Synthesis of *diabetic II* inhibitors based on 2-mercaptobenzimidazole and their molecular docking study

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Abstract

In the search of potent α-amylase inhibitors, we have synthesized seventeen derivatives of 2-mercaptobenzimidazole bearing sulfonamide (**1-17**) and evaluated for their α-amylase inhibitory potential. All compounds display a variable degree of α-amylase activity having IC$_{50}$ values ranging between 0.90 ± 0.05 to $11.20 \pm 0.30 \, \mu$M when compared with the standard drug acarbose having IC$_{50}$ value $1.70 \pm 0.10 \, \mu$M. Compound **1, 2, 11, 12** and **14** having IC$_{50}$ values 1.40 ± 0.10, 1.30 ± 0.05, 0.90 ± 0.05, 1.60 ± 0.05 and $1.60 \pm 0.10 \, \mu$M respectively were found many folds better than the standard drug acarbose. The remaining analogs showed good inhibitory potentials. All the synthesized compounds were characterized by HREI-MS, ^{1}H and ^{13}C-NMR. Structure activity relationship (SAR) has been recognized for all newly synthesized analogs. Through molecular docking study, binding mode of active analogs with α-amylase enzyme was confirmed.

**Keywords:** Synthesis, 2-mercaptabezimidazole, sulfonamide, molecular docking study, α-amylase, SA

1.0. Introduction

α-Amylase is an enzyme (EC.3.2.1.1) that hydrolyses the α-linkage of polysaccharide such as glycogen and starch yielding the dextrin and glucose [1, 2]. α-Amylase can be found in normal serum, human saliva and urine [3]. Design of α- amylase inhibitors can lead to the development of new treatment method for the metabolic disorders like obesity and type II diabetes. α-Amylase preferred for their economical bulk production and malleability for genetic manipulation [4]. α-Amylase were studied extensively due to its broad-spectrum applications in industries oscillating from food to textiles, biofuel, detergents and furthermore in elimination of environment pollutant and had been realized in ground-breaking of medicine [5,6]. These enzyme inhibitors can slow down absorption of carbohydrates, prolonging absorption time of total carbohydrate and reducing glucose absorption which consequently reduce postprandial plasma glucose level.

Benzimidazole is widely spread nitrogen containing bicyclic aromatic heterocyclic compound. Both natural and synthetic benzimidazole derivatives attracted scientific interest due to significant biological activities such as antifungal [7], anti-inflammatory [8], analgesic [9], antibacterial [10], CNS depressant [11], anti-HIV [12] and anthelmintic [13].

2-Mercaptobezimidazole derivatives are one of the most important class of benzimidazole derivatives display a diverse range of interesting biological activities like neutropic [14], analgesic [15] and antihistamine [16] activities. Similarly, sulfone moiety is also the basic functional group and have a variety of biological activities like antibacterial [17], antitumor [18], anti-inflammatory [19] and anhydrase inhibitory [20] activities.

Our research group is continuously in struggle to design and synthesized various heterocyclic scaffold in search of potent therapeutics. In this regard we have already reported various heterocyclic moiety as alpha glucosidase, alpha amylase and beta glucuronidase inhibitor [21-23]. We have previously reported, benzimidazole derivatives as α-glucosidase inhibitors [24], *bis*-indole bearing sulfone as beta-glucuronidase inhibitor and piperazine sulfonamide analogs as alpha amylase inhibitor [25, 26]. Keeping in view the great biological importance of sulfone and benzimidazole, here in this study we have plan to synthesize 2-mercaptobenzimidazole bearing sulfonamide as a potent alpha amylase inhibitor.
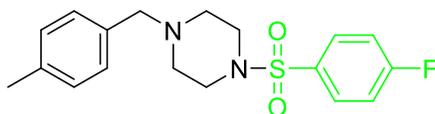
Previously reported analogs

Newly synthesized analogs
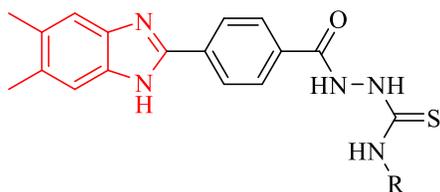
Benzimidazole derivatives
as a-glucosidase inhibitors
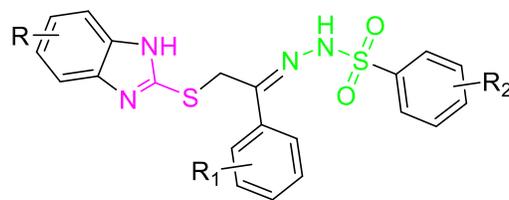
New Synthetic compounds (1-17)
IC_{50} = 1.30 ± 0.05 to 11.20 ± 0.30 μM
New α-amylase inhibitors
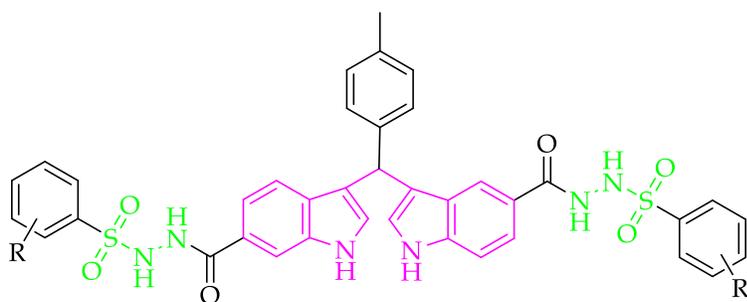
Bis-indole bearing sulfone
as β-glucuronidase inhibitors

Piperazin sulfonamide
as α-amylase inhibitors

Fig-1: Rationalization of the current study

2.0. RESULTS AND DISCUSSION

2.1. Chemistry

Synthesis of 2-mercaptobenzimidazole bearing sulfonamide analogs were carried out in three step.

Step-1:

2-mercaptobenzimidazole was reacted and refluxed with different substituted phenacyl bromide in acetone in the presence of potassium carbonate which yielded 2-((substituted-1*H*-benzo[d]imidazol-2-yl) thio)-1-substituted-phenylethan-1-one as intermediate product (**I**).

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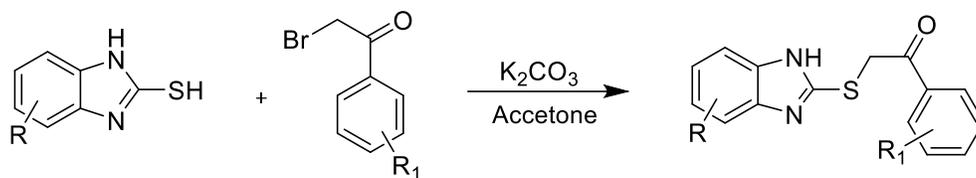
Scheme: Synthesis of 2-((substituted-1*H*-benzo[d]imidazol-2-yl) thio)-1-substituted-phenylethan-1-one

Step-2:
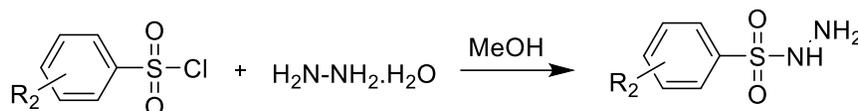
Various sulfonyl chloride was treated and refluxed with hydrazine hydrate in methanol to obtained substituted benzenesulfonohydrazide as intermediate product (**II**).

Scheme: synthesis of substituted benzenesulfonohydrazide

Step-3:
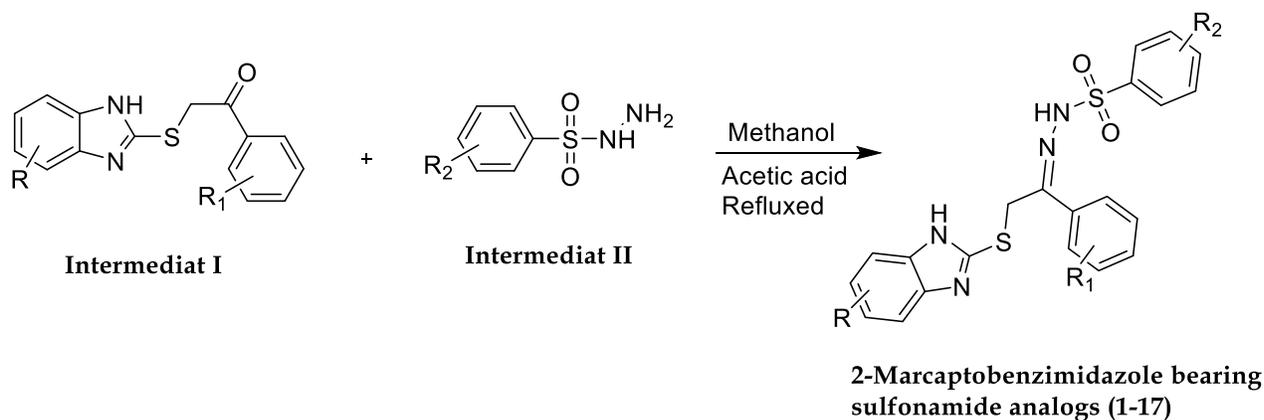
Intermediate product **I** was reacted and refluxed with intermediate product **II** in methanol in the presence of acetic acid to give 2-mercaptobenzimidazole bearing sulfonamide analogs (**1-17**).

Scheme-1: Synthesis of 2-marcaptobenzimidazole bearing sulfonamide analogs (**1-17**)

Table-1: Different substituents of 2-marcaptobenzimidazole analogs and their *α*-amylase inhibitory potential

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S. No	R	R_1	R_2	IC_{50}
1	6-methoxy	3-Nitro	2-methyl	1.40 ± 0.10
2	6-methoxy	4-Nitro	2-methyl	1.30 ± 0.05
3	--	4-phenyl	2-Nitro	2.40 ± 1.0
4	--	4-phenyl	2-chloro	2.80 ± 0.10
5	--	4-bromo	4 -methoxy	7.80 ± 0.30
6	--	4-phenyl	2-bromo	9.30 ± 0.30
7	--	4-phenyl	4 -methoxy	4.30 ± 0.20
8	--	4-bromo	2-bromo	11.20 ± 0.30
9	--	4 -methoxy	2-bromo	4.10 ± 0.20
10	--	3-Nitro	2-bromo	8.60 ± 0.30
11	--	3-Nitro	2,4-difloro	0.90 ± 0.05
12	6-methoxy	4-phenyl	2,4-difloro	1.60 ± 0.05
13	--	4-bromo	2-Nitro	8.40 ± 0.10
14	--	3-Nitro	2-Nitro	1.60 ± 0.10
15	--	2,5 -dimethoxy	2-Nitro	2.30 ± 0.10
16	--	4-Nitro	2-Nitro	3.30 ± 0.10
17	--	4-bromo	2-methyl	8.76 ± 0.05
Acarbose				$\mathbf{1.70 \pm 0.10 \, \mu M}$

2.2. *α-Amylase activity*

Seventeen analogues of 2-mercaptobenzimidazole bearing sulfonamide (**1-17**) was synthesized and screened for *α*-amylase inhibitory potential. All analogs exhibited variable degree of activity having IC_{50} values ranging between 0.90 ± 0.05 to $11.20 \pm 0.30 \, \mu M$ when compared with the standard acarbose having IC_{50} value $1.70 \pm 0.10 \, \mu M$. Compound **1, 2, 11, 12** and **14** having IC_{50} values 1.40 ± 0.10, 1.30 ± 0.05, 0.90 ± 0.05, 1.60 ± 0.05 and $1.60 \pm 0.10 \, \mu M$ respectively were found better inhibitor than standard drug acarbose. The remaining analogues also exhibited good *α*-amylase inhibition.

 The most potent compound among the series is compound **11** ($IC_{50} = 0.90 \pm 0.05 \, \mu M$). The compound **11,** having 3-NO_2 substitution on one phenyl ring and 2,4-difloro substitution on

another phenyl ring. Greater inhibition shown by this analog is seems due to electron withdrawing substitution on both phenyl ring of the compound.

If we compare compound **1** (IC$_{50}$ = 1.40 ± 0.10 μM) having a methoxy group at 6-position of the benzimidazole phenyl ring, 3-nitro group on one phenyl ring and 2-methyl ring on second phenyl ring with compound **2** (IC$_{50}$ = 1.30 ± 0.05 μM) also having a methoxy group at 6-position of the benzimidazole phenyl ring, 4-nitro group on one phenyl ring and 2-methyl ring on second phenyl ring. Little bit difference in inhibitory potential of both the compounds may be due to changing position of nitro group on first phenyl ring.

Similarly, by comparing compound **14** (IC$_{50}$ = 1.60 ± 0.10 μM) with compound **16** (IC$_{50}$ = 3.30 ± 0.10 μM). Both the compounds have nitro groups which is present on different positions of the phenyl rings. In compound **14**, one nitro group is present at 3-position on one phenyl ring and second nitro groups is present on second phenyl ring at 2-position while in compound **16** one nitro group is present at 4-position on one phenyl ring and second nitro groups is present on second phenyl ring at 2-position. Compound **14** show slightly good inhibitory potential as compared to compound **16** which may be due to the changing position of the nitro group on first phenyl ring. The least active compound among the series is analog **8** having bromine substitution on phenyl ring. Although bromine is electron withdrawing group but due to its larger size it might cause steric hindrance which results ultimately in the decline of inhibitory potential of these analogs. It was concluded from the study that nature, position and number of substituents play role in this inhibition.

2.3. Docking study

By using Gold molecular docking software, all synthesized derivatives in this series were docked into active site of target enzyme amylase (pdb: 4W93). The key connections recognized by active analogs were within 4Å radius on the binding site of amylase, were considered as most influential factor for activity. All **17** analogs in this series were active ranging from IC$_{50}$ = 0.90 - 11.20 μM. Here we reveal binding mode of four active analogs (**11, 2, 1** and **12**). The interactive site and the binding mode of standard acarbose had been reported in our previous paper [27].

Compound **11** is most active compound in this series and binding mode exhibit that nitro group attached to the meta position of phenyl ring of this compound is most influential in stabilizing the complex and this nitro moiety forms hydrogen bond with ASP197 and HIS299, also forms

electrostatic interaction with ARG195 and GLU233, also forms π-cation interaction with TYR62. Moreover, the benzimidazole ring forms π- π stacking with HIS201 and π- Alkyl interaction with LEU162 and LYS200. In addition, the ring also forms hydrophobic contact with ILE235. The other stretches of the molecule bearing fluoro group at ortho and para of the phenyl ring forms π- π stacking with the TRP59 (**Fig-2a**).

Fig-2b exhibit the binding mode of the **compound 2**, residue ASP300 and ASP356 forms electrostatic interaction with benzimidazole nitrogen and the methoxy group behaves as acceptor to form hydrogen bond with ASP356. The benzimidazole ring also forms - π stacking with HIS305. The residue ILE235 forms π-sigma interaction with the nitro phenyl ring. Toluene ring of the analog forms - π stacking with HIS201 and π-alkyl interaction with LYS200, ILE235, TYR151, ALA198 and ALA307.

Fig. 2c represents the binding mode of the **compound 1**, a hydrogen bond is established between ASP197 and nitro group attached to the phenyl ring forms π-cation interaction with the TYR62, while the GLU233 forms electrostatic interaction with nitro group. Additionally, toluene ring forms π-alkyl interaction with LYS200 and π- π stacking with HIS201 and hydrophobic interaction with ILE235. On the other hand, the benzimidazole ring also forms π- π stacking with TRP59.

Finally, the binding mode orientation of the **compound 12** exhibit the formation of hydrogen bond of sulfonyl group with ASP197 and HIS299. The ortho-para fluoro moieties on phenyl ring behaves as hydrogen bond acceptor for ASP197 and TRP59. The same ring also forms π - π stacking with TYR62. Likewise, the diphenyl ring forms π - π stacking with HIS305. Finally, the benzimidazole ring was also found to form π - π stacking TRY151 and π-alkyl interaction with LYS200 and hydrophobic contact with ILE235 (**Fig. 2d**).

In general docking studies, shows that the presence aromatic ring of this series forms π- π stacking and π- Alkyl interaction followed by hydrogen bond that were influential to stabilize the complex and reflects in the biological activity index.
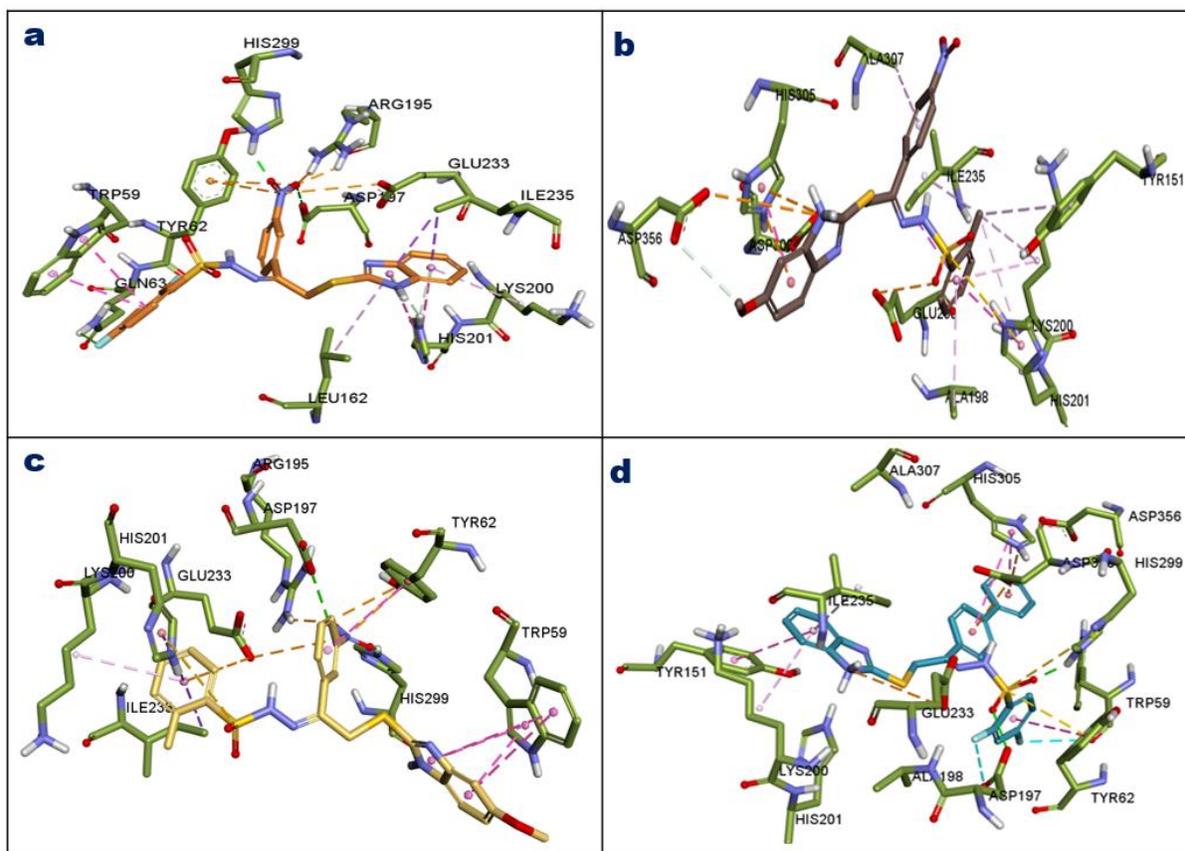
Fig-2: Shows the binding mode of the potent analogues in the amylase active site (a) compound **11** in brown stick, (b) compound **2** in gray stick (c) compound **1** in yellow stick and (d) compound **12** blue stick. Key interacting residues are shown in greenish stick form and the hydrogen bond is represented by dashed green line and the Carbon Hydrogen Bond and π-donor hydrogen bond are represented as pale green dashed line. The π- π are represented as magenta dashed line, electrostatic interaction shown as orange dashed line, and the hydrophobic interaction are shown as pink dashed lines.

3.0. CONCUSION:

In conclusion, we have synthesized seventeen compounds of 2-mercaptobenzimidazole bearing sulfonamide, characterized through ^{1}H, ^{13}C-NMR and HREI-MS and screened for their α-amylase activity. All compounds exhibited varying degree of α-amylase activity having IC$_{50}$ values ranging between 0.90 ± 0.05 to 11.20 ± 0.30 μM when compared with the standard acarbose having IC$_{50}$ value 1.70 ± 0.10 μM. Compound **11** (IC$_{50}$ =0.90 ± 0.05 μM) was found the most potent and compound **8** (IC50 = 11.20 ± 0.30 μM) was found the least potent among the series. SAR has been

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recognized for all analogs based mainly on substitution pattern on phenyl ring. Through molecular docking studies, the interaction of the most potent compounds with the enzyme active sites were confirmed.

4.0. Material and methods

4.1. Synthesis of 2-((substituted-1*H*-benzo[d]imidazol-2-yl)thio)-1-substituted-phenylethan-1-one

2-mercaptobenzimidazole was reacted and refluxed with different substituted phenacyl bromide in acetone in the presence of potassium carbonate which yielded 2-((substituted-1*H*-benzo[d]imidazol-2-yl) thio)-1-substituted-phenylethan-1-one as intermediate product (**I**).

4.2. Synthesis of substituted benzenesulfonohydrazide

Various sulfonyl chloride was treated and refluxed with hydrazine hydrate in methanol to obtained substituted benzenesulfonohydrazide as intermediate product (**II**).

4.3. Synthesis of 2-marcaptobenzimidazole bearing sulfonamide analogs

Intermediate product (**I**) was reacted and refluxed with intermediate product (**II**) in methanol in the presence of acetic acid to give 2-mercaptobenzimidazole bearing sulfonamide analogs (**1-17**).

4.3.1.   *N′*-(2-((6-methoxy-1H-benzo[d]imidazol-2-yl)thio)-1-(3-nitrophenyl)ethylidene)-2-methylbenzenesulfonohydrazide (1)

Yield: 68 %; ^{1}HNMR: (500 MHz, DMSO-d_6): δ 8.76 (s, 1H, Ar), 8.5 (m, 2H, Ar), 7.9 (t, J = 6.6 Hz, 1H, Ar), 7.7 (d, J = 6.1 Hz, 1H, Ar), 7.6 (t, J = 6.7 Hz, 1H, Ar), 7.5 (t, J = 6.7 Hz, 1H, Ar), 7.45 (d, J = 7 Hz, 1H, Ar), 7.4 (d, J = 7.4 Hz, 1H, Ar), 7.0 (s, 1H, Ar), 6.9 (dd, J = 1.9, 7.3Hz 1H, Ar), 5.32 (s, 2H, CH$_2$), 3.8 (s, 3H, OCH$_3$) 2.09 (s, 3H, CH$_3$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 163, 155, 138.9, 137, 137, 133, 131.7, 131.7, 128.7, 128.7, 126, 126, 125, 117.8, 117.8, 115.3, 115.3, 114, 114, 80, 55.8, 27.7, 22.3. HREI-MS: m/z calcd for $C_{23}H_{21}N_5O_5S_2$, [M]+ 511.0984; Found: 511.0979.

4.3.2.   *N′*-(2-((6-methoxy-1H-benzo[d]imidazol-2-yl)thio)-1-(4-nitrophenyl)ethylidene)-2-methylbenzenesulfonohydrazide (2)

Yield: 71 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.4 (d, J = 8.7 Hz, 2H, Ar), 8.3 (d, J = 7.0 Hz, 2H, Ar), 7.7 (d, J = 6 Hz, 1H, Ar), 7.6 (m,1H, Ar), 7.5 (d, J = 4.9 Hz, 1H, Ar), 7.49 (m, 1H, Ar), 7.0 (s, 1H, Ar), 6.9 (m, 1H, Ar), 7.4 (d, J = 6.2 Hz, 1H, Ar), 5.3(s, 2H, CH$_2$), 3.8(s, 3H, OCH$_3$), 2.09 (s, 3H, CH$_3$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 160, 155, 150, 140, 138.9, 138.4, 136.6, 131.8, 131.8, 131.5, 129.7, 129.7, 127.7, 127.7, 127.7, 127, 127, 112.7, 103.5, 80, 55.8, 27.7 22.3. HREI-MS: m/z calcd for C$_{23}$H$_{21}$N$_5$O$_5$S$_2$, [M]+ 511.0984; Found: 511.0970.

4.3.3.   *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-2-nitrobenzenesulfonohydrazide (3)

Yield: 68 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.5 (d, J = 6.4 Hz, 1H, Ar) 8.17 (d, J = 6.8 Hz, 2H, Ar), 8 (d, J = 6.7 Hz, 1H, Ar), 7.95 (t, J = 7 Hz, 2H, Ar) 7.91 (d, J = 6.8 Hz, 2H, Ar), 7.79 (d, J = 6.2 Hz, 2H, Ar), 7.5 (m, J = 6.6, 4H, Ar), 7.4 (d, J = 6 Hz, 1H, Ar), 7.2 (m, 2H, Ar), 4.2 (s, 2H, CH$_2$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 167.5, 159.4, 155.6, 149.2, 139.9, 139.9, 135.3, 135.1, 133, 132.9, 131.9, 129.2, 127.0, 127.0, 127.0, 126.3, 126.3, 125.4, 114.9, 114.9, 114.9, 112.8, 110, 110, 109.6, 109.6, 39.8. HREI-MS: m/z calcd for C$_{27}$H$_{21}$N$_5$O$_4$S$_2$, [M]+ 543.1035; Found; 543.1023.

4.3.4.   *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-2-chlorobenzenesulfonohydrazide (4)

Yield: 71 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.16 (d, J = 6.7, 2H, Ar), 7.93 (d, J = 6.5, 2H, Ar), 7.78 (d, J = 6.9, 2H, Ar), 7.6 (m, 5H, Ar), 7.55 (t, J = 6.8, 2H, Ar), 7.47 (t, J = 6.2, 2H, Ar), 7.13 (d, J = 7, 1H, Ar), 7.0 (dd, J = 1.5, 7 Hz, 1H, Ar) 4.2(s, 2H, CH$_2$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 191.8, 157.3, 148.8, 145.4, 140.7, 138.5, 134.0, 133.6, 133.5, 131.5, 130.2, 129.2, 129.2, 129.1, 129.1, 128.6, 128.1, 127.5, 127.5, 127.1, 127.1, 127, 127, 126, 114.1, 114.1, 41.4. HREI-MS: m/z calcd for C$_{27}$H$_{21}$ClN$_4$O$_2$S$_2$, [M]+ 532.0794; Found; 532.0778.

4.3.5.   *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(4-bromophenyl)ethylidene)-4-methoxybenzenesulfonohydrazide (5)

Yield: 68 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.17 (d, J = 6.95, 2H, Ar), 7.9 (d, J = 6.9, 2H, Ar), 7.8 (d, J = 6.1, 2H, Ar), 7.59 (d, J = 7.4, 1H, Ar), 7.55 (t, J = 6.2, 2H, Ar), 7.4 (d, J = 6, 1H, Ar), 7.1 (d, J = 6, 1H, Ar), 7.1(dd, J = 1.7, 7.1 Hz, 1H, Ar), 5.4 (s, 2H, CH$_2$), 3.8 (s, 3H, OCH$_3$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 163, 155, 138.9, 137, 137, 133, 131.7, 131.7, 128.7, 128.7, 126, 126, 125, 11.8, 117.8, 115.3, 115.3, 114, 114, 80, 55.8, 27.7. HREI-MS: m/z calcd for C$_{22}$H$_{19}$BrN$_4$O$_3$S$_2$, [M]+ 530.0082; Found: 530.0068.

4.3.6.   *N'*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-2-bromobenzenesulfonohydrazide (6)

Yield: 75 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.5 (s, 1H, NH), 8 (d, J = 6.7, 2H, Ar), 7.9(d, J = 6.5, 2H, Ar), 7.79 (d, J = 6.9, 1H, Ar), 7.77 (m, 1H, Ar), 7.75 (d, J = 6.3, 2H, Ar), 7.67 (d, J = 6.8, 1H, Ar), 7.52 (m, 1H, Ar), 7.49 (m, 2H, Ar), 7.4 (m, 1H, Ar), 7 (s, 1H, NH), 6.61 (d, J = 7, 2H, Ar), 6.4 (d, J = 6.2, 2H, Ar), 4.2 (s, 2H, CH$_2$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 191.8, 157.3, 148.8, 145.4, 138.5, 134.9, 134.0, 133.5, 132.3, 129.3, 129.3, 129.3, 129.3, 129.2, 129.1, 128.6, 127.5, 127.1, 127.1, 127.0, 127.0, 127.0, 127.0, 120.8, 114.1, 114.0, 41.3. HREI-MS: m/z calcd for C$_{27}$H$_{21}$BrN$_4$O$_2$S$_2$, [M]+ 576.0289; Found; 576.0274.

4.3.7.   *N'*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-4-methoxybenzenesulfonohydrazide (7)

Yield: 68 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.5 (s, 1H, NH), 8 (d, J = 6.7, 2H, Ar), 7.9 (d, J = 6.5, 2H, Ar), 7.75 (d, J = 6.3, 2H, Ar), 7.70 (d, J = 6.8, 2H, Ar), 7.49 (m, 2H, Ar), ,7.4 (m, 1H, Ar), 7.1 (d, J = 6.6, 2H, Ar), 7 (s, 1H, NH), 6.61 (d, J = 7, 2H, Ar), 6.4 (d, J = 6.2, 2H, Ar), 4.2 (s, 2H, CH$_2$), 3.7 (s, 3H, OCH$_3$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 191.8, 167.4, 157.2, 148.8, 145.4, 138.5, 138.3, 134.2, 133.5, 129.3, 129.3, 129.2 129.2, 129.1, 128.6, 127.8, 127.0, 127.0, 127.0, 127.0, 126, 126, 115.3, 115.3, 114.0, 114.0, 55.8, 41.2. HREI-MS: m/z calcd for C$_{28}$H$_{24}$N$_4$O$_3$S$_2$, [M]+ 528.1290; Found; 528.1277.

4.3.8.   *N'*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(4-bromophenyl)ethylidene)-2-bromobenzenesulfonohydrazide (8)

Yield: 73 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.1 (d, J = 6.9 Hz, 2H, Ar), 7.92 (d, J = 6.9 Hz, 2H, Ar), 7.7 (d, J = 6.2 Hz, 2H, Ar), 7.56 (t, J = 6.3 Hz, 3H, Ar), 7.47 (t, J = 6.1 Hz, 1H, Ar), 7.10 (d, J = 1.7 Hz, 1H, Ar), 7 (dd, J = 1.7, 7.4 Hz, 1H, Ar), 5.3 (s, 2H, CH$_2$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 160, 155, 138.9, 138.4, 136.6, 136.6, 134.9, 134.3, 131.8, 131.8, 131.8, 131.5, 129.7, 129.7, 126.2, 125.6, 120.8, 112.7, 103.5, 55.8, 22.3. HREI-MS: m/z calcd for C$_{21}$H$_{16}$Br$_2$N$_4$O$_2$S$_2$, [M]+ 577.9081; Found: 577.9078.

4.3.9.   *N'*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(4-methoxyphenyl)ethylidene)-2-bromobenzenesulfonohydrazide (9)

Yield: 74 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8 (d, J = 6.8, 2H, Ar), 7.9 (d, J = 6.8, 1H, Ar), 7.85 (m, 1H, Ar), 7.79 (d, J = 7, 1H, Ar), 7.59 (m, 1H, Ar), 7.5 (d, J = 6.3, 2H, Ar), 7.1 (d, J = 7, 2H, Ar), 7 (d, J = 6.2, 2H, Ar), 5.1 (s, 2H, CH$_2$), 3.81 (s, 3H, OCH$_3$). ^{13}CNMR (125 MHz, DMSO-d_6):

δ 162, 155, 142.9, 137, 137, 134, 130, 129, 128.7, 128.7, 128, 126, 120, 117.8 117.8, 115.3, 115.3, 114, 114, 80, 55, 27.7. HREI-MS: m/z calcd for $C_{22}H_{19}BrN_4O_3S_2$, [M]+ 530.0082; Found; 530.0069

4.3.10. *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(4-nitrophenyl)ethylidene)-2-bromobenzenesulfonohydrazide (10)

Yield: 69 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.6 (d, J = 6.9 Hz, 2H, Ar), 7.9 (d, J = 6.9 Hz, 2H, Ar), 7.8 (d, J = 6.1 Hz, 2H, Ar), 7.56 (m, 3H, Ar), 7.4 (d, J = 6.2 Hz, 1H, Ar), 7.1 (d, J = 1.6 Hz, 1H, Ar), 7 (dd J = 1.9, 7.4 Hz, 1H, Ar), 5.3 (s, 2H, CH$_2$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 191.9, 157.1, 148.7, 145.3, 138.5, 134.5, 133.5, 129.2, 129.2, 129.1, 128.6, 128.6, 128.2, 127.0, 127.0, 127, 127, 114.0, 113.8, 96.1, 41.1. HREI-MS: m/z calcd for $C_{21}H_{16}BrN_5O_4S_2$, [M]+ 544.9827; Found: 544.9811.

4.3.11. *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(3-nitrophenyl)ethylidene)-2,4-difluorobenzenesulfonohydrazide (11)

Yield: 65 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.4 (s, 1H, Ar), 8.3 (d, J = 7.2 Hz, 1H, Ar), 8.2 (d, J = 6.7 Hz, 1H, Ar), 7.80 (t, J = 6.8, 1H, Ar), 7.6 (s 1H, Ar), 7.38 (m, 1H, Ar), 7.3 (m, 1H, Ar), 7.0 (d, J = 7.1 Hz, 2H, Ar), 6.7 (m, 2H, Ar), 5.4 (s, 2H, CH$_2$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 174.0, 167.9, 167.6, 155.6, 133.0, 131.5, 131.5, 130.3, 130.3, 128.9, 128.4, 126.3, 126.1, 126.1, 125.4, 122.6, 109.9, 109.9, 109.6, 109.6, 39.8. HREI-MS: m/z calcd for $C_{21}H_{15}F_2N_5O_4S_2$, [M]+ 503.0534; Found; 503.0518.

4.3.12. *N′*-(1-([1,1'-biphenyl]-4-yl)-2-((6-methoxy-1H-benzo[d]imidazol-2-yl)thio)ethylidene)-2,4-difluorobenzenesulfonohydrazide (12)

Yield: 71 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.15 (d, J = 6.9, 2H, Ar), 7.9 (d, J = 6.9, 2H, Ar), 7.8 (d, J = 6.2, 2H, Ar), 7.6 (m, 5H, Ar), 7.4 (t, J = 6.1, 2H, Ar), 7.09 (s, 1H, Ar), 7.0 (dd, J = 1.5, 7.3 Hz, 1H, Ar), 5.2 (s, 2H, CH$_2$) 3.82 (3H, OCH$_3$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 192.1, 171.9, 156.8, 148.7, 145.3, 138.5, 138.5, 135.2, 135.2, 133.6, 133.6, 129.2, 129.2, 129.1, 129.1, 128.5, 127.0, 127.0, 126.9, 126.9, 126.9, 121.9, 120.1, 114.1, 113.3, 111.1, 55.7, 40.9. HREI-MS: m/z calcd for $C_{28}H_{22}F_2N_4O_3S_2$, [M]+ 564.1101; Found; 564.1092.

4.3.13. *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(4-bromophenyl)ethylidene)-2-nitrobenzenesulfonohydrazide (13)

Yield: 69 %; ^{1}HNMR (500 MHz, DMSO-d_6): 8.16 (d, J = 6.9 Hz, 2H, Ar) 7.92 (d, J = 6.9 Hz, 2H, Ar), 7.79 (d, J = 6.2 Hz, 2H, Ar), 7.54 (d, J = 6.9 Hz, 3H, Ar), 7.47 (t, J = 6.1 Hz, 1H, Ar), 7.1(d,

J = 1.7 Hz, 1H, Ar), 7.1 (dd, J = 1.6. 7.4 Hz, 1H, Ar), 5.3 (s, 2H, CH$_2$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 191.8, 157.0, 148.6, 148.2, 136.8, 134.1, 133.9, 133.9, 132.7, 131.9, 131.9, 130.4, 130.4, 128.2, 124.7, 124.7, 116.4, 116.4, 114.0, 113.6, 40.9. HREI-MS: m/z calcd for C$_{21}$H$_{16}$BrN$_5$O$_4$S$_2$, [M]+ 544.9827; Found; 544.9810.

4.3.14.   *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(3-nitrophenyl)ethylidene)-2-nitrobenzenesulfonohydrazide (14)

Yield: 65 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.25 (s, 1H, Ar), 8.19 (d, J = 6.4 Hz, 1H, Ar) 8.07 (d, J = 6 Hz, 1H, Ar), 7.91 (d, J = 7 Hz, 1H, Ar), 7.86 (t, J = 6.8 Hz, 2H, Ar), 7.6 (d, J = 6.3 Hz, 1H, Ar), 7.3 (t, J = 6.6 Hz, 1H, Ar), 7.08 (d, J = 6.9 Hz, 2H Ar), 6.9 (dd, J = 1.9, 7.5, 2H, Ar), 5.2 (s, 2H, CH$_2$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 168.1, 167.5, 155.6, 153, 152, 138.9, 137, 137, 136.6, 131.8, 131.5, 120.8, 118, 117.4, 117, 117, 115, 115, 115, 114, 39.9. HREI-MS: m/z calcd for C$_{21}$H$_{16}$N$_6$O$_6$S$_2$, [M]+ 512.0573; Found; 512.0561.

4.3.15.   *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(2,5-dimethoxyphenyl)ethylidene)-2-nitrobenzenesulfonohydrazide (15)

Yield: 73 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.2 (d, J = 6.8 Hz, 1H, Ar) 7.87 (d, J = 7.3 Hz, 1H, Ar), 7.81 (d, J = 7.4 Hz, 1H, Ar), 7.4 (s, 1H, Ar), 7.29 (s, 1H, Ar), 7.24 (t, J = 7 Hz, 2H, Ar), 7.06 (d, J = 7.1 Hz, 2H, Ar), 6.9 (m, 1H, Ar), 6.7 (s, 1H, Ar), 5.2 (s, 2H, CH$_2$), 3.9 (s, 3H, OCH$_3$), 3.8 (s, 3H, OCH$_3$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 172, 168.0, 155.6, 153.9, 153.0, 147.2, 146.5, 143.5, 133, 126.9, 126.8, 126.7, 124.4, 124.4, 117.3, 115.7, 115.1, 115.8, 55.8, 55.5, 39.7. HREI-MS: m/z calcd for C$_{23}$H$_{21}$N$_5$O$_6$S$_2$, [M]+ 527.0933; Found; 527.0924.

4.3.16.   *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(4-nitrophenyl)ethylidene)-2-nitrobenzenesulfonohydrazide (16)

Yield: 62 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.17 (d, J = 6.9 Hz, 1H, Ar) 7.93 (d, J = 6.9 Hz, 2H, Ar) 7.8 (d, J = 6.1 Hz, 2H, Ar), 7.59 (d, J = 7.4 Hz, 1H, Ar),7.55 (t, J = 6.2 Hz, 3H, Ar), 7.4 (d, J = 6 Hz, 1H, Ar), 7.1 (d, J = 1.8, Hz, 1H, Ar), 7 (dd, J = 1.8, 7.4, 1H, Ar), 5.4 (s, 2H, CH$_2$). ^{13}CNMR (125 MHz, DMSO-d_6): δ 191.8, 157.3, 148.8, 145.4, 138.5, 134.0, 133.5, 129.2, 129.2, 129.1, 129.1, 128.6, 127.5, 127.2, 127.0, 127.0, 127.0, 127.0, 114.1, 114, 41.4. HREI-MS: m/z calcd for C$_{21}$H$_{16}$N$_6$O$_6$S$_2$, [M]+ 512.0573; Found; 512.055.

4.3.17.   *N′*-(2-((1H-benzo[d]imidazol-2-yl)thio)-1-(4-bromophenyl)ethylidene)-2-methylbenzenesulfonohydrazide (17)

Yield: 68 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.17 (d, J= 6.9, 2H, Ar), 7.93 (d, J= 6.9 Hz, 2H, Ar), 7.80 (d, J= 6.2 Hz, 2H, Ar), 7.58(m, 3H, Ar), 7.47(t, J= 6.1 Hz, 1H, Ar), 7.1(d, J= 1.9 Hz, 1H, Ar), 7(dd, J = 1.9, 7.4 Hz, 1H, Ar), 5.38(s, 2H, CH$_2$), 2.60 (s, 3H, CH$_3$). ^{13}CNMR (125 MHz, DMSO-d_6), δ 191.8, 157.2, 148.8, 145.4, 138.5, 134.2, 133.5, 129.3, 129.3, 129.2, 129.2, 129.1, 128.6, 127.8, 127.0, 127.0, 127.0, 127.0, 114.0, 114.0, 41.2, 30.6. HREI-MS: m/z calcd for C$_{22}$H$_{19}$BrN$_4$O$_2$S$_2$, [M]+ 514.0133, Found; 514.0118.

3.4. Assay protocol for *α*-Amylase activity

To determine *α*-amylase inhibitory activity Zhang and coworker method with a slight modification was used [28, 29]. In 1.5 mL centrifuge tube fraction, 40 μL legume extract, 160 μL of distilled H$_2$O, 400 μL 0.5 % starch and individual phenolic compound were assorted and then 200 μL of enzyme solution (30 unit/mL) added. At 25 °C, the tube was incubated for 3 minutes. In a separate tube, 200 μL of mixture was taken and added which already contained 100 μL DNS color reagents solution (96mM 3,5-dinitro-salicylic acid, 5.31M sodium potassium tartrate in 2M NaOH). Tube was retained into 70-95 °C thermo-mixer (Eppendrof, Hamburg Germany) for 10 minutes to activate the enzyme. 0.9 mL of distilled H$_2$O was put into the tube and well mixed. Into a 96 well-plates, 200 μL of mixture was added. Absorbance of reaction mixture was determined at 540nm. To eliminate background absorbance formed by legume extracts, a suitable extract control without enzyme was incorporated. Type-1-amylase inhibitor from triticum aestivum was also analyzed as a positive control. *α*-Amylase activity was determined at five different concentrations and for the calculation of IC$_{50}$ values (mg/mL) logarithmic regression curve was established.

$$\alpha - Amylase\ inhibition = [1 - (A\ sample - A\ blank)] \div A\ test - A control \times 100\%$$

''A sample'' is the absorbance of mixture of enzyme, extract and DNS color reagent, ''A blank'' is absorbance of mixture of extract, starch solution and DNS color reagent, ''A test'' is absorbance of mixture of enzyme, starch and DNS color reagent and ''A control'' is absorbance of mixture of DNS color reagent and starch solution without enzyme.

3.5. Docking study protocol

Docking study was done targeting the crystal structure of amylase (PDB ID: 4W93) [30] in order to reveal the binding modes of synthesized derivatives (**1–17**). For the purpose of docking studies, protein preparation module in discovery studio 2018 (Dassault systemes BIOVIA, USA) was used

to optimize the crystal structure of amylase [31]. From protein data bank (PDB), crystal structure was retrieved and furthermore, structure was optimized by removing co-factors, hetero-atoms and H_2O molecule. Charges, hydrogen bond and missing atom were computed. Built and ligand preparation module implemented in discovery studio 2018 (Dassault systems BIOVIA, USA) was used to prepared and optimized the docking study of the synthesized analogs (**1-17**). Gold docking tool was used for the purpose of docking, ligand preparation comprises stereochemistry, assigning bond order and various tautomer. Moreover, by choosing centroid of complex ligand (Montbretin A), receptor grid was produced around amylase active site. Active sites were defined with a radius of 12Å around Montbretin A binding sites. By using Chem PLP scoring function, docking calculations were skilled [32]. By using Discover studio visualizer, docking result was further evaluated and each derivative, binding mode was visually inspected.

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