d] imidazole **(23)**

Brown solid, 0.245 g (69.8% yield); M.P. 115–118 °C; R_f: 0.44 (ethyl acetate/hexane, 1:1); ^{1}H NMR (500 MHz, DMSO-d6): δ_H 12.73 (br d, 1H, -NH), 7.59 (br s, 1H, H-4), 7.40 (br d, 1H, $J_{7,6} = 8.5$ Hz, H-7), 7.33 (s, 1H, H-3′), 7.30 (s, 1H, H-6′), 7.09 (td, 1H, $J_{6,7} = 9.5$ Hz, $J_{6,4} = 2.0$ Hz, H-6), 3.85 (s, 3H, 5′-OCH_3), 3.81 (s, 3H, 4′-OCH_3); IR (KBr, cm^{-1}): 3168 (N–H), 3004, 2953, 2836 (C–H), 1633 (C=N), 1602, 1504 (C=C), 1443 (C–H), 1267 (C–F), 1136, 1035 (C–O), 900 (C–Br); EI-MS: *m/z* (rel. abund. %), 350 [M^+] (99), 352 [M^++2] (100), 335 (19), 321 (23), 307 (14), 228 (8), 213 (23), 198 (7), 185 (11), 110 (4), 57 (10), 43 (6); HREI-MS: *m/z* Calcd for $C_{15}H_{12}N_2O_2BrF$ [M^+] 350.0066; Found 350.0063.

4.2.24. 2-(2′-Chloro-3′,4′-dimethoxyphenyl)-5-fluoro-1H-benzo[d] imidazole **(24)**

Brown solid, 0.191 g (62.3% yield); M.P. 142–145 °C; R_f: 0.38 (ethyl acetate/hexane, 1:1); ^{1}H NMR (500 MHz, DMSO-d6): δ_H 12.72 (br s, 1H, -NH), 7.63 (d, 1H, $J_{6',5'} = 9.0$ Hz, H-6′), 7.60 (m, 1H, H-4), 7.39 (d, 1H, $J_{7,6} = 8.5$ Hz, H-7), 7.23 (d, 1H, $J_{5',6'} = 8.5$ Hz, H-5′), 7.08 (td, 1H, $J_{6,7} = 10.0$ Hz, $J_{6,4} = 2.5$ Hz, H-6), 3.91 (s, 3H, 4′-OCH_3), 3.80 (s, 3H, 3′-OCH_3); IR (KBr, cm^{-1}): 3151 (N–H), 2945, 2845 (C–H), 1630 (C=N), 1596, 1423 (C=C), 1460 (C–H), 1284 (C–F), 1136, 1041 (C–O); EI-MS: *m/z* (rel. abund. %), 306 [M^+] (66), 308 [M^++2] (25), 291 (11), 263 (30), 248 (13), 228 (7), 185 (39), 83 (100), 44 (15); HREI-MS: *m/z* Calcd for $C_{15}H_{12}ClFN_2O_2$ [M^+] 306.0566; Found 306.0571.

4.2.25. 2′,4′-Dichloro-6′-(5-fluoro-1H-benzo[d]imidazole-2-yl) phenol **(25)**

Brown solid, 0.266 g (89.5% yield); M.P. 313–314 °C; R_f: 0.67 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 13.72 (br s, 1H, -NH), 8.14 (d, 1H, $J_{5',3'} = 2.4$ Hz, H-5′), 7.70 (br s, 1H, H-4), 7.70 (d, 1H, $J_{3',5'} = 2.4$ Hz, H-3′) 7.54 (br d, 1H, $J_{7,6} = 5.2$ Hz, H-7), 7.22 (td, 1H, $J_{6,7} = 9.6$ Hz, $J_{6,4} = 1.6$ Hz, H-6); IR (KBr, cm^{-1}): 3367 (O–H), 3091 (C–H), 1664 (C=N), 1596, 1461 (C=C), 1520 (N–H), 1311

(C–N), 1187 (C–F), 1140 (C–O), 962 (C–Cl); EI-MS: *m/z* (rel. abund. %), 296 [M^+] (100), 298 [M^++2] (67), 300 [M^++4] (11), 261 (6), 233 (31), 197 (8), 148 (3), 134 (4), 83 (3); HREI-MS: *m/z* Calcd for $C_{13}H_7N_2OCl_2F$ [M^+] 295.9919; Found 295.9937.

4.2.26. 2′-Bromo-4′-chloro-6′-(5-fluoro-1H-benzo[d]imidazole-2-yl)phenol **(26)**

Brown solid, 0.300 g (87.8% yield); M.P. 276–278 °C; R_f: 0.69 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 13.79 (br s, 2H, -NH, 1′-OH), 8.18 (d, 1H, $J_{5',3'} = 2.1$ Hz, H-5′), 7.81 (d, 1H, $J_{3',5'} = 2.4$ Hz, H-3′) 7.70 (br s, 1H, H-4), 7.55 (br d, 1H, $J_{7,6} = 8.1$ Hz, H-7), 7.22 (td, 1H, $J_{6,7} = 9.6$ Hz, $J_{6,4} = 1.8$ Hz, H-6); IR (KBr, cm^{-1}): 3382 (O–H), 3086 (C–H), 1632 (C=N), 1597, 1452 (C=C), 1311 (C–N), 1181 (C–F), 1145 (C–O), 885 (C–Br); EI-MS: *m/z* (rel. abund. %), 340 [M^+] (82), 342 [M^++2] (100), 344 [M^++4] (24), 261 (6), 233 (35), 198 (9), 116 (4), 99 (5); HREI-MS: *m/z* Calcd for $C_{13}H_7N_2OBrClF$ [M^+] 339.9414; Found 339.9434.

4.2.27. 4′-(5-Chloro-1H-benzo[d]imidazole-2-yl)-2′-methoxyphenyl acetate **(27)**

Dark-brown solid, 0.196 g (61.9% yield); M.P. 128–129 °C; R_f: 0.38 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 7.89 (d, 1H, $J_{3',5'} = 1.6$ Hz, H-3′), 7.76 (dd, 1H, $J_{5',6'} = 8.4$ Hz, $J_{5',3'} = 1.6$ Hz, H-5′), 7.66 (s, 1H, H-4), 7.63 (d, 1H, $J_{7,6} = 8.8$ Hz, H-7), 7.29 (d, 1H, $J_{6',5'} = 8.4$ Hz, H-6′), 7.26 (dd, 1H, $J_{6,7} = 8.8$ Hz, $J_{6,4} = 2.0$ Hz, H-6), 3.90 (s, 3H, 2′-OCH_3), 2.29 (s, 3H, 1′-$OCOCH_3$); IR (KBr, cm^{-1}): 3076 (N–H), 2935 (C–H), 1758 (C=O), 1656 (C=N), 1600, 1500 (C=C), 1431 (C–H), 1374 (C–N), 1204 (C–O), 1060 (C–Cl); EI-MS: *m/z* (rel. abund. %), 316 [M^+] (36), 318 [M^++2] (13) 274 (100), 259 (15), 245 (23), 231 (27), 203 (15), 168 (16), 137 (12), 90 (11), 63 (21); HREI-MS: *m/z* Calcd for $C_{16}H_{13}ClN_2O_3$ [8^+] 316.0615; Found 316.0621.

4.2.28. 5-Chloro-2-(2′-chloro-3′-methoxyphenyl)-1H-benzo[d]imidazole **(28)**

Brown solid, 0.217 g (74.0% yield); M.P. 145–147 °C; R_f: 0.48 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 7.67 (s, 1H, H-4), 7.64 (d, 1H, $J_{7,6} = 8.7$ Hz, H-7), 7.49–7.42 (m, 2H, H-6′, H-5′), 7.33 (dd, 1H, $J_{6,7} = 7.5$ Hz, $J_{6,4} = 2.4$ Hz, H-6), 7.27 (dd, 1H, $J_{4',5'} = 8.4$ Hz, $J_{4',6'} = 2.1$ Hz, H-4′), 3.92 (s, 3H, 3′-OCH_3); IR (KBr, cm^{-1}): 3097 (N–H), 2969 (C–H), 1573, 1432 (C=C), 1537 (N–H), 1269, 1060 (C–O), 980 (C–Cl); EI-MS: *m/z* (rel. abund. %), 292 [M^+] (100), 294 [M^++2] (68), 296 [M^++4] (12), 277 (4), 256 (15), 167 (3), 152 (2), 147 (3), 141 (3), 124 (6), 90 (3), 43 (9); HREI-MS: *m/z* Calcd for $C_{14}H_{10}N_2OCl_2$ [M^+] 292.0170; Found 292.0165.

4.2.29. 2-(5′-Bromo-2′-methoxyphenyl)-5-chloro-1H-benzo[d]imidazole **(29)**

Brown solid, 0.259 g (76.7% yield); M.P. 115–118 °C; R_f: 0.51 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 8.39 (d, 1H, $J_{6',4'} = 2.4$ Hz, H-6′), 7.68–7.62 (m, 3H, H-6, H-4, H-4′), 7.25 (d, 2H, $J_{7,6} = J_{3',4'} = 9.0$ Hz, H-7, H-3′), 4.02 (s, 3H, 2′-OCH_3); IR (KBr, cm^{-1}): 3324 (N–H), 3173, 2939, 2842 (C–H), 1622, 1470 (C=C), 1517 (N–H), 1431 (C–H), 1338 (C–N), 1257, 1090 (C–O), 1058 (C–Cl), 852 (C–Br); EI-MS: *m/z* (rel. abund. %), 320 [M^+] (100), 322 [M^++2] (94), 302 (4), 292 (30), 227 (23), 211 (48), 198 (18), 192 (15), 137 (77), 127 (15), 107 (11), 111 (13), 99 (13), 89 (7), 63 (10)); HREI-MS: *m/z* Calcd for $C_{14}H_{10}BrClN_2O$ [M^+] 335.9665; Found 335.9659.

4.2.30. 5-Chloro-2-(2′-fluoro-4′-methoxyphenyl)-1H-benzo[d]imidazole **(30)**

Brown solid, 0.175 g (63.2%yield); M.P. 158–159 °C; R_f: 0.55 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 8.16 (t, 1H, $J_{6',5'} = 8.7$ Hz, H-6′), 7.62 (s, 1H, H-4), 7.60 (d, 1H, $J_{7,6} = 9.0$ Hz, H-7), 7.22 (dd, 1H, $J_{6,7} = 8.4$ Hz, $J_{6,4} = 1.8$ Hz, H-6), 7.09 (dd, 1H, $J_{3',2'F} = 13.5$ Hz, $J_{3',5'} = 2.4$ Hz H-3′), 6.99 (dd, 1H, $J_{5',6'} = 8.7$ Hz, $J_{5',3'} = 2.4$ Hz, H-5′), 3.85 (s, 3H, 4′-OCH_3); IR (KBr, cm^{-1}): 3065, 3015, 2968 (C–H), 1627, 1580, 1494 (C=C), 1442 (C–H), 1321 (C–N), 1283, 1094 (C–O), 1153 (C–F), 1027 (C–Cl); EI-MS: *m/z* (rel. abund. %), 276 [M^+] (100), 278 [M^++2] (84), 261 (96), 241 (4), 213 (24), 138 (7) 124 (6), 63 (8); HREI-MS: *m/z* Calcd for $C_{14}H_{10}ClFN_2O$ [M^+] 276.0466; Found 276.0459.

4.2.31. 2-(2′-Bromo-4′,5′-dimethoxyphenyl)-5-chloro-1H-benzo[d]imidazole **(31)**

Brown solid, 0.216 g (58.8% yield); M.P. 113–115 °C; R_f: 0.46 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 7.66 (s, 1H, H-4), 7.62 (d, 1H, $J_{7,6} = 8.4$ Hz, H-7), 7.34 (s, 1H, H-3′), 7.31 (s, 1H, H-6′), 7.26 (dd, 1H, $J_{6,7} = 8.4$ Hz, $J_{6,4} = 1.6$ Hz, H-6), 3.86 (s, 3H, 5′-OCH_3), 3.81 (s, 3H, 4′-OCH_3); IR (KBr, cm^{-1}): 3350 (N–H), 3092, 2939, 2840 (C–H), 1602, 1497 (C=C), 1435 (C–H), 1333 (C–N), 1257, 1213 (C–O), 1028 (C–Cl), 859 (C–Br); EI-MS: *m/z* (rel. abund. %), 366 [M^+] (81), 368 [M^++2] (100), 370 [M^++4] (26), 353 (21), 337 (31), 322 (16), 272 (5), 229 (29), 215 (7), 201 (11), 184 (4), 166 (5), 43 (3); HREI-MS: *m/z* Calcd for $C_{15}H_{12}N_2O_2BrCl$ [M^+] 365.9771; Found 365.9804.

4.2.32. 4′-(5-Chloro-1H-benzo[d]imidazole-2-yl)-2′-iodo-6′-methoxyphenol **(32)**

Brown soild, 0.341 g (85.1% yield); M.P. 202–204 °C; R_f: 0.62 (ethyl acetate/hexane, 3:7); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 10.25 (s, 1H, 1′-OH), 8.11 (d, 1H, $J_{3',5'} = 1.6$ Hz, H-3′), 7.76 (d, 1H, $J_{5',3'} = 1.6$ Hz, H-5′), 7.65 (d, 1H, $J_{4,6} = 1.6$ Hz, H-4), 7.61 (d, 1H, $J_{7,6} = 8.4$ Hz, H-7), 7.27 (dd, 1H, $J_{6,7} = 8.4$ Hz, $J_{6,4} = 2.0$ Hz, H-6), 3.92 (s, 3H, 6′-OCH_3); IR (KBr, cm^{-1}): 3459 (O–H), 3375 (N–H), 3087, 2933, 2848 (C–H), 1630, 1588 (C=C), 1472 (C–H), 1351 (C–N), 1281, 1039 (C–O), 600 (C–I); EI-MS: *m/z* (rel. abund. %), 400 [M^+] (100), 402 [M^++2] (57), 385 (6), 275 (5), 273 (14), 260 (6), 258 (14), 243 (20), 200 (10), 152 (3), 129 (5); HREI-MS: *m/z* Calcd for $C_{14}H_{10}N_2O_2ClI$ [M^+] 399.9475; Found 399.9488.

4.2.33. 2′-Bromo-4′-chloro-6′-(5-chloro-1H-benzo[d]imidazole-2-yl)phenol **(33)**

Dark-brown, 0.296 g (82.7% yield); M.P. 265–267 °C; R_f: 0.70 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 13.77 (br s, 2H, -NH, 1′-OH), 8.18 (d, 1H, $J_{5',3'} = 2.1$ Hz, H-5′), 7.82 (d, 1H, $J_{3',5'} = 2.1$ Hz, H-3′), 7.78 (br s, 1H, H-4), 7.72 (br d, 1H, $J_{7,6} = 8.4$ Hz, H-7), 7.36 (dd, 1H, $J_{6,7} = 8.4$ Hz, $J_{6,4} = 1.5$ Hz, H-6); IR (KBr, cm^{-1}): 3380 (O–H), 3076 (C–H), 1657 (C=N), 1585, 1451 (C=C), 1517 (N–H), 1303 (C–N), 1178 (C–O), 981 (C–Cl), 866 (C–Br); EI-MS: *m/z* (rel. abund. %), 356 [M^+] (67), 358 [M^++2] (100), 360 [M^++4] (48), 362 [M^++6] (8), 323 (4), 277 (5), 249 (30), 214 (7), 179 (5), 107 (5), 125 (5); HREI-MS: *m/z* Calcd for $C_{13}H_7N_2OBrCl_2$ [M^+] 355.9119; Found 355.9141.

4.2.34. 5-Chloro-2-(2′-chloro-3′,4′-dimethoxyphenyl)-1H-benzo[d]imidazole **(34)**

Dark-brown solid, 0.243 g (75.2% yield); M.P. 129–131 °C; R_f: 0.40 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 7.65 (d, 1H, $J_{6',5'} = 8.8$ Hz, H-6′), 7.65 (s, 1H, H-4), 7.61 (d, 1H, $J_{7,6} = 8.4$ Hz, H-7), 7.24 (dd, 1H, $J_{6,7} = 8.4$ Hz, $J_{6,4} = 2.4$ Hz, H-6), 7.23 (d, 1H, $J_{5',6'} = 8.8$ Hz, H-5′), 3.91 (s, 3H, 4′-OCH_3), 3.80 (s, 3H, 3′-OCH_3); IR (KBr, cm^{-1}): 3179 (N–H), 3140, 2938, 2841 (C–H), 1594, 1479 (C=C), 1457 (C–H), 1282, 1040 (C–O); EI-MS: *m/z* (rel. abund. %), 322 [M^+] (100), 324 [M^++2] (59), 326 [M^++4] (11), 307 (14), 279 (46), 264 (16), 244 (13), 201 (17), 185 (54), 83 (48), 85 (27), 44 (22); HREI-MS: *m/z* Calcd for $C_{15}H_{12}Cl_2N_2O_2$ [M^+] 322.0270; Found 322.0282.

*4.2.35. 2-(4′-Bromo-3′,5′-dimethoxyphenyl)-5-chloro-1H-benzo[d] imidazole **(35)***

Dark-brown solid, 0.346 g (94.0% yield); M.P. 254–256 °C; R_f: 0.50 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 7.69 (d, 1H, $J_{4,6} = 1.5$ Hz, H-4), 7.66 (d, 1H, $J_{7,6} = 8.7$ Hz, H-7), 7.53 (s, 2H, H-6′, H-2′), 7.28 (dd, 1H, $J_{6,7} = 8.4$ Hz, $J_{6,4} = 1.8$ Hz, H-6), 3.96 (s, 6H, 5′-OCH_3, 3′-OCH_3); IR (KBr, cm^{-1}): 3384 (N–H), 3094, 2943, 2847 (C–H), 1631 (C=N), 1587 (C=C), 1465 (C–H), 1240, 1124 (C–O), 1036 (C–Cl), 846 (C–Br); EI-MS: *m/z* (rel. abund. %), 366 [M^+] (100), 368 [M^++2] (97), 370 [M^++4] (24), 337 (8), 257 (20), 229 (39), 227 (27), 201 (12), 183 (5), 44 (26); HREI-MS: *m/z* Calcd for $C_{15}H_{10}BrClN_2O_2$ [M^+] 365.9765; Found 365.9768.

*4.2.36. 3′-Bromo-2′-(5-chloro-1H-benzo[d]imidazole-2-yl)-6′-methoxyphenol **(36)***

Brown solid, 0.296 g (83.7% yield); M.P. 214–216 °C; R_f: 0.52 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 11.50 (br s, 2H, -NH, 1′-OH), 7.65 (s, 1H, H-4), 7.61 (d, 1H, $J_{7,6} = 8.4$ Hz, H-7), 7.25 (dd, 1H, $J_{6,7} = 8.4$ Hz, $J_{6,4} = 2.0$ Hz, H-6), 7.17 (d, 1H, $J_{4',5'} = 8.8$ Hz, H-4′), 7.08 (d, 1H, $J_{5',4'} = 8.8$ Hz, H-5′), 3.85 (s, 3H, 6′-OCH_3); ^{13}C NMR (75 MHz, DMSO-d6): δ_C 149.6 (C-2), 147.7 (C-6′), 147.5 (C-1′), 138.5 (C-9), 120.3 (C-8), 126.2 (C-5), 122.4 (C-5′, C-4′), 122.1 (C-6), 118.8 (C-2′), 114.3 (C-7, C-4), 112.6 (C-3′), 56.1 (7′); IR (KBr, cm^{-1}): 3336 (O–H), 3100, 2837 (C–H), 1587 (C=C), 1510 (N–H), 1458 (C–H), 1344 (C–N), 1247 (C–O), 1053 (C–Cl), 875 (C–Br); EI-MS: *m/z* (rel. abund. %), 352 [M^+] (80), 354 [M^++2] (100), 356 [M^++4] (28), 336 (25), 334 (19), 274 (9), 229 (14), 215 (11), 201 (36.5), 126 (4); HREI-MS: *m/z* Calcd for $C_{14}H_{10}BrClN_2O_2$ [M^+] 351.9614; Found 351.9602.

*4.2.37. 4′-(5-Chloro-1H-benzo[d]imidazole-2-yl)-2′,6′-dimethoxyphenol **(37)***

Dark-brown solid, 0.253 g (83.0% yield); M.P. 284–286 °C; R_f: 0.45 (ethyl acetate/hexane, 3:7); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 9.24 (br s, 1H, 1′-OH), 7.68 (s, 1H, H-4), 7.64 (d, 1H, $J_{7,6} = 8.4$ Hz, H-7), 7.49 (s, 2H, H-3′, H-5′), 7.32 (dd, 1H, $J_{6,7} = 8.4$ Hz, $J_{6,4} = 2.0$ Hz, H-6), 3.88 (s, 6H, 2′-OCH_3, 6′-OCH_3); IR (KBr, cm^{-1}): 3500 (O–H), 3200 (N–H), 3114, 2941, 2844 (C–H), 1627, 1504 (C=C), 1467 (C–H), 1361 (C–N), 1234, 1118 (C–O); EI-MS: *m/z* (rel. abund. %), 304 [M^+] (100), 306 [M^++2] (34), 289 (10), 273 (14), 258 (14), 246 (8), 218 (10), 177 (4), 44 (13); HREI-MS: *m/z* Calcd for $C_{15}H_{13}ClN_2O_3$ [M^+] 304.0615; Found 304.0616.

*4.2.38. 2′-Methoxy-4′-(5-nitro-1H-benzo[d]imidazole-2-yl)phenyl acetate **(38)***

Yellow solid, 0.231 g (70.6% yield); M.P. 203–207 °C; R_f: 0.31 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 13.61 (br s, 1H, -NH), 8.50 (br s, 1H, H-6), 8.15 (d, 1H, $J_{7,6} = 8.4$ Hz, H-7), 7.94 (s, 1H, H-3′), 7.83 (d, $J_{5',6'} = 8.4$ Hz, H-5′), 7.77 (br s, 1H, H-4), 7.34 (d, 1H, $J_{6',5'} = 8.4$ Hz, H-6′) 3.91 (s, 3H, 2′-OCH_3), 2.29 (s, 3H, 1′-$OCOCH_3$); IR (KBr, cm^{-1}): 3315 (N–H), 3101, 2959 (C–H), 1760 (C=O), 1501 (C=C), 1338 (N=O), 1214 (C–O); EI-MS: *m/z* (rel. abund. %), 327 [M^+] (8), 285 (100), 297 (3), 255 (25), 239 (20), 212 (10), 90 (11), 69 (14), 51 (5); HREI-MS: *m/z* Calcd for $C_{16}H_{13}N_3O_5$ [M^+] 327.0855; Found 327.0856.

*4.2.39. 2-(2′-Fluoro-4′-methoxyphenyl)-5-nitro-1H-benzo[d] imidazole **(39)***

Yellow solid, 0.177 g (61.6% yield); M.P. 222–224 °C; R_f: 0.52 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 13.03 (s, -NH), 8.51 (br d, 1H, H-6), 8.20 (br s, 1H, H-4), 8.13 (br d, 1H, $J_{6',5'} = 7.2$ Hz, H-6′), 7.73 (br s, 1H, H-7), 7.13 (d, 1H, $J_{3',2'F} = 14$ Hz, H-3′), 7.03 (dd, 1H, $J_{5',6'} = 8.8$ Hz, $J_{5',3'} = 2.0$ Hz, H-5′), 3.87 (s, 3H, 4′-OCH_3); IR (KBr, cm^{-1}): 3425 (N–H), 3096, 2940, 2844 (C–H), 1626, 1485 (C=C), 1508, 1333 (N=O), 1442 (C–H), 1230 (C–F), 1154, 1066 (C–O); EI-MS: *m/z* (rel. abund. %), 287 [M^+] (100), 288 [M^++1] (15), 257 (18), 241 (24), 214 (18), 90 (5), 63 (5); HREI-MS: *m/z* Calcd for $C_{14}H_{10}FN_3O_3$ [M^+] 287.0706; Found 287.0705.

*4.2.40. 2-(2′-Chloro-3′-methoxyphenyl)-5-nitro-1H-benzo[d] imidazole **(40)***

Yellow solid, 0.259 g (85.3% yield); M.P. 206–208 °C; R_f: 0.45 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 13.40 (br d, 1H, -NH), 8.54 (br s, 1H, H-4), 8.17 (d, 1H, $J_{7,6} = 7.5$ Hz, H-7), 7.80 (br d, 1H, H-6), 7.53–7.46 (m, 2H, H-6′, H-5′), 7.38 (dd, 1H, $J_{4',5'} = 7.2$ Hz, $J_{4',6'} = 2.4$ Hz, H-4′), 3.94 (s, 3H, 3′-OCH_3); IR (KBr, cm^{-1}): 3105, 2938, 2850 (C–H), 1666 (C=N), 1573, 1435 (C=C), 1518, 1338 (N=O), 1467 (C–H), 1275, 1059 (C–O), 979 (C–Cl); EI-MS: *m/z* (rel. abund. %), 303 [M^+] (100), 305 [M^++2] (30), 273 (23), 258 (23), 242 (4), 230 (7), 222 (5), 207 (2), 179 (4), 168 (3), 90 (9), 63 (8); HREI-MS: *m/z* Calcd for $C_{14}H_{10}N_3O_3Cl$ [M^+] 303.0411; Found 304.0391.

*4.2.41. 3′-Bromo-6′-methoxy-2′-(5-nitro-1H-benzo[d]imidazole-2-yl)phenol **(41)***

Yellow solid, 0.299 g (82.1% yield); M.P. 272–274 °C; R_f: 0.39 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 13.29 (br s, 1H, -NH), 9.89 (br s, 1H, 1′-OH), 8.51 (br s, 1H, H-6), 8.15 (d, 1H, $J_{7,6} = 8.8$ Hz, H-7), 7.76 (br s, 1H, H-4), 7.20 (d, 1H, $J_{4',5'} = 8.8$ Hz, H-4′), 7.11 (d, 1H, $J_{5',4'} = 8.8$ Hz, H-5′), 3.86 (s, 3H, 6′-OCH_3); IR (KBr, cm^{-1}): 3348 (O–H), 3095, 2933, 2839 (C–H), 1591 (C=C), 1517, 1334 (N=O), 1461 (C–H), 1249 (C–O), 881 (C–Br); EI-MS: *m/z* (rel. abund. %), 363 [M^+] (99), 365 [M^++2] (100), 348 (14), 334 (42), 320 (28), 290 (14), 274 (29), 212 (5), 166 (22), 90 (6); HREI-MS: *m/z* Calcd for $C_{14}H_{10}BrN_3O_4$ [M^+] 362.9855; Found 362.9853.

*4.2.42. 2-(2′-Bromo-4′,5′-dimethoxyphenyl)-5-nitro-1H-benzo[d] imidazole **(42)***

Orange solid, 0.256 g (67.7% yield); M.P. 168–171 °C; R_f: 0.39 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 13.30 (s, 1H, -NH), 8.56 (br d, 1H, H-6), 8.15 (s, 1H, H-4), 7.86 (br d, 1H, H-7), 7.39 (s, 1H, H-3′), 7.34 (s, 1H, H-6′), 3.87 (s, 3H, 5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3); IR (KBr, cm^{-1}): 3350 (N–H), 3102, 2937, 2841 (C–H), 1600, 1493 (C=C), 1436 (C–H), 1335 (N=O), 1257, 1213 (C–O), 869 (C–Br); EI-MS: *m/z* (rel. abund. %), 377 [M^+] (100), 379 [M^++2] (99), 362 (27), 348 (23), 333 (34), 318 (20), 240 (9), 194 (21), 90 (34), 63 (65); HREI-MS: *m/z* Calcd for $C_{15}H_{12}N_3O_4Br$ [M^+] 377.0011; Found 376.9998.

*4.2.43. 2′-Iodo-6′-methoxy-4′-(5-nitro-1H-benzo[d]imidazole-2-yl) phenol **(43)***

Yellow solid, 0.368 g (89.5% yield); M.P. 258–261 °C; R_f: 0.58 (ethyl acetate/hexane, 3:7); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 13.43 (s, 1H, -NH), 10.25 (s, 1H, 1′-OH), 8.48 (br d, 1H, H-6), 8.15 (s, 1H, H-3′), 8.11 (d, 1H, $J_{7,6} = 8.8$ Hz, H-7), 7.80 (s, 1H, H-5′), 7.69 (br s, 1H, H-4), 3.94 (s, 3H, 6′-OCH_3); IR (KBr, cm^{-1}): 3607 (O–H), 3346 (N–H), 3095, 2939 (C–H), 1627, 1591 (C=C), 1473 (C–H), 1335 (N=O), 1282 (C–O), 629 (C–I); EI-MS: *m/z* (rel. abund. %), 411 [M^+] (100), 412 [M^++1] (60), 381 (18), 365 (30), 269 (10), 254 (19), 223 (9), 90 (7), 64 (9), 44 (3); HREI-MS: *m/z* Calcd for $C_{14}H_{10}N_3O_4I$ [M^+] 410.9716; Found 410.9717.

*4.2.44. 2′-Bromo-4′-chloro-6′-(5-nitro-1H-benzo[d]imidazole-2-yl) phenol **(44)***

Orange solid, 0.362 g (98.2% yield); M.P. 320–323 °C; R_f: 0.61 (ethyl acetate/hexane, 1:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 13.95 (br s, 1H, -NH), 8.58 (s, 1H, H-4), 8.23–8.19 (m, 2H, H-6, H-5′), 7.88 (d, 1H, $J_{3',5'} = 2.4$ Hz, H-3′), 7.88 (d, 1H, $J_{7,6} = 9.3$ Hz, H-7); IR (KBr, cm^{-1}): 3514 (N–H), 3347 (O–H), 3084 (C–H), 1639, 1455 (C=C), 1519, 1337 (N=O), 1183 (C–O), 982 (C–Cl), 871 (C–Br); EI-MS: *m/z*

(rel. abund. %), 367 [M^+] (76), 369 [M^++2] (100), 371 [M^++4] (23), 339 (5), 323 (39), 179 (4), 90 (5), 63 (7); HREI-MS: *m/z* Calcd for $C_{13}H_7BrClN_3O_3$ [M]$^+$ 336.9359; Found 366.9353.

4.2.45. 2-(5′-Bromo-2′-fluorophenyl)-5-nitro-1H-benzo[d] imidazole (45)

Brown solid, 0.168 g (50.2% yield); M.P. 228–230 °C; R_f: 0.55 (ethyl acetate/hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 13.33 (br s, 1H, -NH), 8.54 (s, 1H, H-4), 8.39 (dd, 1H, $J_{6,7} = 6.4$ Hz, $J_{6,4} = 2.4$ Hz, H-6), 8.18 (dd, 1H, $J_{4',3'} = 8.8$ Hz, $J_{4',6'} = 2.0$ Hz, H-4′), 7.83–7.81 (m, 2H, H-7, H-6′), 7.52 (td, 1H, $J_{3',4'} = 8.8$ Hz, H-3′); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 160.0 (C-2′), 157.5 (C-2), 149.5 (C-5), 143.0 (C-9, C-8), 135.4 (C-6′), 135.3 (C-4′), 132.3 (C-6), 119.3 (C-7), 119.1 (C-1′), 119.0 (C-3′), 118.3 (C-4), 116.8 (C-5); IR (KBr, cm^{-1}): 3615 (N–H), 3106 (C–H), 1629 (C=N), 1591 (N=O), 1474 (C=C), 1523, 1343 (N=O), 885 (C–Br); EI-MS: *m/z* (rel. abund. %), 335 [M^+] (100), 337 [M^++2] (98), 305 (24), 289 (25), 210 (23), 105 (6), 90 (15), 63 (18); HREI-MS: *m/z* Calcd for $C_{13}H_7BrFN_3O_2$ [M^+] 334.9706; Found 334.9703.

4.3. α-Amylase inhibition assay

The α-amylase inhibitory activity was determined by an assay modified from Kwon, Apostolidis & Shetty [36,37]. A volume of 500 μL of test sample (100 μg/mL, 200 μg/mL, 400 μg/mL, 800 μg/mL, 1000 μg/mL) and 500 μL of α-amylase solution (0.5 mg/mL) in 0.2 mM phosphate buffer (pH 6.9) were incubated at 25 °C for 10 min. After pre-incubation, 500 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at 25 °C for 10 min. The reaction was arrested with 1 mL of dinitrosalicylic acid colour reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The solutions were diluted after adding 10 mL distilled water and the absorbance was measured at 540 nm [38]. Acarbose was used as standard [11] and assay was carried out in triplicate.

The percentage of inhibition was calculated as illustrated, % Inhibition = (Absorbance $_{Control}$ − Absorbance $_{Sample}$)/Absorbance $_{Control}$ x 100.

The IC_{50} values, concentration required to inhibit the α-amylase activity by 50% were calculated by a non-linear regression graph plotted between percentage inhibition (x axis) versus concentrations (y axis), using a Graph Pad Prism Software (Version 5).

4.4. Molecular docking

In silico molecular docking study was conducted by MOE to explore the binding interactions of the 2-aryl benzimidazole derivatives toward the α-amylase enzyme. Three dimensional crystal structure of the target protein α-amylase was downloaded from Protein Databank (ID: 1HNY). The structures of the 2-aryl benzimidazole derivatives were built in MOE and energy minimized using the default parameters of the MOE (www.chemcomp.com). α-Amylase was allowed to dock to the 2-aryl benzimidazole compounds using MOE by the default parameters *i.e.* Placement: Triangle Matcher, Rescoring: London dG. For each ligand ten conformations were generated. The top-ranked conformation of each compound was used for further analysis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.03.011.

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