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Synthesis of benzimidazole derivatives as potent inhibitors for α-amylase and their molecular docking study in management of type-II diabetes

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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 synthesized 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. While others derivatives of the series showed good inhibitory potentials. All the synthesized compounds were characterized by HREI-MS, ^1H and ^{13}C NMR spectroscopy. Structure activity relationship (SAR) has been established for all newly synthesized analogs. Binding interactions between ligands and active residues of the enzyme were confirmed through molecular docking study.

1. Introduction

Benzimidazole is fused aromatic heterocyclic compound and a privileged scaffold with active pharmacophore in medicinal chemistry. Until the present by moiety of choice it displayed versatile character, and in nature *N*-ribosyldimethylbenzimidazol is the most prominent compound which act as axial ligand in vitamin B_{12} for cobalt [1]. Aimed at the structural modifications for biological potentials, various derivatives of benzimidazole have been synthesized to increase the stability, bioavailability and promising biological profiles [2,3]. In view of their affinity towards enzymes and proteins, medicinal chemist employed it as sub-structure in drug designing and synthesis. Due to its chemotherapeutic values, benzimidazole and its analogs received much attention by the researchers [4]. Benzimidazole derivatives proved to be useful therapeutics such as diuretics [5], antimicrobial [6], anticancer [7], antiprotozoal [8], antidiabetic [9], antioxidant [10], and anticonvulsant [11], respectively. Moreover, many other benzimidazole derivatives were synthesized from 2-marcatobenzimidazole (1*H*-benzo[d]imidazole-2-thiol), while these derivatives demonstrated their biological activities such as antidiabetic [12], antiviral [13] and antimicrobial [14], respectively.

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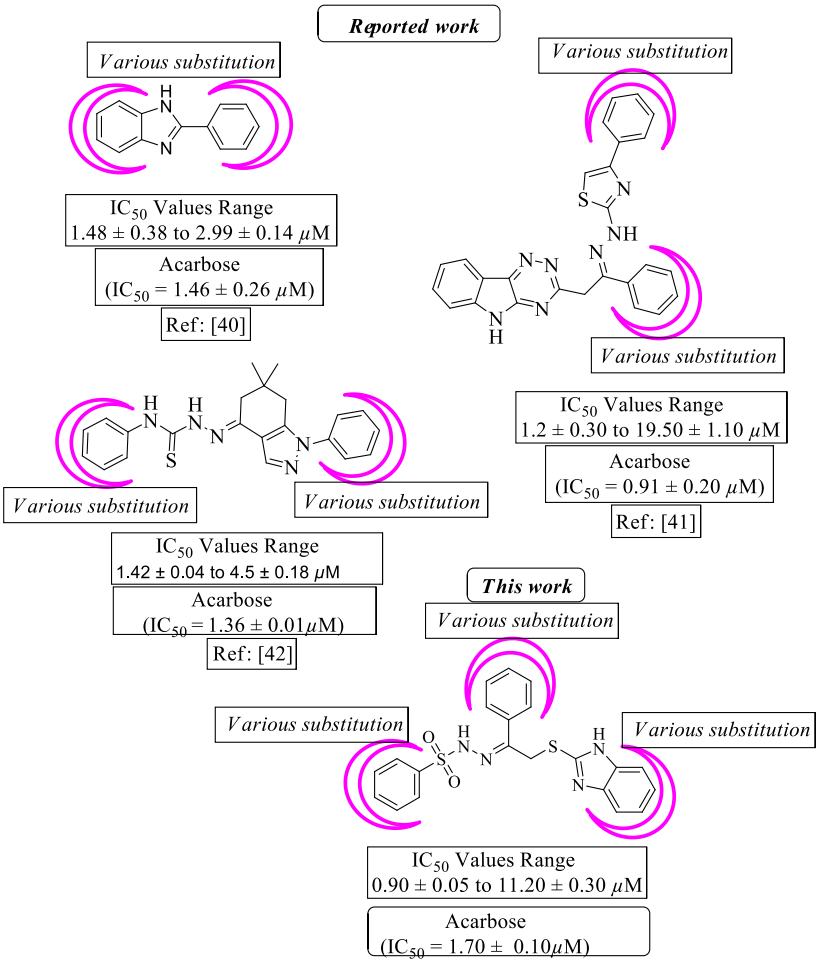


Fig. 1. Current work with previously reported work.

Diabetes mellitus (DM) is chronic metabolic disorder characterized by hyperglycemia ensuing by defects in insulin secretion and insulin action [15]. The current estimate and forecast about diabetic patient indicate that the figure may reach from 383 million to 592 million by the year 2035 [16]. Defects in insulin secretion and action prompt the glucose level in blood which particularly damages the blood vessels and initiates some other complications like neuropathy, retinopathy, ulceration, cardiovascular and nephropathy, respectively [17]. The detail innovative study showed that postprandial hyperglycemia kept in normal level by inhibiting intestinal hydrolyse enzyme α-amylase and α-glucosidase are the practical solutions for glycemic control [18,19]

α-Amylase (E.C.3.2.1.1) is hydrolyse enzyme comprises of Ca^{+2} ion in its active pocket. It catalyzes the conversion of starch into glucose and maltose by involving water molecule respectively. Alpha-amylase catches considerable attention due to potential capability on attacking of $\alpha-1,4$-glycosidic linkage by hydrolysis [20–23]. Drugs such as voglibose, acarbose and miglitol have the function to inhibit the hydrolyzing ability of α-amylase enzyme and consequently stopped the absorption of glucose. Although, these drug found with some side effects and therefore to minimize its adverse effect other antidiabetic agents are employed in combination during treatment [24–27]. In this context, medicinal and synthetic chemists reported variety of heterocyclic scaffolds for promising antidiabetic activity [28–31].

With continuing efforts, our group has reported numerous heterocycles for potent therapeutics potentials [32–36] including some potent antidiabetic agents as well [37–39]. In this manuscript,

Based on our earlier reports on benzimidazole class of [40–42], we have synthesized some novel benzimidazole derivatives bearing sulfonamide moiety and evaluated them for their α-amylase inhibitory activity in search potent candidates (Fig. 1).

2. Material and methods

All chemicals and reagents which used in this protocol were purchased from sigma Aldrich. The purity of chemicals and reagents which were employed for synthesis of intermediate and benzimidazole derivatives were checked by TLC analysis. Moreover, the structures of all derivatives were confirmed *via* spectroscopic techniques like ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopy respectively.

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

2-Mercaptobenzimidazole/6-methoxy-2-mercaptobenzimidazole (5 mmol), different substituted phenacyl-bromides (5 mmol) were reacted and stirred the mixture at room temperature in acetone (20 ml) in the presence of potassium carbonate which yielded intermediate product (**I**). The progress of reaction was monitored with the help of TLC. After reaction completion, reaction mixture was cooled at low temperature and filtered the precipitate to achieve crude product. The product was recrystallized in methanol to obtained pure product.

2.2. Synthesis of substituted benzenesulfonohydrazide

Various sulfonyl chlorides were treated (5 mmol) with equivalent quantity of hydrazine hydrate and refluxed the reaction blend in methanol (20 ml) to obtain substituted benzenesulfonohydrazide as intermediate product (**II**). The progress of reaction was monitored with the help of TLC. After reaction completion, the solvent was evaporated under vacuum to obtain crude product. The product was recrystallized in ethyl acetate to obtained pure product.

2.3. Synthesis of 2-marcaptobenzimidazole bearing sulfonamide analogs (1–17)

Equivalent quantity of product (**I**) was reacted with intermediate product (**II**) and refluxed in methanol in the presence of acetic acid to give 2-mercaptobenzimidazole bearing sulfonamide analogs (**1–17**). The progress of reaction was monitored with the help of TLC. After reaction completion, the solvent was allowed to evaporate to achieve crude product. The crude product was washed with hexane and ethyl acetate to obtained pure product

2.3.1. 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 12.51 (s, 1H, NH), 11.49 (s, 1H, NH), 8.53 (J = 1.2 Hz, 1H, Ar), 8.16 (J = 6.9 Hz, 2H, ArH), 7.93 (d, J = 7.3 Hz, 2H, ArH), 7.80 (d, J = 6.8 Hz, 2H, ArH), 7.56 (m, 3H, ArH), 7.47 (d, J = 6.2 Hz, 1H, ArH), 7.12 (d, J = 7.5 Hz, 1H, ArH), 7.05 (dd, J = 1.9, 6.8 Hz, 1H, ArH), 5.36 (s, 2H, CH_2), 3.88 (s, 3H, O-Me), 2.09 (s, 3H, CH_3). ^{13}CNMR (125 MHz, DMSO-d_6): δ 157.15, 156.42, 148.79, 145.37, 138.57, 134.57, 133.57, 131.28, 129.25, 129.25, 129.11, 128.60, 128.22, 127.02, 127.00, 125.80, 125.42, 114.06, 113.83, 96.16, 55.80, 30.64, 21.01; HREI-MS: m/z calcd for $C_{23}H_{21}N_5O_5S_2$, [M]+ 511.0984; Found: 511.0979.

2.3.2. 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 12.49 (s, 1H, NH), 11.92 (s, 1H, NH), 8.76 (d, J = 6.7 Hz, 2H, Ar), 8.54 (dd, J = 1.5, 6.6,7 Hz, 2H, ArH), 7.92 (t, J = 6.6 Hz, 2H, ArH), 7.48 (d, J = 1.4 Hz, 1H, ArH), 7.06 (d, J = 6.2 Hz, 2H, ArH), 6.96 (dd J = 1.8, 7.2 Hz, 2H, ArH), 5.32 (s, 2H, CH_2), 3.82 (s, 3H, O-Me), 2.09 (s, 3H, CH_3). ^{13}CNMR (125 MHz, DMSO–d_6): δ 157.15, 156.42, 151.03, 147.9, 145.37, 141.08, 138.57, 131.2, 129.25, 129.11, 128.60, 128.22, 128.11, 128.11, 127.61, 127.61, 127.50, 114.06, 113.83, 96.16, 55.8, 32.51, 21.02; HREI-MS: m/z calcd for $C_{23}H_{21}N_5O_5S_2$, [M]+ 511.0984; Found: 511.0970.

2.3.3. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-([1,1′-biphenyl]−4-yl)ethylidene)−2-nitro benzenesulfonohydrazide (3)

Yield: 68%; ^{1}HNMR (500 MHz, DMSO–d_6): δ 11.58 (s, 1H, NH), 10.51 (s, 1H, NH), 8.78 (dd, J = 1.2, 7.4 Hz, 1H, ArH), 8.46(dd, J = 6.6 Hz, 1H, ArH), 8.03 (t, J = 6.4 Hz, 1H, ArH), 7.48 (m, 5H, ArH), 7.25 (m, 4H, ArH), 6.89 (m, 5H, Ar), 5.29 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO-d_6): δ 157.37, 151.32, 148.89, 145.42, 139.92, 136.43, 134.97, 134.07, 133.54, 133.08, 129.36, 129.36, 129.25, 129.25, 129.11, 129.11, 128.61, 128.61, 128.05, 127.57, 127.19, 127.02, 127.02, 125.41, 114.19, 114.19, 32.50; HREI-MS: m/z calcd for $C_{27}H_{21}N_5O_4S_2$, [M]+ 543.1035; Found; 543.1023.

2.3.4. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-([1,1′-biphenyl]−4-yl)ethylidene)−2-chloro benzenesulfonohydrazide (4)

Yield: 71%; ^{1}HNMR (500 MHz, DMSO–d_6): δ 12.34 (s, 1H, NH), 12.31 (s, 1H, NH), 7.62–7.58 (m, 4H, ArH), 7.45 (d, J = 7.2 Hz, 2H, ArH), 7.43 (d, J = 7.2 Hz, 2H, Ar), 7.36 (d, J = 7.6 Hz, 2H, ArH), 6.98 (t, J = 7.6 Hz, 2H, ArH), 6.78 (t, J = 7.1 Hz, 2H, ArH), 6.67–6.63 (m, 3H, ArH) 4.63 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO–d_6): δ 157.37, 148.89, 145.42, 140.33, 139.92, 134.97, 134.97, 133.54, 133.17, 132.2, 130.41, 129.36, 129.36, 129.25, 129.25, 129.11, 129.11, 128.7, 128.61, 128.61, 127.57, 127.19, 127.02, 127.02, 127.00, 114.19, 114.19, 32.51; HREI-MS: m/z calcd for $C_{27}H_{21}ClN_4O_2S_2$, [M]+ 532.0794; Found; 532.0778.

2.3.5. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(4-bromophenyl) ethylidene)−4-methoxy benzenesulfonohydrazide (5)

Yield: 68%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 12.55 (s, 1H, NH), 11.99 (s, 1H, NH), 8.17 (d, J = 6.9 Hz, 2H, ArH), 7.93 (d, J = 6.9 Hz, 2H, ArH), 7.80 (d, J = 6.1 Hz, 2H, ArH), 7.59 (d, J = 7.4 Hz, 1H, ArH), 7.55 (t, J = 6.2 Hz, 2H, ArH), 7.47 (d, J = 6 Hz, 1H, ArH), 7.14 (d, J = 6.4 Hz, 1H, ArH), 7.08 (dd, J = 1.9, 7.4, Hz, 1H, ArH), 5.41 (s, 2H, CH_2), 3.87 (s, 3H, O-Me). ^{13}CNMR (125 MHz, DMSO-d_6): δ 164.31, 157.33, 148.84, 145.41, 138.56, 138.56, 133.50, 129.27, 129.27, 129.11, 128.61, 128.61, 127.58, 127.58, 127.02, 127.02, 114.17, 114.17, 114.01, 114.01, 55.84, 32.52; HREI-MS: m/z calcd for $C_{22}H_{19}BrN_4O_3S_2$, [M]+ 530.0082; Found: 530.0068.

2.3.6. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-([1,1′-biphenyl]−4-yl)ethylidene)−2-bromo benzenesulfonohydrazide (6)

Yield: 75%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 11.46 (s, 1H, NH), 11.08 (s, 1H, NH), 8.03–7.99 (m, 3H, ArH), 7.80–7.76 (m, 3H, ArH), 7.52 (t, J = 6.8 Hz, 3H, ArH), 7.20–7.14 (m, 5H, ArH), 7.02–6.98 (m, 3H, ArH), 5.38 (s, 2H, CH_2); ^{13}CNMR (125 MHz, DMSO-d_6): δ 157.37, 148.89, 145.42, 141.70, 139.92, 134.97, 134.97, 133.54, 132.5, 131.41, 129.36, 129.36, 129.25, 129.25, 129.12, 129.11, 129.11, 128.61, 128.61, 128.01, 127.57, 127.19, 127.02, 121.03, 114.19, 114.19, 32.50; HREI-MS: m/z calcd for $C_{27}H_{21}BrN_4O_2S_2$, [M]+ 576.0289; Found; 576.0274.

2.3.7. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-([1,1′-biphenyl]−4-yl)ethylidene)−4-methoxy benzenesulfonohydrazide (7)

Yield: 68 %; ^{1}HNMR (500 MHz, DMSO-d_6): δ 12.62 (s, 1H, NH), 11.98 (s, 1H, NH), 8.09 (d, J = 6.7 Hz, 2H, ArH), 7.98–7.95 (m, 3H, ArH), 7.92–7.89 (m, 3H, ArH), 7.56–7.51 (m, 4H, ArH), 7.46–7.41 (m, 2H, ArH), 7.06 (d, J = 6.1 Hz, 1H, ArH), 7.02 (t, J = 6.7, Hz 1H, ArH), 5.34 (s, 2H, CH_2), 3.81 (s, 3H, O-Me). ^{13}CNMR (125 MHz, DMSO-d_6): δ 166.41,157.37, 148.89, 145.42, 139.92, 138.58, 134.97, 134.97, 133.54, 129.36, 129.36, 129.25, 129.25, 129.11, 129.11, 128.61, 128.61, 127.57, 127.19, 127.04, 127.04, 127.02, 127.02, 120.88, 120.88, 114.19, 114.19, 55.80, 32.50; HREI-MS: m/z calcd for $C_{28}H_{24}N_4O_3S_2$, [M]+ 528.1290; Found; 528.1277.

2.3.8. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(4-bromophenyl) ethylidene)−2-bromo benzenesulfonohydrazide (8)

Yield: 73%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 12.03 (s, 1H, NH), 11.25 (s, 1H, NH), 8.20 (d, J = 7.2 Hz, 2H, ArH), 7.93 (d, J = 6.9 Hz, 2H, ArH), 7.80 (d, J = 6.2 Hz, 2H, ArH), 7.58 (m, 3H, ArH), 7.47 (t, J = 6.1 Hz, 1H, ArH), 7.13 (d, J = 6.1 Hz, 1H, ArH), 7.07 (dd, J = 1.8, 7.2 Hz, 1H, ArH), 5.38 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO-d_6): δ 157.33, 148.84, 145.41, 138.56, 138.56, 135.34, 133.50, 130.0, 129.53, 129.27, 129.27, 129.11, 128.61, 128.61, 128.11, 127.03, 127.03, 120.08, 114.17, 114.17, 32.50; HREI-MS: m/z calcd for $C_{21}H_{16}Br_2N_4O_2S_2$, [M]+ 577.9081; Found: 577.9078.

2.3.9. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(4-methoxyphenyl) ethylidene)−2-bromo benzenesulfonohydrazide (9)

Yield: 74%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.50 (s, 2H, NH), 8.01 (d, J = 6.3 Hz, 2H, ArH), 7.78 (m, 2H, ArH), 7.66 (m, 2H, ArH), 7.50 (d, J = 6.7 Hz, 2H, ArH), 7.25 (m, 2H, ArH), 6.68 (m, 2H, ArH), 5.19 (s, 2H, CH_2), 3.88 (s, 3H, O-Me). ^{13}CNMR (125 MHz, DMSO-d_6): δ 166.41, 157.33, 148.84, 145.41, 138.56, 138.56, 135.34, 130.02, 129.53, 129.11, 128.61, 128.61, 128.0, 127.03, 127.03, 120.06, 116.43, 116.43, 114.17, 114.17, 55.81, 32.53; HREI-MS: m/z calcd for $C_{22}H_{19}BrN_4O_3S_2$, [M]+ 530.0082; Found; 530.0069

2.3.10. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(4-nitrophenyl) ethylidene)−2-bromo benzenesulfonohydrazide (10)

Yield: 69%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 12.32 (s, 1H, NH), 11.26 (s, 1H, NH), 8.78 (d, J = 2.0 Hz, 1H, ArH), 8.14 (d, J = 6.6 Hz, 2H, ArH), 7.86 (d, J = 6.1 Hz, 1H, ArH), 7.51 (d, 1H, J = 6.1 Hz, ArH), 7.38–7.32 (m, 2H, ArH), 7.08–7.02 (m, 4H, ArH), 6.69 (dd J = 1.9, 7.4 Hz, 1H, ArH), 6.47–6.42 (m 2H, ArH), 4.91 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO-d_6): δ 157.33, 151.62, 148.79, 145.37, 138.57, 134.57, 133.57, 130.24, 129.25, 129.11, 128.60, 128.60, 128.22, 128.22, 127.96 127.02, 127.00. 119.00, 114.06, 113.83, 32.51; HREI-MS: *m/z* calcd for $C_{21}H_{16}BrN_5O_4S_2$, [M]+ 544.9827; Found: 544.9811.

2.3.11. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(3-nitrophenyl) ethylidene)−2,4-difluoro benzenesulfonohydrazide (11)

Yield: 65%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 11.92 (s, 1H, NH), 11.88 (s, 1H, NH), 8.48 (d, J = 1.4 Hz, 1H, ArH), 8.01 (dd J = 1.2,8.2 Hz, 1H, ArH), 7.78 (dd, J = 1.6, 6.4 Hz, 1H, ArH), 7.51 (m, 1H, ArH), 7.46 (t, J = 6.2 Hz, 1H, ArH), 7.24 (dd, J = 1.9, 8.2 Hz, 1H, ArH), 6.91 (t, J = 7.2 Hz, 1H, ArH), 6.54 (m, 4H, ArH), 5.48 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO-d_6): δ 167.98, 167.66, 157.33, 148.84, 138.56, 138.56, 133.50, 133.03, 130.31, 129.25, 129.25, 129.11, 127.21, 127.03, 127.03, 125.40, 114.17, 114.17, 109.95, 109.66, 32.51; HREI-MS: *m/z* calcd for $C_{21}H_{15}F_2N_5O_4S_2$, [M]+ 503.0534; Found; 503.0518.

*2.3.12. 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 12.66 (s, 1H, NH), 11.48 (s, 1H, NH), 8.31 (d, J = 7.2 Hz, 2H, ArH), 8.19 (m, 3H, ArH), 7.91 (m, 3H, ArH), 7.59 (m, 3H, ArH), 7.49 (dd, J = 1.6, 6.9 Hz, 2H, ArH), 6.92 (d, J = 1.4 Hz, 1H, ArH), 6.88 (dd, J = 1.2, 8.3 Hz, 1H, ArH), 5.47 (s, 2H, CH_2) 3.88 (3H, O-Me). ^{13}CNMR (125 MHz, DMSO-d_6): δ 167.98, 167.66, 157.37, 148.89, 145.42, 139.92, 134.97, 133.54, 130.30, 129.36, 129.36, 129.25, 129.25, 129.11, 129.11, 128.61, 128.61, 127.57, 127.19, 127.02, 127.02, 121.44, 110.39, 110.19, 105.95, 100.66, 55.8, 32.53; HREI-MS: *m/z* calcd for $C_{28}H_{22}F_2N_4O_3S_2$, [M]+ 564.1101; Found; 564.1092.

2.3.13. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(4-bromophenyl) ethylidene)−2-nitro benzenesulfonohydrazide (13)

Yield: 69%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 12.44 (s, 1H, NH), 12.19 (s, 1H, NH), 8.15 (d, J = 6.9 Hz, 2H, ArH) 7.92 (d, J = 6.9 Hz, 2H, ArH), 7.80 (d, J = 6.2 Hz, 2H, ArH), 7.60 (m, 3H, ArH), 7.47 (t, J = 6.1 Hz, 1H, ArH), 7.09 (d, J = 6.3 Hz, 1H, ArH), 7.00 (dd, J = 1.7. 6.4 Hz, 1H, ArH), 5.46 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO-d_6): δ 157.33, 150.03, 148.84, 145.11, 138.56, 138.56, 135.02, 133.50, 132.82, 129.27, 129.27, 129.11, 129.11, 128.61, 128.61, 127.03, 127.03, 124.04, 114.17, 114.17, 32.57; HREI-MS: *m/z* calcd for $C_{21}H_{16}BrN_5O_4S_2$, [M]+ 544.9827; Found; 544.9810.

2.3.14. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(3-nitrophenyl) ethylidene)−2-nitro benzenesulfonohydrazide (14)

Yield: 65%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.79 (s, 1H, NH), 9.94 (s, 1H, NH), 8.16 (d, J = 6.9 Hz, 2H, ArH), 7.92 (d, J = 6.9 Hz, 2H, ArH) 7.79 (d, J = 6.2 Hz, 2H, ArH), 7.55 (m, 3H, ArH), 7.48 (t, J = 6.1 Hz, 1H, ArH), 7.10 (d, J = 1.7 Hz, 1H, ArH), 7.00 (dd, J = 1.9, 6.3 Hz, 1H, ArH), 5.48 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO-d_6): δ 157.33, 150.04, 148.84, 145.53, 138.56, 138.56, 136.11, 135.03, 134.31, 133.01, 129.8, 129.8, 129.11, 127.02, 127.02, 126.54, 125.03, 123.60, 114.17, 114.17, 32.57; HREI-MS: *m/z* calcd for $C_{21}H_{16}N_6O_6S_2$, [M]+ 512.0573; Found; 512.0561.

2.3.15. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(2, 5-dimethoxyphenyl)ethylidene)−2-nitro benzenesulfonohydrazide (15)

Yield: 73%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 11.49 (s, 2H, NH), 8.00 (d, J = 7.1 Hz, 2H, ArH) 7.84 (d, J = 7.0 Hz, 2H, ArH), 7.53 (d, J = 7.4 Hz, 1H, ArH), 7.10 (d, J = 1.8 Hz, 1H, ArH), 7.00 (m, 4H, ArH), 6.51 (d, J = 8.6 Hz, 1H, ArH), 5.47 (s, 2H, CH_2), 3.89 (s, 6H, O–CH_3). ^{13}CNMR (125 MHz, DMSO-d_6): δ 157.33, 154.4, 153.34, 150.21, 148.84, 145.5, 138.56, 138.56, 136.11, 133.22, 129.11, 127.13, 127.13, 123.61, 121.32, 119.71, 116.05, 115.34, 114.17, 114.17, 55.80, 55.07, 32.57; HREI-MS: *m/z* calcd for $C_{23}H_{21}N_5O_6S_2$, [M]+ 527.0933; Found; 527.0924.

2.3.16. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(4-nitrophenyl)ethylidene)−2-nitro benzenesulfonohydrazide (16)

Yield: 62%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 11.19 (s, 1H, NH), 10.37 (s, 1H, NH), 8.25 (d, J = 7.4 Hz, 2H, ArH), 8.07 (d, J = 7.1 Hz, 2H, ArH), 7.61 (d, J = 6.2 Hz, 2H, ArH), 7.51 (m, 3H, ArH), 7.39(dd, J = 1.6, 6.3 Hz, 1H, ArH), 6.89 (dd, J = 1.6, 6.6 Hz, 1H, ArH), 6.77 (dd, J = 1.3, 8.2 Hz, 1H, ArH), 5.48 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO-d_6): δ 157.33, 150.41, 148.84, 145.41, 140.17, 138.56, 138.56, 134.05, 133.50, 129.27, 129.11, 129.11, 128.61, 127.58, 127.58, 127.02, 127.02, 123.6, 114.17, 114.17, 32.53; HREI-MS: *m/z* calcd for $C_{21}H_{16}N_6O_6S_2$, [M]+ 512.0573; Found; 512.055.

2.3.17. N'-(2-((1H-benzo[d]imidazol-2-yl)thio)−1-(4-bromophenyl)ethylidene)−2-methyl benzenesulfonohydrazide (17)

Yield: 68%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 12.52 (s, 1H, NH), 10.91 (s, 1H, NH), 8.22 (d, J = 7.8 Hz, 2H, ArH), 7.87 (d, J = 7.7 Hz, 2H, ArH), 7.76 (d, J = 6.2 Hz, 2H, ArH), 7.56 (dd, J = 1.4, 6.6 Hz, 1H, ArH), 7.49 (d, J = 6.8 Hz, 2H, ArH), 7.42 (dd J = 1.6, 7.4 Hz, 1H, ArH), 7.11 (d, J = 6.9 Hz, 1H, ArH), 76.99 (dd J = 1.8, 8.2 Hz, 1H, ArH), 5.48 (s, 2H, CH_2), 2.25 (s, 3H, CH_3). ^{13}CNMR (125 MHz, DMSO d_6): δ 164.31, 157.33, 148.84, 139.21, 138.56, 138.56, 136.06, 133.50, 132.31, 131.61, 130.05, 129.27, 129.27, 129.11, 128.61, 128.61, 127.03, 127.03, 114.17, 114.17, 32.50, 21.07; HREI-MS: *m/z* calcd for $C_{22}H_{19}BrN_4O_2S_2$, [M]+ 514.0133, Found; 514.0118.

3. Docking study protocol

Docking study was done targeting the crystal structure of amylase (PDB ID: 4W93) [38] 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 [43]. 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 tautomers. 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 12 Å around Montbretin A binding sites. By using Chem PLP scoring function, docking calculations were skilled [44]. By using Discover studio visualizer, docking result was further evaluated and each derivative, binding mode was visually inspected.

3.1. Alpha-amylase assay protocol

a-Amylase inhibition activity had been determined using assay slightly modified from Kwon et al. [45]. All compounds were highly dissolved in dimethyl sufoxide (DMSO). A total of 40 μl

of sample solution and 40 μl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing a-amylase solution (Porcine pancreatic a-amylase) (0.5 mg/ml) were incubated at 25 C for 10 min. Forty μl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was then added to each tube every 5 s intervals. The reaction mixtures were then incubated at 25 C for another 10 min. The reaction was stopped using 100 μl of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 900 μl distilled water and the absorbance was measured at 540 nm.% inhibition was calculated using below formula.

$$\%\text{inhibition} = [(A_c - A_s)/Ac] \times 100\%$$

Where:

Ac = absorbance of the control.

As = absorbance of the sample.

4. Results and discussion

4.1. Chemistry

Synthesis of 2-mercaptobenzimidazole/6-metnoxy-2-mercaptobenzimidazole derivatives **(1–17)** bearing sulfonamide moiety were carried out in three steps.

Step-1: 2-mercaptobenzimidazole/6-metnoxy-2-mercaptobenzimidazole, various substituted phenacylbromides were dissolved in acetone (20 ml), followed by addition of catalytic quantity of potassium carbonate and then stirred the reaction mixture for 2 hrs at room temperature. Upon completion of the reaction, reaction mixture was cooled at 10–15 °C temperature and filtered the precipitate which yielded intermediate (**I**) as a product having white cotton like texture (Scheme 1) [46,47].

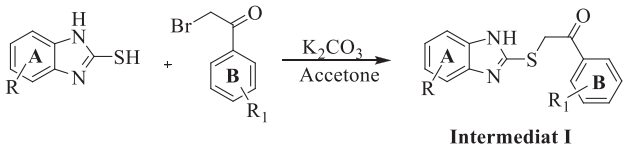
Step-2: Various substituted sulfonyl chlorides were reacted with equivalent quantity of hydrazine hydrate in methanol and refluxed the reaction blend for 3 hrs. Upon reaction completion, solvent was evaporated under vacuum to yield intermediate product (**II**) (Scheme 2).

Step-3: Intermediate product (**I**) was reacted with intermediate product (**II**) in methanol, acidified the reaction medium by 3–7 drops of glacial acetic and then the mixture was refluxed for 4 hrs. When the reaction was completed, precipitate formed in reaction medium. These precipitates were filtered to afford benzimidazole bearing sulfonamide moiety (**1–17**) (Scheme 3; Table 1).

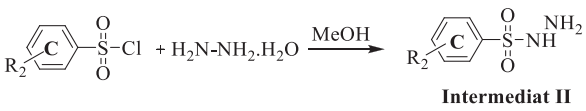
4.2. Activity

4.2.1. In vitro *alpha-amylase inhibitory activity*

4.2.1.1. α-Amylase activity. Seventeen (**17**) derivatives of 2-mercaptobenzimidazole bearing sulfonamide moety (**1–17**) were evaluated for α-amylase inhibitory potential. All analogs displayed



Scheme 1. Synthesis of intermediate product (**I**).



Scheme 2. Synthesis of intermediate product (**II**).

Table 1
Different substituents of 2-mercaptobenzimidazole analogs and α-amylase inhibitory potential.

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				1.70 ± 0.10μM

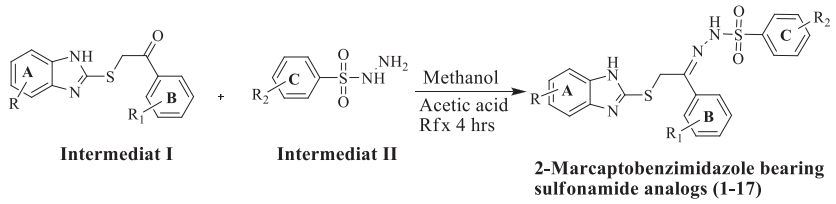
varying degree of inhibitory potential with IC_{50} values ranging between 0.90 ± 0.05 to 11.20 ± 0.30 μM when compared with the standard acarbose having IC_{50} value 1.70 ± 0.10 μM. Compound **1, 2, 11, 12** and **14** having IC_{50} values 1.40 ± 0.10, 1.30 ± 0.05, 0.90 ± 0.05, 1.60 ± 0.05 and 1.60 ± 0.10 μM respectively were displayed inhibitory activity than standard drug acarbose. The remaining analogues also exhibited good α-amylase inhibition.

The limited SAR study revealed that the most potent compound in the series is **11** having IC_{50} value 0.90 ± 0.05 μM. This is may be due to more electronegative substituents such as NO_2 group on phenyl ring **B** and difluoro substituents on phenyl ring **C** which create more polarity in derivative **11**, and directly prompt the binding interaction with active site of residues. By this rationality derivative **11** exhibited more potent potential.

SAR study also revealed that relocation of same substituent on phenyl ring affects the inhibitory activity of derivatives. The effect of this relocation of groups on inhibitory activity was found when we compared the potential of derivative **1** (IC_{50} = 1.40 ± 0.10 μM) with **2** (IC_{50} = 1.30 ± 0.05 μM) (Fig. 2). Derivative **2** displayed slightly better inhibitory activity than derivative **1,** even they have same basic skeleton but the difference found in their structure was due to the position of NO_2 on phenyl ring **B.** The same change in activity was also found in derivatives **14** and **16** (Fig. 2). This clearly indicates that electron withdrawing substituent like NO_2 creates more polarity within molecules when it relocate from one position to other position on phenyl ring, and by increasing the polarity the binding interactions of derivatives were also increased with active site of an enzyme and therefore derivatives **1** displayed greater inhibitory activity than **2** . Similarly, derivatives **14** displayed greater inhibitory activity than derivatives **16** due to more polarity index.

It was found through SAR study that some time less electronegative characters influences the efficacy of derivatives in enzyme inhibition study because of their large sizes, which do not easily coordinate with active site of an enzyme, and vice versa. By this consideration derivatives 9 (IC_{50} = 4.10 ± 0.20 μM) exhibited greater potential than derivatives **8** (IC_{50} = 11.20 ± 0.30 μM) (Fig. 2), because derivatives **9** have 4-OMe on phenyl ring **B** and 2-Br on phenyl ring **C** while derivative **8** have Br substituents on phenyl ring **B** and **C.** This clearly indicates that declination in inhibitory activity is due large size and less electronegative character of Br atom.

Here, it was concluded from the SAR study that electron-withdrawing substituents on phenyl ring deactivates the phenyl ring *via* attracting pi electronic clouds, but induced polarity within



Scheme 3. Synthesis of 2-mercaptobenzimidazole bearing sulfonamide analogs (**1–17**).

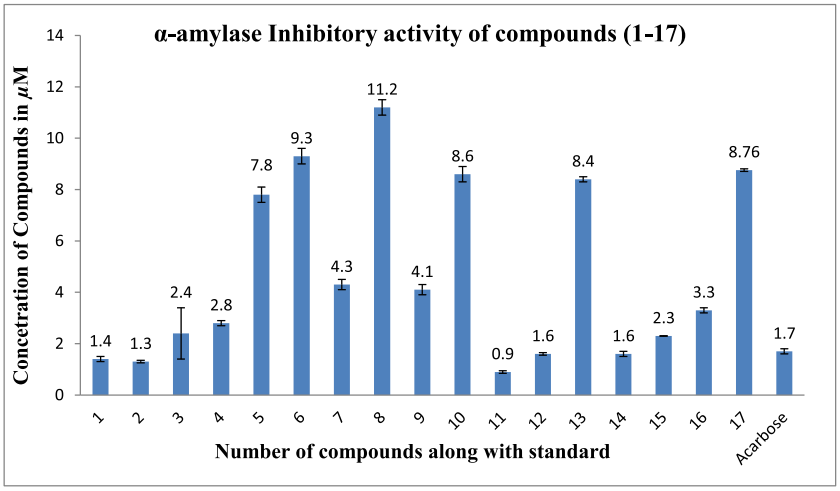


Fig. 2. Inhibitory activity of derivatives, acarbose and their concentrations.

molecules and as result increased the binding interactions between derivatives (ligands) and active site of enzyme which directly increased the inhibitory activity of derivatives. This hypothesis was further supported by comparison the inhibitory activity of derivative **13** ($IC_{50} = 8.40 \pm 0.10\,\mu M$) with derivative **17** ($IC_{50} = 8.76 \pm 0.05\,\mu M$) (Fig. 2). Herein this approach, almost both derivative have the same basic structure but derivative **13** have 2-NO_2 substitution on phenyl ring **C**, while derivative **17** have 2-CH_3 substitution on phenyl ring **C** respectively. The slight better inhibitory activity of derivative **13** was attributed due to 2-NO_2 substitution which has electron with-drawing nature as compare to 2-CH_3 substitution in derivative **17** which has electron donating nature. This comparison absolutely supports our hypothesis, which proves that electron withdrawing substituents enhances the activity by increasing the polarity within/around the molecules respectively.

Although, among the series **(1–17)** all noticeable proton and carbon peaks in the spectra were compared with literature, aiming to confirm the exact range of their respective peaks. In this context, the protons of S-CH_2 group give singlet at 5.3 ppm, while in literature S-CH_2 proton give singlet at 4.6 to 4.9 ppm respectively. Similarly within our synthesized derivatives the proton of SO_2NH–N=C group give peak at 12.5 ppm but the same proton of CONH–N=C group in our reported work give peak at 12.1 to 12.7 ppm respectively. In the same way, carbon atom of S-C-N_2H in imidazole ring in our synthesized derivatives give peak at 148.7 to 148.7 but the carbon atom in the same S-C-N_2H group in imidazole ring which we have already reported gives peak at 149.3 to 149.5 ppm respectively. Moreover, carbon of SCH2-C=N–N=NH group give peak at 156.6 to 157.3 ppm, but the same type of carbon in SCH2-C=N–N=NH group in our reported work give peak in 164.3 to 164.3 ppm. The study about carbon atom of S-CH_2 group in our current work give peak at the range of 32.5 ppm, while in case of our reported work carbon atom of S-CH_2 group give peak in the range of 33.5 and 34.1 to 34.5 ppm respectively [43,44]. This rational study of present work based on proton and carbon-NMR displayed great correlation with already reported work.

4.3. Docking

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 4 Å radius on the binding site of amylase, were considered as most influential factor for activity. In this series all **17** analogs were active with IC_{50} value ranging in between 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 was reported in our previous paper [48].

Compound **11** is most potent 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 hydrogen bond is formed by this nitro group. The nitro group has electrostatic interacts with ASP197 and HIS299, with ARG195 and GLU233. Also with TYR62 π-cation interaction formed as well as C-cation interaction with TYR62. Moreover, with HIS201π- π stacking, with LEU162 and LYS200 π-Alkyl interaction forms with benzimidazole ring. the benzimidazole ring forms π- π stacking with HIS201 and π- Alkyl interaction with LYS200 and LEU162. 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. 3a).

In the case of compound **2**, methoxy group at R2 behaves as acceptor to form hydrogen bond with acidic residue on the other hand R_1 nitro group bearing phenyl ring forms π-sigma interac-

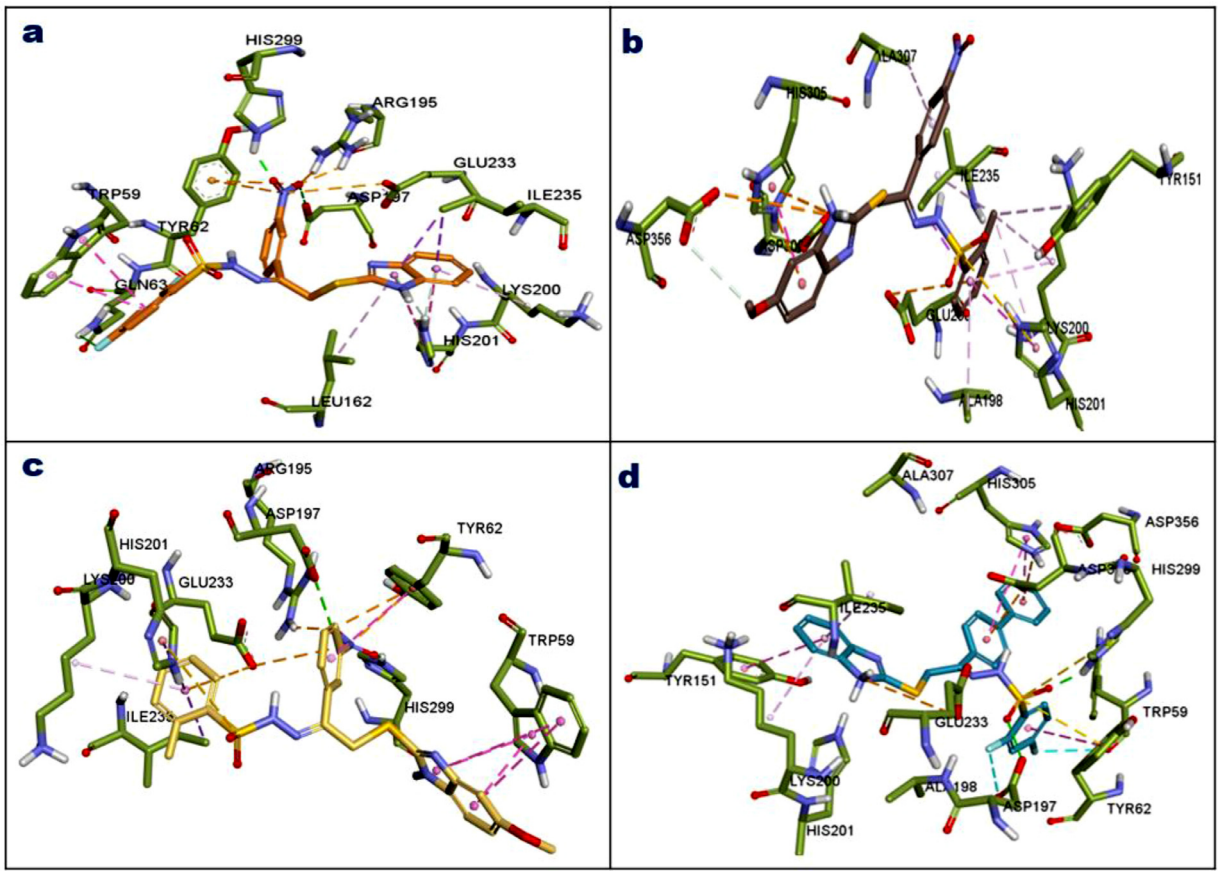


Fig. 3. Shows the binding interaction of the potent derivative in the α-amylase active site (a) compound **11** in brown stick, (b) compound **2** in gray stick(c) compound **1** in yellow stick and (d) compound **12** blue stick. Key interacting residues are shown in greenish stick form and the hydrogen 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.

tion responsible for activity. In the case of compound **1** the nitro group at the R_2 position forms hydrogen bond with acidic residue contribute for activity. Finally, the compound **12** the ortho-para fluoro moieties at R_2 position have contact with acidic and aromatic residue of **12** influencing in the activity. Likewise, the phenyl group at R_1 forms π - π stacking with histidine also plays role in activity Fig. 3b show the binding mode of compound **2**.

Fig. 3c represents the binding mode of the compound **1**, a hydrogen bond is established between ARG195, ASN298, and HIS299 with nitro group attached to the phenyl ring and the same group also forms electrostatic contact with ARG195 and ASP300. The linker sulfonyl nitrogen forms hydrogen bond with ASP300. The toluene group attached the sulfonyl group forms π- π stacking and hydrophobic contact with HIS305. The methoxy attached to the benzodiazole forms hydrogen bond with THR163 and π contact with THY151.

Finally, the binding mode orientation of the compound **14** shows that the benzonitro group forming hydrogen with ASP197 and ARG195 as well π-ionic interaction with TYR62, GLU233 and ARG195. The benzonitro group forms hydrogen bond with HIS305 and the ring also forms π-anion contact with ASP300 and π-alkyl contact. Next the sulfonyl nitrogen forms hydrogen bond with ASP300. Next the nitro group forms hydrogen bond with TRP59 and the benzene group forms π- π stacking HIS305. These contacts was influential to stabilize the complex and reflects in the biological activity index.

The docking studies binding mode show that in the presence of methoxy group at R position in compound **1**, **2**, forms a hydrogen bond with active residues such as Thr163 in compound **1** and Asp356 in compound **2** is shown as an example are important for activity. Similarly, the presence of methyl at R_2 position stabilizes the complex further with the presence of hydrophobic interactions as in **1** and **2**. Finally, the presence of the nitro group at R_1 is highly preferred to increase the activity that is crucial for hydrogen bonding and π-anion interaction as seen in compound **1**, **2**, **11** and **14**. Collectively these sort of interaction predicted by docking studies is key for the activity. On the other hand as seen in the compound **13** and **17** the presence of bromo group at R_1 position didn't bring any interaction and therefore there was loss in activity.

5. Conclusion

In the present work, we have synthesized 2-marcaptobenzimidazole derivatives **(1–17)** bearing sulfonamide moiety. The inhibitory activity derivatives against alpha-amylase enzyme revealed that almost all derivatives of the series showed good inhibitory activity in the presence of standard drug acarbose. Some of the derivatives like **1**, **2**, **11**, and **14** emerged with most potent inhibitory activity as compared to standard drug. Moreover, derivative **11** is the most potent derivative among the series and exhibited many folds better potential than standard drug. As derivatives **11** have NO2-group at 3 position on phenyl ring B and 2/4-diflouro on phenyl ring, and these groups are very electronegative which draw electronic density from phenyl rings and at result increased the polarity of derivatives, due to which

it strongly coordinated with active site of an enzyme as shown **in fig-2a**, therefore exhibited better potential than standard drug. Overall potential of the series displayed that it is most potent class of alpha-amylase inhibitors, and may provide the base for further investigation of alpha-amylase inhibitors in future in search of lead candidates.

Author statement

All authors equally contributed in the manuscript.

Declaration of Competing Interest

None.

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