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Synthesis, antidiabetic activity and *in silico* studies of benzo[*b*]thiophene based small molecule α-amylase inhibitors

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ABSTRACT

Benzo[*b*]thiophene has been implicated as molecular framework in the drug discovery against broad spectrum of biological targets. In the antidiabetic drug regime, benzo[*b*]thiophene based SGLT2 and ALR2 inhibitors have been recently developed but their potential towards α-amylase inhibition remained unexplored to date. In this context, a series of novel small molecule benzo[*b*]thiophene-2-carboxylic acid derivatives **(3a-p)** was synthesized, characterized, and evaluated for antidiabetic activity as α-amylase inhibitors. We found that, all benzo[*b*]thiophene derivatives exhibited significant α-amylase inhibition with IC_{50} value ranging from 5.37 ± 0.25 μM to 29.89 ± 0.68 μM. The SAR studies showed benzo[*b*]thiophene carboxylate bearing bis(2-hydroxyethyl)amino group (**3b**) was most potent with IC_{50} of 5.37 ± 0.25 μM compared to standard drug Acarbose (IC_{50} = 6.40 ± 0.14 μM). Further, the enzyme inhibition mechanism study regarded **3b** as competitive inhibitor of α-amylase with Ki value of 1.76 μM. A detailed *in silico* study was also performed in order to estimate binding properties, drug likeness and predict toxicity profile of these agents. It was demonstrated that novel small molecule benzo[*b*]thiophene derivative (**3b**) can effectively bind through H-bonding, hydrophobic and π-stacking interactions within α-amylase active site. Moreover, drug likeness and toxicity prediction studies suggested compound **3b** as potential & safter α-amylase inhibitor. Overall, our present study disclosed a novel class of benzo[*b*]thiophene based α-amylase inhibitors and opened a template for further lead optimization and development.

1. Introduction

According to the International Diabetes Federation (IDF), the number of people with diabetes is steadily increasing, with 415 million currently affected globally. As estimated, by 2040, the diabetes mellitus (DM) cases could rise to 642 million [1]. Diabetes mellitus (DM) is a long-term metabolic disease associated with the persistence of high blood glucose level (hyperglycemia) condition which often leads to other health issues such as heart diseases, nerve damage, eye issues and kidney failure [2]. Thus, efficacious, and safer drug against this global disease remained as key area of research.

In this context, enzyme inhibition-based drug discovery emerged as an effective strategy for metabolic disorders like Diabetes mellitus (DM) [3]. Especially, α-amylase and α-glucosidase has been extensively explored for the treatment of type 2 diabetes (DM2) since both are vital metabolic enzymes for the efficient digestion of carbohydrates and glycogen to provide free glucose for absorption [4]. Of these, the enzyme amylase, which is secreted by the salivary glands, hydrolyze the α-(1, 4)-D-glycosidic bonds found in carbohydrates and disintegrate into smaller components like glycogen and monosaccharides, which the body would then absorb further [5–8]. Hence, suppressing the α-amylase enzyme there by reducing the postprandial glycaemia proved to be an effective treatment option for DM2 [9–12]. Presently, the FDA-approved α-amylase/glucosidase inhibitors such as Acarbose, Voglibose, and Miglitol (Fig. 1) are being used in the clinics to treat DM2 [13,14]. Nevertheless, a number of side effects, including diarrhea, flatulence, skin reactions, abdominal pain, and abnormal liver functions, are associated to these antidiabetic medications [15]. Consequently,

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development of effective and safter α-amylase inhibitors remained as main areas of research in the antidiabetic regime.

Thiophenes are extensively explored bioactive scaffolds among the sulfur containing heterocycles providing plethora of new lead molecules for the drug design and discovery in last two decades [16–19]. This resulted in several clinical drugs based on thiophene framework for the treatment of various types of diseases with high therapeutic potency [20–22]. Particularly, the benzo[*b*]thiophene-2-carboxylic acid derivatives (amide and ester) demonstrated broad spectrum of biological activities including anticancer, antifungal, antibacterial, anti-inflammatory and antidiabetic [23–30]. For instance, benzo[b] thiophene carboxamide-benzimidazole conjugate (**A**) (Fig. 2) showed potent antiproliferative activities against HeLa cells with IC_{50} in low micromolar range [31]. Similarly, quinazolinone-benzothiophene conjugate (**B**) found to be potential candidate against mycobacterium tuberculosis H37Rv strain [32] while benzo[b]thiophene carboxamide (**C**) exhibited potent anti-inflammatory activity as COX-2 inhibitor. Furthermore, hybrid of quinoxaline-benzo[b]thiophene moiety (**D**) reported as potent and selective aldose reductase (ALR2) inhibitor [33]. Recently, benzo[b]thiophene based drug candidates viz. Ipragliflozin (**E**) has been approved for the treatment of DM2 which is selective inhibitor of sodium-glucose cotransporter-2 (SGLT2) [34]. Thus, there is considerable renewed interest towards synthesis of novel benzo[*b*] thiophene based antidiabetic agents.

In the quest of searching novel and safer antidiabetic agents, currently a number of heterocycles such as isatin, coumarin, chromene, indole, benzimidazole and triazole have been extensively used as core skeletons for the development of α-glucosidase/α-amylase inhibitors [35–39]. However, potential of benzo[*b*]thiophene scaffolds towards a-amylase inhibition is yet to explore. Hence, inspired by the aforementioned therapeutic applications of benzo[*b*]thiophenes and our continuous research efforts towards the development of new leads as antidiabetic agents [40], we sought to utilize bioactive benzo[*b*]thiophene framework and develop a library of small molecule α-amylase inhibitors. For this purpose, a series of novel benzo[*b*]thiophene-2-carboxylic acid derivatives have been synthesized by incorporating functionalized aromatic or aliphatic (cyclic/acyclic) units through amide or ester linkages and evaluated for their inhibition potential against α-amylase enzyme along with *in silico* studies. The present study revealed new benzo[*b*]thiophene analogs as potent and competitive α-amylase inhibitors along with favorable drug-likeness profile which is reported herein.

2. Materials and methods

2.1. Chemistry

All the chemicals and reagent were used for the experiment, were purchased from Loba Chemie. Pvt Ltd., Sigma Pvt. Ltd. & Meark India Ltd. (Mumbai, India). and were used without further purification. The reactions were monitored through TLC using E. Merck 0.25 mm silica gel plates and spots were visualized through UV light. The uncorrected melting points of the compounds were measured in one end open capillary method. The characterization of synthesized compound was carried out by FT-IR (Shimadzu, Japan) spectrophotometer in frequency range of 4000-400 cm^{-1} using KBr griding method, ^{1}H NMR (400 MHz, Bruker, Germany) and ^{13}C NMR (100 MHz, Bruker, Germany). The chemical shift of the ^{1}H and ^{13}C NMR spectrum was expressed in parts per million (ppm) relative to tetramethylsilane (TMS) using DMSO as solvent. The elemental analysis of the compounds was performed on a Elementar vario MICRO cube instrument. 3-Chlorobenzo[*b*]thiophene-2-carboxylchloride **(1a-c)** were prepared from cinnamic acid and thionyl chloride using pyridine as the catalyst by following the reported procedure [41]. 2,2′-((4-Hydroxyphenyl)azanediyl)bis(ethan-1-ol) **(2a)** was prepared from 4-amino phenol and 2-chloro ethanol using Na_2CO_3 as described in the literature [42]. Intermediate **2b-n** were purchased from sigma Aldrich and utilized without further purification.

2.2. General procedure for the synthesis of *3a-p*

To a solution of 2,2′-((4-hydroxyphenyl)azanediyl)bis(ethan-1-ol) **(2a)** or amines **(2b-n)** (1 mmol) in ethyl acetate (2 mL), a solution of corresponding 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (1 mmol) in ethyl acetate (3 mL) was added dropwise followed by the addition of triethyl amine (TEA) (1.5 mmol) at 0–5 °C. After addition, the reaction mixture was brought to ambient temperature and stirred further for 60 min. The resulting products **(3a-p)** were filtered off, washed with water and recrystallized from MeOH to give analytically pure products **(3a-p)**.

(4-(Bis(2-hydroxyethyl)amino)phenyl-3-chlorobenzo [*b*] thiophene-2-carboxylate) (3a)

Compound **3a** was prepared by following above general method from 2,2′-((4-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (**2a**, 0.19 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a,** 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.37 g (97%) as white powder; m.p.= 128–131 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 8.14 (d, J = 8 Hz, 1H, ArH), 8.00 (d, J = 7.6 Hz, 1H, ArH), 7.66 (m, 2H, ArH), 7.06 (d, J = 8.8 Hz, 2H, ArH), 6.71 (d, J = 9.2 Hz, 2H, ArH), 4.92 (brs, 2H, 2×OH), 3.56 (m, 4H, 2×CH_2), 3.42 (m, 4H, 2×CH_2); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 160.1, 146.7, 140.1, 138.5, 136.5, 129.4, 127.5, 126.7, 125.3, 123.9, 123.8, 122.4, 112.0, 58.6, 53.8; IR (KBr, υ_{max}, cm^{-1}): 3371 (O-H), 3267 (O-H), 3057 (C-H), 2879 (C-C), 1711 (C-O), 1608 (C=C), 1504 (C=C), 1325 (C-N Ar), 1205 (C-N aliphatic), 1042 (C-O primary alcohol), 991 (C=C), 934 (C=C), 748 (C-Cl), 727 (C=C) cm^{-1}; Anal. calc. for $C_{19}H_{18}ClNO_4S$: C, 58.24; H, 4.63; N, 3.57; S, 8.18; Found: C, 58.38; H, 4.75; N, 3.32; S, 8.05.

(4-(Bis(2-hydroxyethyl)amino)phenyl-3,6-dichlorobenzo [*b*] thiophene-2-carboxylate) (3b)

Compound **3b** was prepared by following above general method from 2,2′-((4-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (**2a**, 0.19 g, 1 mmol), 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl chloride (**1b**, 0.26 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.38 g (92%) as yellow powder; m.p.= 165–168 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 8.29 (s, 1H, ArH), 7.96 (d, J = 8.8 Hz, 1H, ArH), 7.63 (d, J = 8.8 Hz, 1H, ArH), 7.06 (d, J = 8.8 Hz, 2H, ArH), 6.73 (d, J = 9.2 Hz, 2H, ArH), 4.92 (brs, 2H, OH) 3.56 (t, J = 4.0 Hz, 4H, 2×CH_2), 3.42 (t, J = 5.6 Hz, 4H,

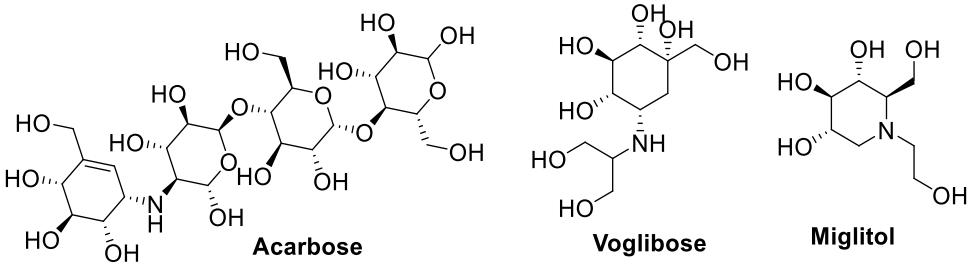


Fig. 1. FDA approved α-amylase/glucosidase inhibitors.

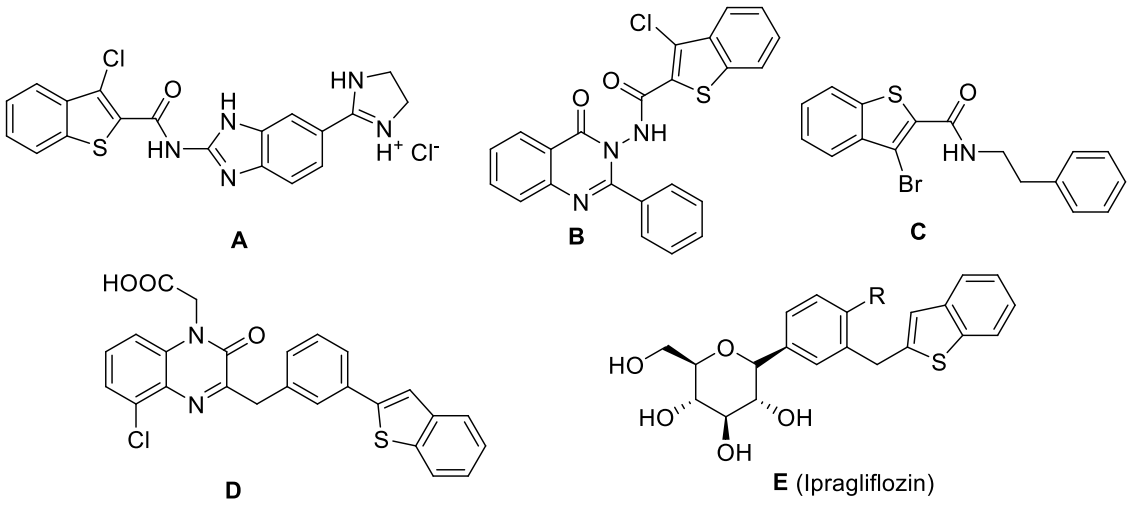


Fig. 2. Representative examples of bioactive benzo[b]thiophene derivatives (**A–E**).

2×CH_2); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 159.8, 146.7, 140.1, 139.6, 135.3, 134.4, 127.4, 127.2, 126.2, 125.3, 123.5, 122.4, 112.1, 58.5, 53.8; IR (KBr, υ_{max}, cm^{-1}): 3284 (O-H), 3076 (O-H), 3055 (C-H), 2875 (C-C), 1708 (C-O), 1611 (C=C), 1503 (C=C), 1323 (C-N Ar), 1208 (C-N aliphatic), 1044 (C-O primary alcohol), 1004 (C=C), 937 (C=C), 867 (C=C), 750 (C-Cl) cm^{-1}; Anal. calc. for $C_{19}H_{17}C_{l2}NO_4S$: C, 53.53; H, 4.02; N, 3.29; S, 7.52; Found: C, 53.36; H, 4.32; N, 3.12; S, 7.45.

(4-(Bis(2-hydroxyethyl)amino)phenyl-4-bromo-3-chlorobenzo [*b*] thiophene-2-carboxylate) (3c)

Compound **3c** was prepared using above described method, from 2,2′-((4-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (**2a**, 0.19 g, 1 mmol), 4-bromo-3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1c,** 0.30 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.43 g (95%) as yellow crystals; m.*p* = 163–166 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 8.16 (d, *J* = 8.0 Hz, 1H, ArH), 7.82 (d, *J* = 7.6 Hz, 1H ArH), 7.50 (t, *J* = 8.0 Hz, 1H, ArH), 7.05 (d, *J* = 9.2 Hz, 2H, ArH), 6.71 (d, *J* = 9.2 Hz, 2H, ArH), 4.92 (brs, 2H, OH), 3.55 (t, *J* = 6.4 Hz, 4H, 2×CH_2), 3.42 (t, *J* = 6.0 Hz, 4H, 2×CH_2); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 159.7, 146.8, 141.2, 140.0, 132.8, 131.7, 129.6, 127.4, 127.2, 123.9, 122.3, 117.2, 112.0, 58.6, 53.8; IR (KBr, υ_{max}, cm^{-1}): 3572 (O-H), 2952 (C-C), 2921 (C-C), 1693 (C-O), 1608 (C=C), 1513 (C=C), 1315 (C-N Ar), 1203 (C-N aliphatic), 1038 (C-O primary alcohol), 934 (C=C), 748 (C-Cl), 522 (C-Br) cm^{-1}; Anal. calc. for $C_{19}H_{17}BrClNO_4S$: C, 48.48; H, 3.64; N, 2.98; S, 6.81; Found: C, 48.25; H, 3.50; N, 2.88; S, 6.96.

(3-Chlorobenzo [*b*] thiophen-2-yl)(1*H*-pyrazol-1-yl)methanone (3d)

Compound **3d** was prepared by following above general method from 1*H*-pyrazole (**2b**, 0.06 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.26 g (89%) as white powder; m.p.= 192–195 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 13.12 (s, 1H, NH), 8.10 (d, *J* = 6.8 Hz, 1H, ArH), 7.93 (d, *J* = 8.4 Hz, 1H, ArH), 7.60 (m, 2H, ArH), 7.53 (t, *J* = 4.0 Hz, 1H, ArH), 7.21 (t, *J* = 3.6 Hz, 1H, ArH); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 137.8, 137.0, 128.1, 126.2, 123.8, 123.1, 122.3, 122.9; IR (KBr, υ_{max}, cm^{-1}): 3383 (C-H), 3092 (C-H), 2851 (C-H), 2359 (C-H), 1931 (C-H), 1684 (C-O), 1531 (N-H), 1282 (C-N), 935 (C=C), 760 (C-Cl) cm^{-1}; Anal. calc. for $C_{12}H_7ClN_2OS_2$: C, 48.90; H, 2.39; N, 9.50; S, 21.75; Found: C, 48.82; H, 2.15; N, 9.66; S, 21.88.

3-Chloro-N-(thiazol-2-yl)benzo [*b*] thiophene-2-carboxamide (3e)

Compound **3e** was prepared by following above general method from thiazol-2-amine (**2c**, 0.10 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.26 g (89%) as white powder; m.p.= 194–196 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 13.12 (brs, 1H, NH), 8.10 (d, *J* = 6.8 Hz, 1H, ArH), 7.93 (d, *J* = 8.4 Hz, 1H, ArH), 7.60 (m, 2H, ArH), 7.53 (t, *J* = 4.0 Hz, 1H, ArH), 7.21 (d, *J* = 3.6 Hz, 1H, ArH); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 137.8, 137.0, 128.1, 126.2, 123.8, 123.1, 122.3, 122.9; IR (KBr, υ_{max}, cm^{-1}): 3092 (C-H), 2851 (C-H), 2359 (C-H), 1931 (C-H), 1684 (C-O), 1536 (N-H), 1282 (C-N), 935 (C=C), 763 (C-Cl) cm^{-1}; Anal. calc. for $C_{12}H_7ClN_2OS_2$: C, 48.90; H, 2.39; N, 9.50; S, 21.75; Found: C, 48.82; H, 2.15; N, 9.66; S, 21.88.

N-Benzyl-3-chloro-N-methylbenzo [*b*] thiophene-2-carboxamide (3f)

Compound **3e** was prepared by following above general method from N-methyl-1-phenylmethanamine (**2d**, 0.12 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.30 g (96%) as white powder; m.p.>300 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 8.13 (d, *J* = 8.0 Hz, 1H, ArH), 7.87 (m, 1H, ArH), 7.59 (q, 2H, ArH), 7.37 (d, *J* = 16.0 Hz,4H, ArH), 4.67 (m, 2H, ArH), 2.93 (d, *J* = 8.0 Hz, 3H, CH_3), 2.55 (s, 2H, CH_2); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 162.4, 137.1, 128.1, 127.3, 126.4, 123.9, 122.4; IR (KBr, υ_{max}, cm^{-1}): 3242 (C-H), 3067 (C-H), 2854 (C-H), 1696 (C-O), 1285 (C-N), 940 (C=C), 767 (C-Cl) cm^{-1}; Anal. calc. for $C_{17}H_{14}ClNOS$: C, 64.65; H, 4.47; N, 4.44; S, 10.15; Found: C, 64.55; H, 4.30; N, 4.56; S, 10.28.

3-Chloro-N-(4-fluorobenzyl)benzo [*b*] thiophene-2-carboxamide (3g)

Compound **3g** was prepared by following above general method from (4-fluorophenyl)methanamine (**2e**, 0.12 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.31 g (97%) as white powder; m.p.= 114–119 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 9.02 (t, *J* = 4.0 Hz, 1H, NH), 8.11 (m, 1H, ArH), 7.91 (m, 1H, ArH), 7.60 (m, 2H, ArH), 7.42 (m, 2H, ArH), 7.19 (m, 2H, ArH), 4.51 (d, *J* = 4.0 Hz, 2H, CH_2); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 162.9, 160.7, 160.5, 137.1, 136.5, 135.5, 132.6, 129.7, 127.9, 126.4, 123.8, 123.0, 119.4,115.6, 115.4, 42.8; IR (KBr, υ_{max}, cm^{-1}): 3265 (C-H), 3068 (C-H), 1624 (C-O), 1510 (N-H), 1277 (C-N), 1223 (C-F), 932 (C=C), 757 (C-Cl); Anal. calc. for $C_{16}H_{11}ClFNOS$: C, 60.10; H, 3.47; N, 4.38; S, 10.03; Found: C, 60.25; H, 3.35; N, 4.42; S, 10.11.

Methyl4-((3-chlorobenzo [*b*] thiophene-2-carboxamido)methyl) benzoate (3h)

Compound **3h** was prepared by following above general method from methyl 4-(aminomethyl)benzoate (**2f**, 0.16 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.33 g (97%) as white powder; m.p.= 143–146 °C; ^{1}H NMR (400 MHz. DMSO-d_6) δ(ppm) 9.10 (t, *J* = 4.0 Hz, 1H, NH), 8.12 (m, 1H, ArH), 7.96 (d, *J* = 8.0 Hz, 2H, ArH), 7.92 (m, 1H, ArH), 7.61 (m, 2H, ArH), 7.52 (d, 2H, *J* = 8.0 Hz, ArH), 4.61 (d, *J* = 4.0

Hz, 2H, CH_2), 3.85 (s, 3H, CH_3); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 166.5, 160.9, 145.0, 137.1, 136.5, 132.4, 129.6, 128.7, 127.9, 126.4, 123.8, 123.0, 119.6; IR (KBr, υ_{max}, cm^{-1}): 3338 (C-H), 3052 (C-H), 1723 (C=O ester), 1626 (C-O), 1509 (N-H), 1273 (C-N), 917 (C=C), 775 (C-Cl); Anal. calc. for $C_{18}H_{14}ClNO_3S$: C, 60.08; H, 3.92; N, 3.89; S, 8.91; Found: C, 60.20; H, 3.85; N, 3.98; S, 8.81.

N-((1*H*-Benzo [*d*] imidazol-2-yl)methyl)-3-chlorobenzo [*b*] thiophene-2-carboxamide (3i)

Compound **3i** was prepared by following above general method from (1H-benzo[*d*]imidazol-2-yl)methanamine (**2 g**, 0.14 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.31 g (96%) as white powder; m.p.= 220–223 °C; ^{1}H NMR (400 MHz. DMSO-d_6) δ(ppm): 8.98 (s, 1H, NH), 8.13 (m, 1H, NH), 7.61 (m, 3H, ArH), 7.52 (m, 2H, ArH), 7.16 (m, 3H, ArH), 4.77 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ(ppm) 160.9, 152.1, 137.3, 136.6, 132.4, 128.1, 126.4, 123.8, 123.1, 121.9, 119.9; IR (KBr, υ_{max}, cm^{-1}): 3052 (C-H), 1723 (C=O ester), 1626 (C-O), 1509 (C=N), 1496 (N-H), 1275 (C-N), 919 (C=C), 754 (C-Cl) Anal. calc. for $C_{17}H_{12}ClN_3OS$: C, 59.74; H, 3.54; N, 12.29; S, 9.38; Found: C, 59.66; H, 3.40; N, 12.36; S, 9.49.

3-Chloro-N-(3-methoxybenzyl)benzo [*b*] thiophene-2-carboxamide (3j)

Compound **3j** was prepared by following above general method from (3-methoxyphenyl)methanamine (**2h**, 0.13 g, 1 mmol), 3-chlorobenzo [*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.30 g (96%) as white powder; m.p.= 120–124 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 9.03 (t, 1H, J = 4.0 Hz, NH), 8.11 (m, 1H, ArH), 7.92 (m, 1H, ArH), 7.60 (m, 2H, ArH), 7.27 (q, 1H, ArH), 6.95 (m, 2H, ArH), 6.85 (m, 1H, ArH), 4.51 (d, J = 4.0 Hz, 2H, CH_2), 3.37 (s, 3H, OCH_3); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 160.7, 159.8, 140.9, 137.1, 136.4, 132.6, 129.9, 127.9, 126.4, 123.8, 123.0, 119.8, 119.3, 113.2, 112.80, 55.4, 43.3; IR (KBr, υ_{max}, cm^{-1}): 3318 (C-H), 3000 (C-H), 1632 (C-O), 1507 (N-H), 1283 (C-N), 924 (C=C), 747 (C-Cl); Anal. calc. for $C_{17}H_{14}ClNO_2S$: C, 61.54; H, 4.25; N, 4.22; S, 9.66; Found: C, 61.33; H, 4.32; N, 4.11; S, 9.79.

3-Chloro-N-((1R,4R)−4-hydroxycyclohexyl)benzo [*b*] thiophene-2-carboxamide (3k)

Compound **3k** was prepared by following above general method from (1R,4R)−4-aminocyclohexan-1-ol (**2i**, 0.11 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.27 g (93%) as white powder; m.p.= 209–212 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 8.25 (d, J = 7.6 Hz, 1H, NH), 8.06 (d, J = 4.4 Hz, 1H, ArH), 7.86 (d, J = 4.8 Hz, 1H, Ar-H), 7.57 (t, J = 7.6 Hz, 2H), 4.78 (d, J = 3.6 Hz, 1H, OH), 3.41 (s, 2H, CH_2), 2.50 (s, 2H, CH_2) 1.86 (d, J = 9.6 Hz, 5H, CH_2); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 136.9, 136.3, 132.8, 127.8, 126.4, 122.9, 119.0, 68.5, 48.9, 34.1, 30.2; IR (KBr, υ_{max}, cm^{-1}): 3291 (C-H), 3055 (C-H), 2929 (C-H), 2872 (C-H), 1948 (C-H overtone), 1625 (C-O), 1551 (N-H), 1513 (C=C), 1315 (C-N Ar amine), 1081 (C-O primary alcohol), 935 (C=C), 751 (C-Cl) cm^{-1}; Anal. calc. for $C_{15}H_{16}ClNO_2S$: C, 58.15; H, 5.21; N, 4.52; S, 10.35; Found: C, 58.30; H, 5.10; N, 4.44; S, 10.22.

(3-Chlorobenzo [*b*] thiophen-2-yl)(4-methylpiperazin-1-yl) methanone (3l) [43]

Compound **3l** was prepared by following above general method from 1-methylpiperazine (**2j**, 0.10 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.27 g (93%) as white powder; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 8.07 (d, J = 7.6 Hz, 1H, ArH), 7.83 (d, J = 6.8 Hz, 1H, ArH), 7.56 (m, 2H, ArH), 3.36 (s, 1H), 2.40 (s, 2H), 2.34 (s, 4H), 2.17 (s, 1H).

3-Chloro-N-((tetrahydrofuran-2-yl)methyl)benzo [*b*] thiophene-2-carboxamide (3m)

Compound **3m** was prepared by following above general method from (tetrahydrofuran-2-yl)methanamine (**2k**, 0.10 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.27 g (93%) as white powder; m.p.= 100–104 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ(ppm) 8.35 (t, J = 5.2 Hz, 1H, NH), 8.05 (q, 1H, Ar-H), 7.87 (q, 1H, Ar-H), 7,57 (q, 2H, Ar-H), 4.02 (q, 1H), 3.81 (m, 2H), 3.38 (t, J = 5.6 Hz, 2H), 1.93 (m, 1H), 1.85 (m, 2H), 1.61 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ(ppm) 160.8, 137.1, 136.4, 132.7, 127.9, 126.4, 123.7, 123.0, 119.1, 77.2, 67.8, 43.8, 28.9, 25.6; IR (KBr, υ_{max}, cm^{-1}): 3249 (C-H), 2978 (C-H), 2340 (C-H), 1969 (C-H), 1643 (C-O), 1273 (C-N Ar), 1236 (C-N Aliphatic), 1432 (C=C), 1168 (C=C), 761 (C-Cl) cm^{-1}; Anal. calc. for $C_{14}H_{14}ClNO_2S$: C, 56.85; H, 4.77; N, 4.74; S, 10.84; Found: C, 56.72; H, 4.90; N, 4.905; S, 10.71.

(3-Chloro-N-(3-(dimethylamino)propyl)benzo [*b*] thiophene-2-carboxamide) (3n) [44]

Compound **3m** was prepared by following above general method from N^1,N^1-dimethylpropane-1,3-diamine (**2l**, 0.10 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.25 g (86%) as white powder; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 8.30 (s, 1H, NH), 8.05 (q, 1H, Ar-H), 7.88 (q, 1H, Ar-H), 7.57 (q, 2H, Ar-H), 3.40 (d, J = 4.4 Hz, 2H), 2.47 (s, 2H) 2.44 (t, J = 6.8 Hz, 2H), 2.19 (s, 6H).

(*R*)−3-Chloro-N-(2-hydroxypropyl)benzo [*b*] thiophene-2-carboxamide (3o)

Compound **3o** was prepared by following above general method from (*S*)-1-aminopropan-2-ol (**2m**, 0.075 g, 1 mmol), 3-chlorobenzo[*b*] thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.25 g (96%) as white powder; m.p.> 300 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 8.24 (t, J = 5.2 Hz, 1H, NH), 8.05 (q, 1H, ArH), 7.87 (q, 1H, ArH), 7.56 (q, 2H, ArH), 5.02 (d, J = 3.6 Hz,1H, OH), 3.83 (d, J = 5.2 Hz, 1H), 3.29 (m, 2H), 1.11 (d, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 160.7, 137.1, 136.5, 132.9, 127.9, 126.3, 123.7, 123.0, 118.9, 65.3, 47.4, 21.4; IR (KBr, υ_{max}, cm^{-1}): 3059 (C-H), 2956 (C-H), 2359 (C-H), 1945 (C-H overtone), 1613 (C-O), 1547 (N-H), 1429 (C=C), 1075 (C-O secondary alcohol), 934 (C=C), 753 (C-Cl) cm^{-1}; Anal. calc. for $C_{12}H_{12}ClNO_2S$: C, 53.43; H, 4.48; N, 5.19; S, 11.89; Found: C, 53.20; H, 4.59; N, 5.05; S, 11.98.

3-Chloro-N-(2,4,4-trimethylpentan-2-yl)benzo [*b*] thiophene-2-carboxamide (3p)

Compound **3p** was prepared by following above general method from 2,4,4-trimethylpentan-2-amine (**2n**, 0.12 g, 1 mmol), 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a**, 0.23 g, 1 mmol) and TEA (0.20 ml, 1.5 mmol). Yield: 0.28 g (93%) as white powder; m.p.= 105–107 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 8.02 (m, 1H, NH), 7.80 (m, 2H, ArH), 7.51 (m, 2H, ArH), 1.79 (s, 2H, CH_2), 1.37 (s, 6H), 0.94 (s, 9H); ^{13}C NMR (100 MHz. DMSO-d_6): δ(ppm) 160.0, 136.7, 127.6, 126.3, 123.8, 122.7, 118.0, 55.8, 50.4, 31.9, 31.6, 29.6; IR (KBr, υ_{max}, cm^{-1}): 3327 (C-H), 3056 (C-H), 1643 (C-O), 1503 (N-H), 1292 (C-N), 924 (C=C), 755 (C-Cl); Anal. calc. for $C_{17}H_{22}ClNOS$: C, 63.04; H, 6.85; N, 4.32; S, 9.90; Found: C, 63.15; H, 6.75; N, 4.25; S, 9.77.

2.3. α-Amylase inhibition assay

The *in vitro* α-amylase inhibition study was carried out by following our previously reported test procedure [40]. Briefly, stock solutions (1 mg/mL) of synthesized compounds (**3a-p**) and α-amylase (barley malt procured from HIMEDIA) were prepared in DMSO. A mixture of 200 μL of α-amylase, 20 mM sodium phosphate buffer (pH 6.9) and 200 μL of test solution (50, 100, 150, 200, and 250 μg/mL) were incubated at 30 °C for 10 min. Further, 200 μL of the starch solution (1 % w/v) in deionized water was added to each tube, and the tubes were then incubated for 10 min at 30 °C. The reagent DNSA (3,5-Dinitrosalicylic acid) (200 μL) was added and incubated for further five minutes at 85–87 °C, cooled to room temperature and deionized water (5 mL) was added. The absorbance of the resulting solution was measured at 540 nm using a UV–Visible spectrophotometer and compared with blank and control solutions. The % inhibition of α-amylase was calculated by following formula:

$$\%\ \boldsymbol{\alpha}-\textbf{amylase inhibition} = 100\ \mathbf{X}\ \frac{\mathbf{A_{Control}} - \mathbf{A_{Sample}}}{\mathbf{A_{Control}}}$$

The IC_{50} values were determined as mean ± SD in triplicates from a non-linear regression graph using Graph Pad Prism software that showed concentrations (y-axis) versus percentage inhibition on (x-axis).

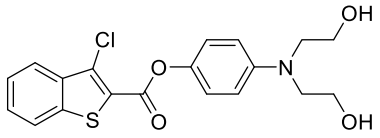
2.4. Enzyme kinetic studies

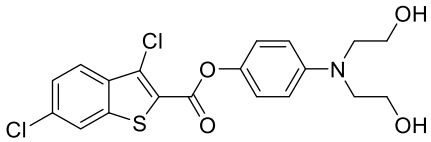
In order to study the mode of inhibition for α-amylase enzyme, the most active compound **3b** was investigated with different concentration of starch as a substrate (1–4 μM) in the absence and presence of (**3b**) at different concentration (0, 1.3, 2.6, 3.9 and 5.2 mM). A Lineweaver-Burk plot [45–47] was generated to identify the mode of inhibition and the Michaelis-Menten constant (K_m) value was determined from the plot between the reciprocal of the subtract concentration (1/[S]) and reciprocal of the enzyme rate (1/V) over the various inhibitor concentrations. The experimental inhibitor constant (K_i) value was obtained from secondary plots of the inhibitor concentration (**3b**) versus K_m. Graphs was plotted and Km and K_i value obtained directly from the software Graph Pad Prism 5.

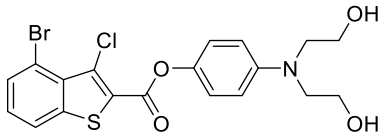
2.5. Computational studies

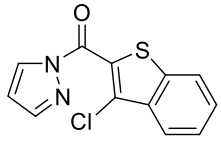
The interactions of the synthesized compounds **3a-p** within the active site of the crystal structure of barley (malt) α-amylase (PDB 1RPK) [48] obtained from the Protein Data Bank [49] were studied by molecular docking using *AutoDock Vina* (*v. 1.2.5*) [50]. The protein was prepared for docking using *UCSF Chimera (v. 1.17.3*) [51], where all solvent and ions were deleted, the highest occupancy conformations were retained, incomplete side chains were replaced using Dunbrack 2010 rotamer library [52], hydrogens were added to complete the valences and Kollman charges were added to the protein. The docking grid with 20 Å extents in x, y, and z directions was defined around the co-crystallized ligand Acarbose. The synthesized compounds and bound molecule Acarbose were prepared for docking using *Openbabel* (*v. 3.1.1*) [53], where the 3D conformations were generated, hydrogens were added to complete the valences at pH 7.4 and necessary ionization states, subsequently adding the EEM partial charges [54] to the structures. The interactions of the inhibits protein complexes were mapped using *Protein Ligand Interaction Profiler Web server* [55]. The pharmacokinetic profiling of the targeted compounds including different parameters such as drug-likeness properties, ADME and toxicity profiles was studied on a web-based server viz. pkCSM [56–58].

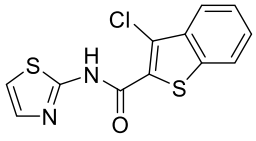
Table 1
Structure and yield of newly synthesized benzo[*b*]thiophene-2-carboxylic acid derivatives (**3a-p**).

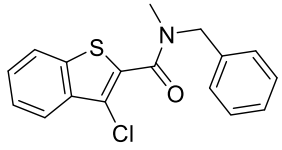

3a (97%)[a]

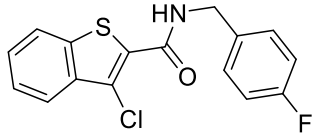

3b (92%)

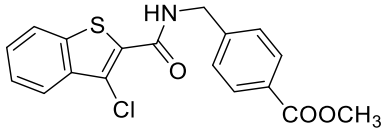

3c (95%)

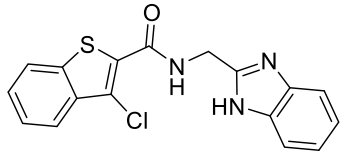

3d (96%)

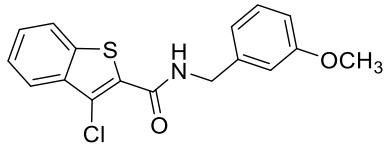

3e (89%)

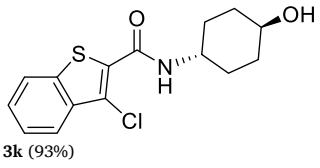

3f (96%)

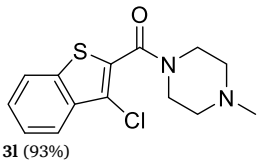

3g (97%)

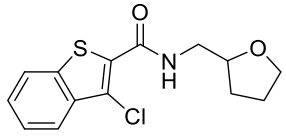

3h (97%)

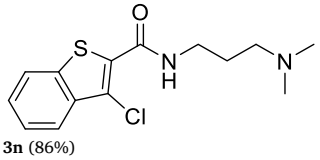

3i (96%)

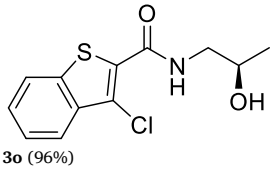

3j (96%)

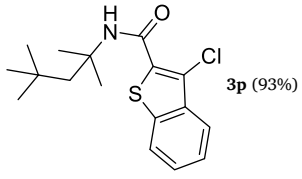

3k (93%)


3l (93%)


3m (96%)


3n (86%)


3o (96%)


3p (93%)

[a]The number in parenthesis shows the isolated yield (%) of synthesized compounds.

3. Result and discussion

3.1. Synthesis and characterization

The targeted benzo[*b*]thiophene-2-carboxylic acid derivatives were synthesized as shown in Scheme 1. Initially the required intermediates 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride (**1a-c)** were synthesized by reported synthetic method from cinnamic acid as starting material [41]. The intermediate 2,2′-((4-hydroxyphenyl)azanediyl)bis (ethan-1-ol) **(2a)** was prepared by our earlier reported method from 4-amino phenol and 2-chloroethanol [42]. Then, the coupling reaction of 3-chlorobenzo[*b*]thiophene-2-carbonyl chloride **(1a-c)** with 2, 2′-((4-hydroxyphenyl)azanediyl)bis(ethan-1-ol) **(2a)** or variety of amines **(2b-n)** was carried out in ethyl acetate using TEA as base at 0–5 °C. As expected, chlorobenzo[*b*]thiophene-2-carbonyl chloride **(1a-c)** readily reacted with 2,2′-((4-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (**2a**) along with several amines such as heteroaryl amines **(2b-c, 2g)**, benzyl amines **(2d-2f, 2h)**, cyclic amines **(2i-k)** and alkyl amines **(2l-m)** to furnish corresponding benzo[b]thiophene-2-carboxylates (**3a-c**) and carboxamide derivatives (**3d-p**) in excellent yields (89–97%) (Table 1).

The molecular structures of all newly synthesized compounds **(3a-p)** were confirmed by FTIR spectroscopy, NMR spectroscopy (^{1}H and ^{13}C NMR) and elemental analysis. The chemical shifts, H-H coupling constants and stretching frequencies of different functional groups were used to determine the structure of newly synthesized compounds. For example, the FTIR spectrum of compounds **3a** showed broad peaks at 3371 cm^{-1}, 3267 cm^{-1} which have been assigned to O-H stretching vibrations of -OH functional group. Bands ranging from 3057 cm^{-1} to 2879 cm^{-1} were identified as aromatic C-H stretching modes. The strong band at 1711 cm^{-1} in the spectrum corresponds to the C=O stretching frequency. Further, C=C stretching band appeared at 1608 cm^{-1} to 1504 cm^{-1} while the bands at 1325 cm^{-1} & 1205 cm^{-1} indicated the stretching of aromatic and aliphatic C-N respectively. The C-Cl vibrations observed 748 cm^{-1}.

In the ^{1}H NMR spectrum of **3a**, the aromatic proton of benzo[*b*] thiophene fused ring system gave three-signal pattern in the form of doublet ($J = 8$ Hz) at 8.14 δ ppm, doublet ($J = 7.6$ Hz) at 8.00 δ ppm and multiplet at 7.61 to 7.70 δ ppm. The phenyl ring protons exhibited the pattern of two doublets at 6.71 and 7.06 δ ppm with *o*-coupling constant ($J = 8.8$ Hz) confirmed the 1,4-di-substitutions on the ring. Protons of the -OH groups displayed broad signal at 4.92 δ ppm as a singlet while methylene protons of the bis(2-hydroxyethyl)amino moiety appeared as multiplet at 3.60–3.54 δ ppm and 3.44–3.41 δ ppm. Moreover, the presence of 12 distinct carbons in the aromatic part of the spectrum is consistent with the structure and carbonyl carbon appeared at 160.11 δ ppm. Further, two distinctive signals were detected at 58.61, 53.84 δ ppm attributed to the N-CH_2, O-CH_2 groups of the bis(2-hydroxyethyl) amino moiety, respectively. Additionally, the elemental analysis of **3a** supported the molecular formula of $C_{19}H_{18}ClNO_4S$.

3.2. In vitro α-Amylase inhibitory activity

Having sixteen derivatives of benzo[*b*]thiophene-2-carboxylic acid (**3a-p**) in hand, next we carried out *in vitro* α-amylase inhibition potential studies of these agents using Acarbose as reference standard. The results of the five-dose *in vitro* α-amylase inhibitions are shown in Table 2 and dose-dependent comparative % inhibition analysis is depicted in Fig. 3. As evident, several of the tested compounds (**3b, 3h-i, 3m**, and **3o**) exhibited significant α-amylase inhibition activities in a dose dependent manner with IC_{50} value ranging from 5.37 to 15.02 μM. Particularly, benzo[*b*]thiophene-2-carboxylate (**3b**) reveled to be more potent ($IC_{50} = 5.37 \pm 0.25$ μM) than Acarbose standard ($IC_{50} = 6.40 \pm 0.14$ μM) under given conditions. Likewise, among the carboxamide thiazole bearing benzo[*b*]thiophene-2-carboxamide (**3o**) turned out as potent derivative with IC_{50} (7.56 ± 0.31 μM) comparable with Acarbose.

Table 2
In vitro α-amylase inhibitory activity of the synthesized compounds (**3a-p**).

Compound	Inhibition of α-amylase(%)[a] *In Vitro* Dose(μg/mL)					IC_{50} (μM)
	50	100	150	200	250	
3a	19.70 ±1.03	29.74 ± 0.93	42.71 ± 0.83	50.09 ± 0.86	54.70 ± 1.01	19.95 ± 0.32
3b	50.09 ± 0.64	60.80 ± 1.02	71.02 ± 0.69	80.78 ± 0.21	85.79 ± 1.04	5.37 ± 0.25
3c	24.62 ± 0.46	30.78 ± 1.14	35.61 ± 0.97	45.93 ± 0.78	54.45 ± 0.99	19.20 ± 0.23
3d	39.02 ± 0.67	46.50 ± 1.22	50.38 ± 1.03	58.71 ± 1.69	63.07 ± 0.58	20.08 ± 1.50
3e	50.76 ± 0.78	61.74 ± 0.84	73.20 ± 1.20	81.53 ± 1.06	90.34 ± 0.78	7.56 ± 0.31
3f	34.19 ± 1.09	38.83 ± 0.41	46.78 ± 1.06	50.76 ± 0.85	60.32 ± 0.37	24.03 ± 0.45
3 g	28.31 ± 0.24	33.62 ± 0.83	40.34 ± 0.11	48.11 ± 0.29	57.10 ± 1.08	26.40 ± 0.21
3h	39.58 ± 0.56	45.17 ± 0.41	52.37 ± 0.08	59.66 ± 0.35	65.15 ± 0.73	14.97 ± 0.18
3i	39.11 ± 1.04	45.45 ± 0.25	53.69 ± 1.36	59.75 ± 0.37	64.39 ± 0.68	15.02 ± 0.61
3j	29.36 ± 0.75	36.55 ± 0.66	42.05 ± 0.64	42.06 ± 0.21	56.63 ± 0.56	26.05 ± 0.04
3k	14.58 ± 0.47	30.78 ± 0.75	45.27 ± 0.49	53.79 ± 1.21	66.10 ± 0.47	23.13 ± 0.50
3l	17.33 ± 1.29	25.95 ± 1.64	35.13 ± 0.66	46.12 ± 0.93	55.97 ± 0.52	29.89 ± 0.68
3m	36.74 ± 0.61	48.11 ± 1.07	60.13 ± 0.70	74.34 ± 0.82	81.82 ± 1.01	14.83 ± 0.46
3n	19.70 ± 1.42	31.44 ± 0.25	40.15 ± 0.55	53.60 ± 0.67	60.04 ± 0.60	25.23 ± 0.42
3o	40.06 ± 0.67	53.69 ± 0.52	65.15 ± 0.48	76.23 ± 0.85	85.70 ± 0.55	12.82 ± 0.06
3p	32.20 ± 1.71	40.53 ± 0.51	46.31 ± 0.99	55.87 ± 0.68	61.93 ± 0.84	20.30 ± 0.72
Acarbose	40.53 ± 0.35	49.05 ± 0.36	59.47 ± 0.20	68.75 ± 0.34	81.16 ± 0.12	6.40 ± 0.14

[a] Each value is the mean ± *S*. D, standard deviation.

The structure activity relationship study (SAR) revealed that among the synthesized carboxylate compounds (**3a-c**), 3,6-dichloro substituted derivatives (**3b**) found to be more potent than 3-chloro (**3a**) or 3-chloro 4-bromo compounds (**3c**). Comparison of inhibition activities of benzo [*b*]thiophene-2-carboxamide (**3d-p**) showed that carboxamide of benzyl amines when substituted with -COOMe group increased the activity ($IC_{50} = 14.97 \pm 0.18$ μM) while -F or OCH_3-substitution led to significant decrease in potency with IC_{50} of 26.40 ± 0.21 μM & 26.05 ± 0.04 μM respectively. On the other hand, benzo[*b*]thiophene-2-carboxamide bearing *N*-Me benzylamine group was less potent ($IC_{50} = 24.03 \pm 0.45$ μM). Interestingly, comparison of a-amylase inhibition activities of carboxamides having benzylamines (**3f, 3g, 3j**) with heteroaryl amine derivative, it was found that benzimidazolyl group (**3i**) increased inhibition activity ($IC_{50} = 15.02 \pm 0.61$ μM). Similarly, among the pyrazole and thiazole carboxamide, the later compound (**3e**) exhibited excellent α-amylase inhibition with IC_{50} of 7.56 ± 0.31 μM. Moreover, carboxamide of alkyl amine bearing tetrahydrofuran ring (**3m**) or -OH as substituent (**3o**) showed two-fold increased α-amylase inhibition compared to their counterparts bearing cyclic amines like piperazine or cyclohexylamine functionality (**3l or 3k**). While carboxamide of alkyl amine having $N(Me)_2$ group (**3n**) or branched alkyl groups (**3p**) found to be less effective against α-amylase. These results underline the importance of -OH group on the alkyl chain of carboxamide unit. Overall, benzo[*b*]thiophene conjugation with 2,2′-((4-hydroxyphenyl)azanediyl) bis(ethan-1-ol) moiety resulted in the potent inhibition of α-amylase. This finding is in line with our previous research results in which 2-amino-4H chromene ring bearing bis(2-hydroxyethyl)amino group exhibited significant α-amylase inhibition [40]. Similar results were also obtained when pyrimidine ring was combined with bis(2-hydroxyethyl)

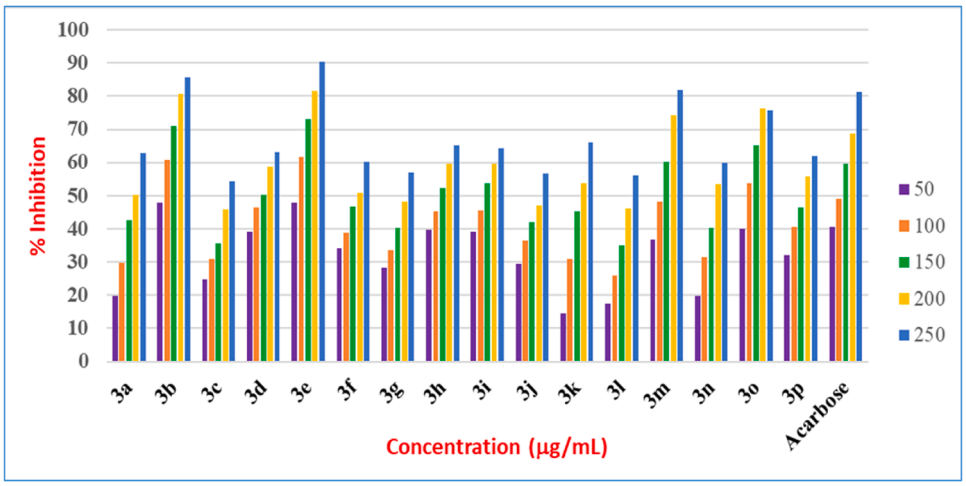


Fig. 3. Comparative analysis of % inhibition of compound **3a-p** and Acarbose.

amino moiety [59]. These corroborative results established the importance of bis(2-hydroxyethyl)amino moiety as a pharmacophore for the inhibition of α-amylase.

3.3. Kinetic mechanism of inhibition of α-Amylase

To study the mode of enzyme inhibition, a kinetics study of **3b** was carried out at varying concentration of substrate as well as inhibitor and was analyzed through Lineweaver-Burk plot [45–47]. As depicted in LB plot (Fig. 4A), upon increasing concentration of **3b**, the value of Km also found increased however Vmax remain constant. Further, the interception of inhibited enzyme and control resided at the same point on Y-axis. These results demonstrated that compound **3b** contested with the substrate to bind with the active site of α-amylase leading to the inhibition in a competitive manner. Moreover, inhibition constant was obtained from secondary plot of concentrations of inhibitor vs Km as shown in Fig. 4B. The value of inhibition constant K_i for compound **3b** was found to be 1.76 mM with r^2 value of 0.9737. Thus, *in vitro* results and enzyme kinetic study has regarded **3b** as potent competitive inhibitor of α-amylase.

3.4. In silico studies

Nowadays, investigation of binding mode of ligands with enzyme active sites through molecular docking has become an integral part of drug discovery processes [50–52]. Hence, a detailed computational analysis was carried out to evaluate a-amylase-binding interactions, assessing physicochemical, pharmacokinetic, and toxicity characteristics of the synthesized compounds **3a-p**.

3.4.1. Molecular docking

To evaluate their potential binding mode and interactions, the synthesized compounds (**3a-p**) were examined for molecular docking simulation with the binding sites of α-amylase (PDB ID: 1rpk). The resultant, binding energy of compounds (**3a-p**) and docking pose of compound **3b, 3e** & **3o** along with Acarbose within the active site of α-amylase is represented in Table 3 and Fig. 5. The docking analysis revealed that, majority of synthesized benzo[*b*]thiophene derivatives effectively bind with the active site with similar range of docking score (7-8 kcal/mol) as of Acarbose. Further, protein binding interactions of **3b** and **3e** are enlisted in Table 4. As compared to Acarbose, besides H-bond interactions (His93, Arg178, His290, Arg183), these derivatives were able to produce additional interactions in the form of π-Stacking (Trp207) and hydrophobic binding with Phe181, Trp207, Trp299 within docking site of a-amylase. Thus, low docking scores along with favorable binding affinity of active structures corroborated *in vitro* results indicating a stable enzyme-ligand complex formation by these agents (**3b** and **3e**) resulted in potent α-amylase inhibition.

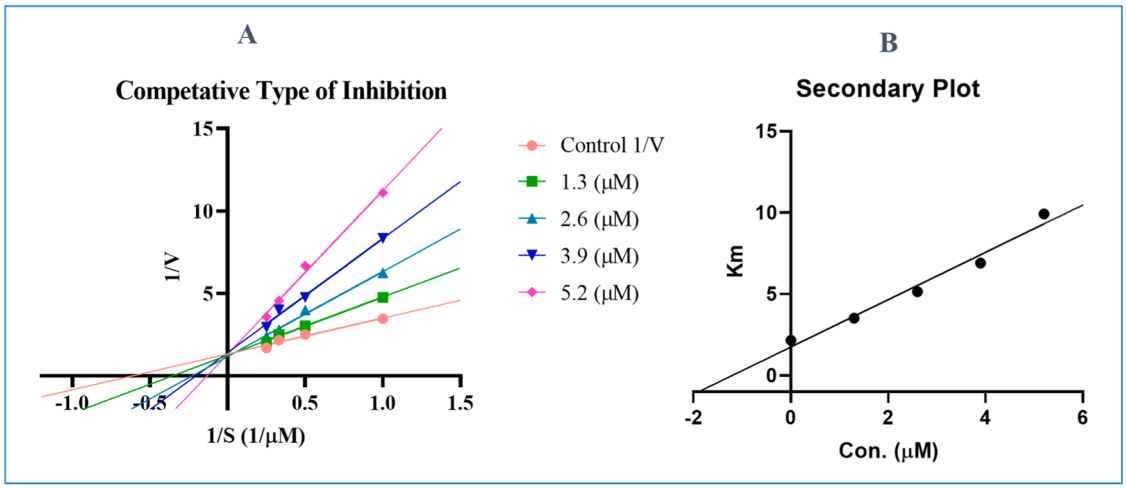


Fig. 4. Enzyme kinetics of α-amylase inhibition by **3b**. (**A**) The Lineweaver–Burk plot in the absence and presence of different concentrations of the **3b**; (**B**) The secondary plot between Km and various concentrations of **3b**.

Table 3
The docking scores for the synthesized compounds **3a-p** and **Acarbose** with α-amylase.

Compound	Docking Score (kcal/mol)	Compound	Docking Score (kcal/mol)
3a	-7.7	**3i**	-7.3
3b	-8.5	**3j**	-6.8
3c	-8.0	**3k**	-7.2
3d	-6.7	**3l**	-6.8
3e	-7.9	**3m**	-7.3
3f	-7.7	**3n**	-6.6
3g	-6.3	**3o**	-6.8
3h	-6.3	**3p**	-7.7
		Acarbose	-7.75

Post docking the protein-inhibitor complexes were subjected to *100 ns* molecular dynamics simulations to establish the stability or change in interactions which were gauged with RMSD (Fig. 6), RMSF (Fig. 7) and interaction energies (Fig. 8) were calculated for the *100 ns* trajectory snapshots. During the simulations it was observed that the protein backbone RMSD ranged between 0.7 and 1.5 Å with SD ± 2.0 Å indicative of very low motion from the initial positions during simulations. The bound inhibitors exhibited high RMSD over the initial simulation position demonstrating that the compounds moved from the initial docked position into a more snugly fit site with more interactions in-between the ligand and the protein (Table 5). The compound **3b** gradually moved by 10 Å over *20 ns* of the simulation and thereafter remained stable while **3e** exhibited motion for initial *30 ns* and subsequently remained stable after being displaced by 11 Å. Furthermore, compound **3o** remained stable in the docked pose for 60 ns before it was displaced by 10 Å into the new binding site. The changes in the interaction pattern are tabulated in Table 5. The interactions between compounds **3b, 3e**, and **3o** were extremely favorable as seen from the numbers in the Table 5 for their corresponding interactions energies.

3.4.2. *Drug-likeness and ADMET studies*

The pharmacokinetic characteristic properties of synthesized compounds were assessed using the web server pkCSM [55,56]. Several molecular properties have been explored for drug likeness prediction, including MW (mass) ≤ 500, number of hydrogen bond donors ≤ 5 & acceptors ≤ 10, and partition coefficient (LogP) ≤ 5. As demonstrated in Table 6, all the evaluated compounds (**3a-p**) exhibited strong correlations with drug-likeness properties, with no violations Lipinski rule was observed whereas Acarbose control group exhibited three violations. It is noteworthy that stand out drug likeness parameters of **3b** such as higher polar surface area, LogP, rotatable bonds, H-acceptor/donor bonds along with low molecular weight might be responsible for-α-amylase inhibitory activity. Moreover, P450 (CYP) enzyme inhibition has been linked to drug-induced toxicity and poor drug elimination [60]. Hence, *in silico* analyses of the majority of active substances involving CYP450 may offer their drug transformation and excretion routes from the body. The present *in silico* investigation toward the inhibition of the cytochrome P450 using most active compounds (**3b, 3e** & **3o**) is revealed in Table 7. As indicated, compound **3b** and **3e** were not substrate or inhibitor of CYP2D6 and showed no potential to inhibit P450 (CYP) enzyme except for the isoform CYP2C9. The findings suggested that compounds **3b** and **3e**, which have minimal P450 isoform

Table 4
List of interactions of the active compound **3b & 3o** with α-amylase.

Interactions with the target	α-amylase		
	3b	**3e**	**Acarbose**
Hydrophobic Interactions	Phe181, Trp207, Trp299	Phe144, Phe181	—
H-bond Interactions	His93, Arg178, Asp180, His290	Arg183	His93, Arg178, Asp180, Arg183, Glu205, Asn209, His290
π-Stacking	Trp207	Trp207	—

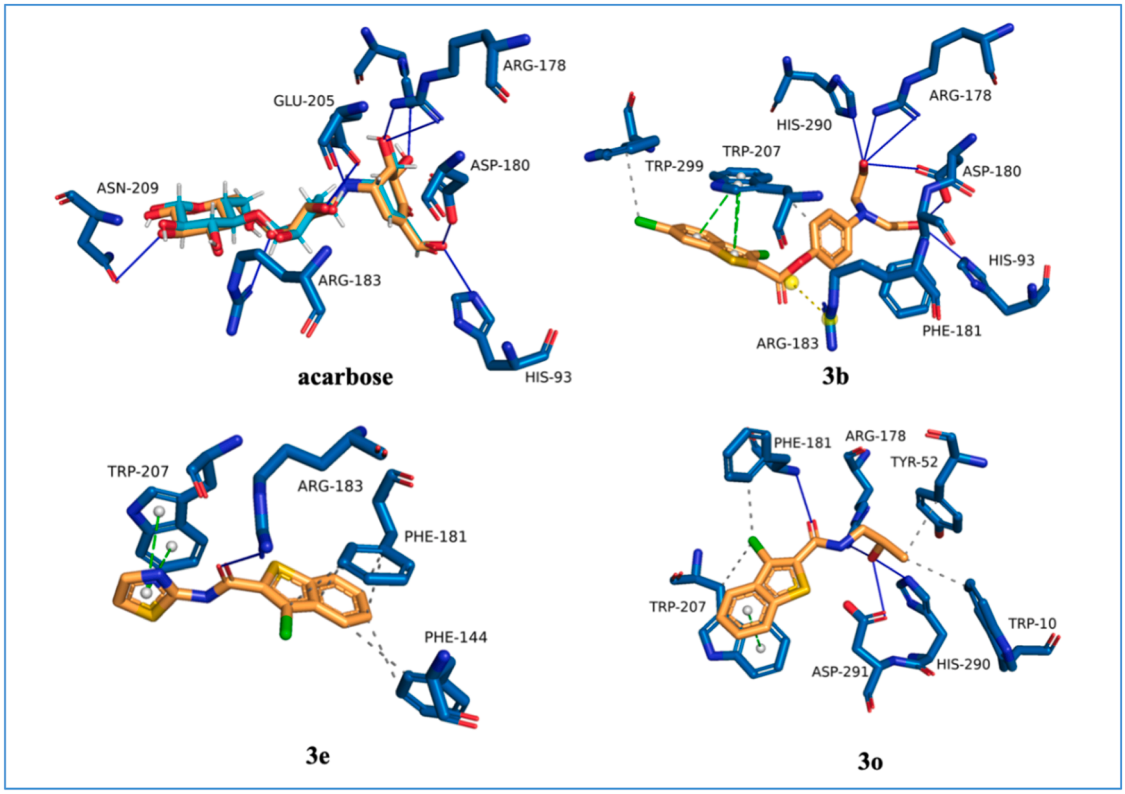


Fig. 5. Binding pose and interactions of the compounds (**3b, 3e, 3o** and **Acarbose**) with α-amylase binding site (PDB ID:1rpk).

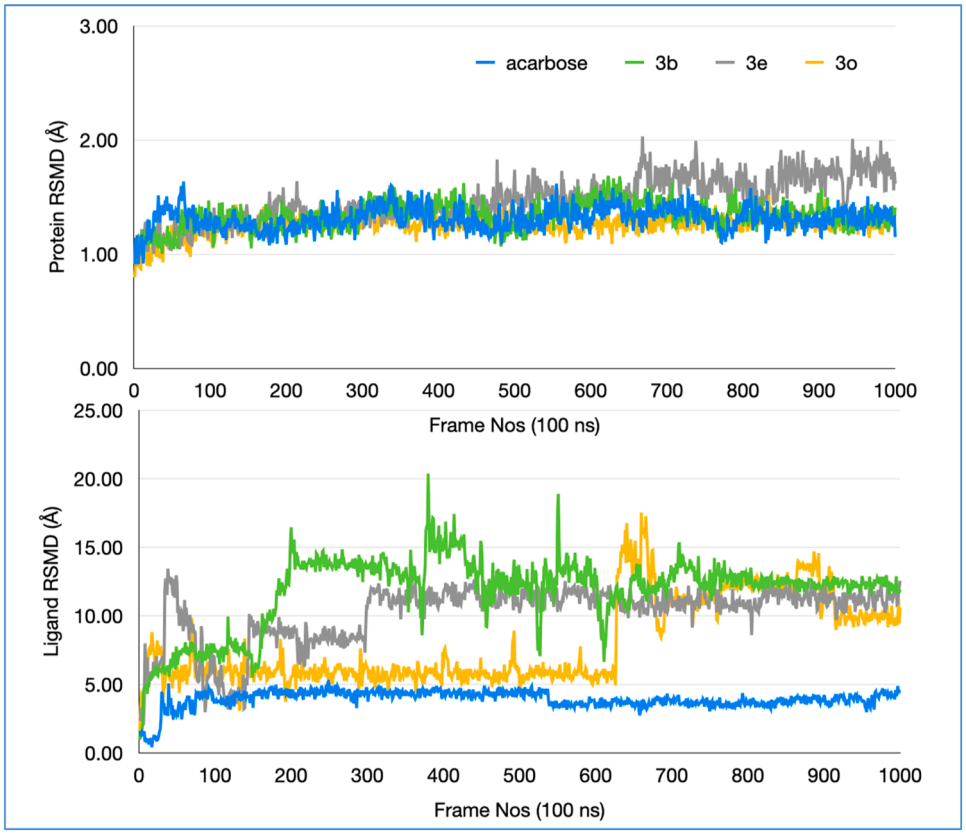


Fig. 6. Plot of RMSD of the ligand and protein backbone in complex with four ligands.

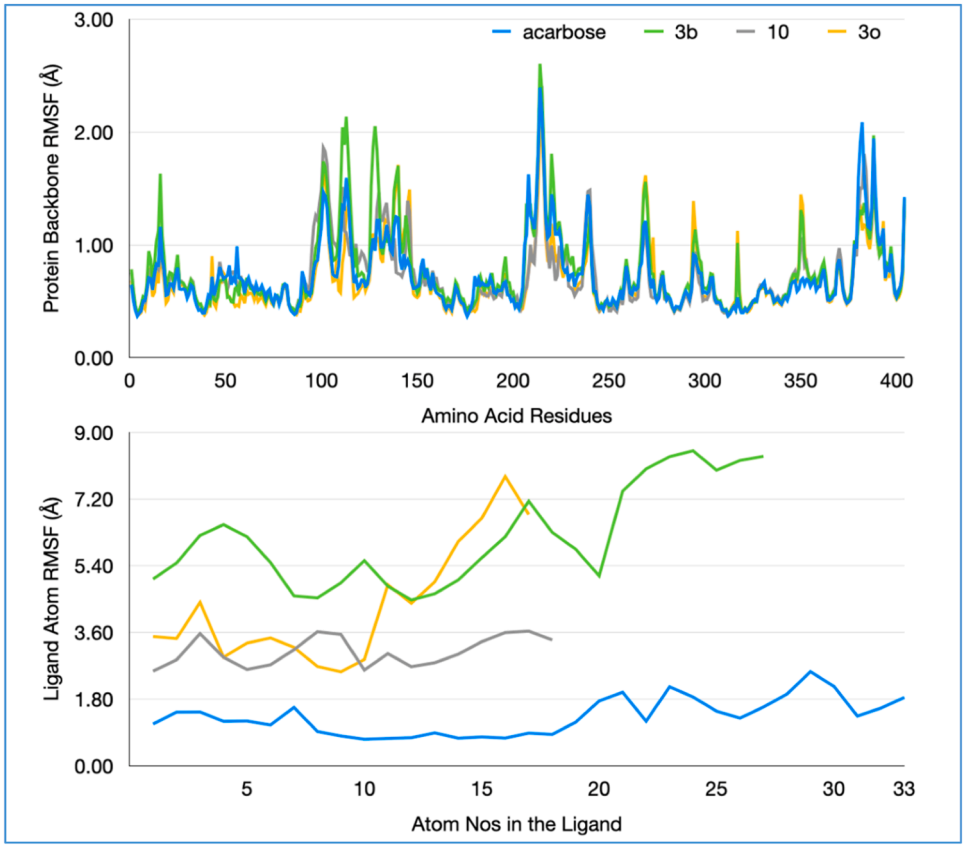


Fig. 7. Plot of RMSF of ligand atoms and the protein backbone in complex with four ligands.

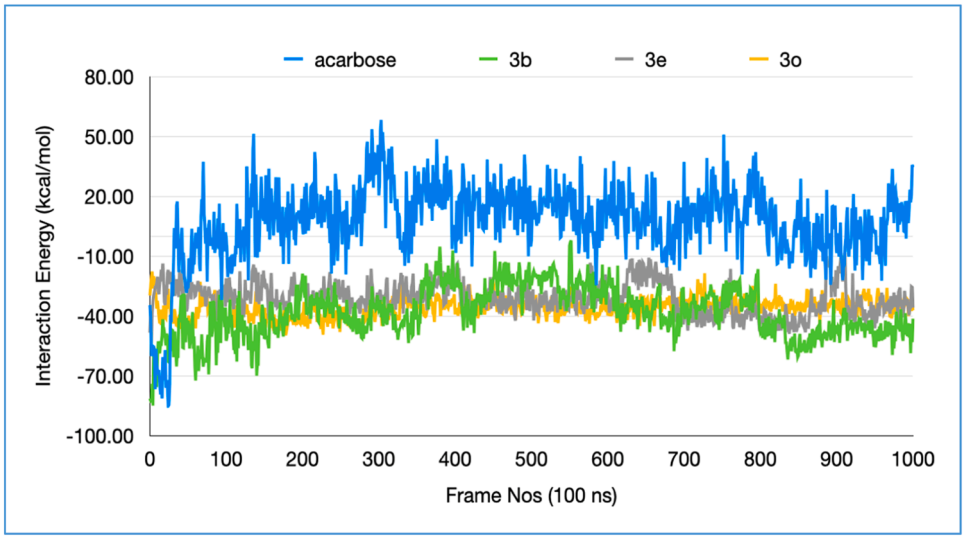
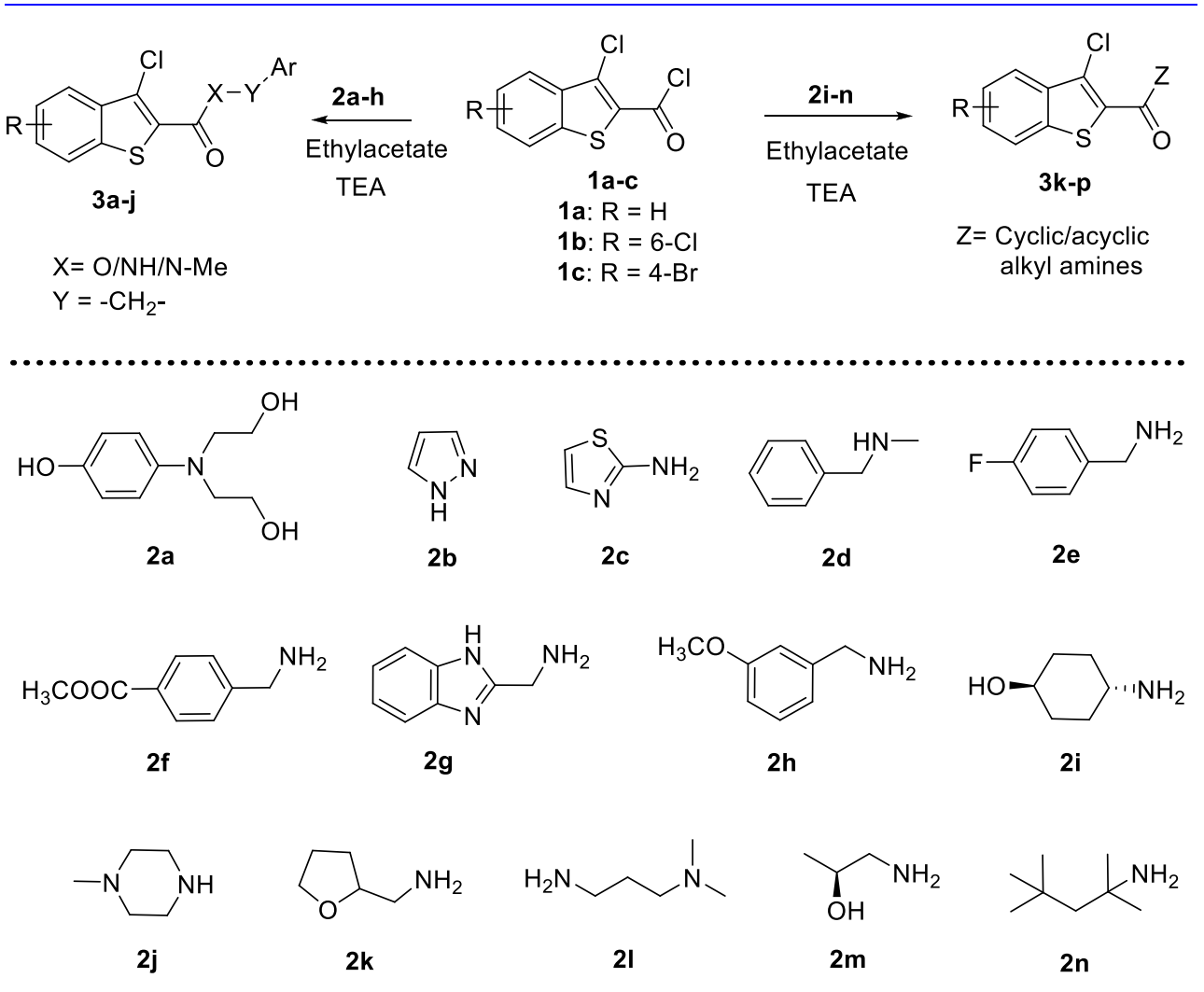


Fig. 8. Plot of interaction energies for protein backbone in complex with ligands throughout 100 ns molecular dynamics simulations.



Scheme 1. Synthesis of various new benzo[*b*]thiophene-2-carboxylic acid derivatives (**3a-p**).

Table 5
RMSD (Å), RMSF (Å) and interaction energy (Kcal/mol) calculated for the protein-inhibitor complexes for trajectory snapshots captured over 100 ns molecular dynamics simulations.

Parameters		Acarbose	3b	3e	3o
Protein Backbon RMSD	Min	0.91	0.92	0.80	0.91
	Max	1.64	1.69	1.53	2.03
	Average	1.31	1.34	1.26	1.47
	SD	0.10	0.12	0.09	0.20
Ligand RMSD	Min	0.42	0.96	1.57	2.04
	Max	5.22	20.39	17.55	13.45
	Average	3.92	11.81	8.07	10.20
	SD	0.66	2.62	3.08	2.12
Protein Backbone RMSF	Min	0.37	0.40	0.38	0.37
	Max	2.39	2.61	2.04	2.41
	Average	0.73	0.78	0.69	0.72
	SD	0.31	0.36	0.29	0.31
Ligand atom RMSF	Min	0.72	4.47	2.54	2.56
	Max	2.54	8.51	7.81	3.64
	Average	1.33	6.17	4.35	3.09
	SD	0.49	1.36	1.62	0.40
Interaction Energies	Min	-85.10	-84.92	-53.90	-49.73
	Max	58.35	-2.12	-8.39	-17.70
	Average	7.68	-38.31	-31.12	-35.90
	SD	19.40	12.52	7.86	4.89

inhibitory properties, may result in an excellent metabolic and elimination profile in the organism without drug-induced toxicity. To further evaluate the toxicity profiles of the inhibitors (**3b, 3e** & **3o**) against a variety of targets and criteria, including skin sensitization, hepatotoxicity, oral rat acute and chronic toxicity (LD_{50} & LOAEL), hERG I/II inhibition, AMES toxicity (Salmonella/microsome mutagenicity), and minnow toxicity were selected for predictions using pkCSM webserver. As illustrated in Table 8, the selected compounds did not exhibit any toxicity towards the assessed parameters or skin sensitization. Furthermore, compounds **3b, 3e** and **3o** were found to have an oral rate acute and chronic toxicity profile that was comparable to that of the standard Acarbose, with LD_{50} values ranging from 2.53 to 2.47 for synthesized compounds and 2.45 for the standard. Similarly, predicted oral rat chronic toxicity (LOAEL) for selected compounds appeared in the range of 1.31−1.92, whereas the standard was 5.32. Additionally, it was discovered that these substances did not block the genes linked to cardiac activity, hERG-I and hERG-II, suggesting that it may not have cardiotoxic profile.

4. Conclusion

In conclusion, a new series of small molecule benzo[*b*]thiophenes was discovered with potent α-amylase inhibition efficacy. The targeted benzo[*b*]thiophene-2-carboxylic acid analogues **(3a-p)** were synthesized in excellent yields and characterized by various spectroscopy methods like ^{1}H & ^{13}C NMR, FT-IR and elemental analysis. All benzo[*b*]thiophene derivatives revealed to have excellent inhibition profile against the targeted α-amylase with IC_{50} value ranging from 5.37 ± 0.25 μM to 29.89 ± 0.68 μM. Remarkably, out of the entire series, compound **3b** was found to be more potent than standard drug Acarbose with IC_{50} = 5.37 ± 0.25 μM. The enzyme kinetic study showed **3b** as competitive inhibitor of α-amylase having K_i of 1.76 mM. The molecular docking analysis & simulation study corroborated the *in vitro* findings and demonstrated that compound **3b** effectively binds within active site of α-amylase and make stable ligand-protein backbone complex. Further, *in silico* studies towards drug likeness and toxicity profiling reveled that compound **3b** possessed drug safety attributes such as no

Table 7
The predicted properties as cytochrome inhibitors for the compounds **3b, 3e & 3o**.

Compound	CYP2D6 substrate/ inhibitor	CYP1A2 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
3b	No	No	Yes	No	Yes
3e	No	Yes	Yes	No	No
3o	No	Yes	No	No	No
Acarbose	No	No	No	No	No

Table 6
The calculated drug-likeness properties for the synthesized compounds **3a-p**.

Compound	Molecular Weight	LogP	Rotatable Bonds	H-bond Acceptor	H-bond Donors	Surface Area	Lipinski Violation
3a	391.87	3.56	7	6	2	159.56	No
3b	426.32	4.21	7	6	2	169.86	No
3c	470.77	4.32	7	6	2	173.42	No
3d	262.72	3.43	1	4	0	106.24	No
3e	294.78	4.26	2	4	1	116.05	No
3f	315.82	4.82	3	2	0	132.13	No
3g	319.78	4.62	3	2	1	129.72	No
3h	359.83	4.27	4	4	1	147.56	No
3i	341.82	4.36	3	3	2	140.66	No
3j	331.82	4.49	4	3	1	137.03	No
3k	309.81	3.58	2	3	2	126.11	No
3l	294.80	2.94	1	3	0	120.92	No
3m	295.79	3.46	3	3	1	120.06	No
3n	296.82	3.23	5	3	1	121.71	No
3o	269.75	2.66	3	3	2	108.02	No
3p	323.88	5.49	3	2	1	135.05	No
Acarbose	645.61	-8.56	9	19	14.00	250.23	Yes

Table 8
The predicted toxicity properties for the compounds **3b, 3e & 3o**.

Compound	AMES toxicity	hERG I/ II inhibitor	Oral Rat Acute Toxicity (LD_{50})	Oral Rat Chronic Toxicity (LOAEL)	Hepato-toxicity	Skin Sensitization	Minnow toxicity
3b	No	No/Yes	2.53	1.31	No	No	0.39
3e	Yes	No/No	2.24	0.94	No	No	0.57
3o	No	No/No	2.47	1.95	No	No	0.58
Acarbose	No	No /Yes	2.45	5.32	No	No	16.82

violation of Lipinski rule, poor inhibitor/substrate profile against P450 (CYP) enzyme and showed no toxicity against variety of targets (AMES, hERG I, hepato, skin or minnow). Thus, our study disclosed a small molecule benzo[*b*]thiophene derivative **3b** as potential antidiabetic agent with favorable drug safety profile which warrant its further pre-clinical studies.

CRediT authorship contribution statement

Rupal J. Joshi: Methodology, Conceptualization. **Monil P. Dholariya:** Methodology, Formal analysis, Data curation. **Savankumar R. Chothani:** Methodology, Investigation. **Chirag A. Chamakiya:** Data curation. **Hardik L. Varu:** Validation, Software. **Manisha B. Karmur:** Visualization. **Deepika Maliwal:** Validation, Software. **Raghuvir R.S. Pissurlenkar:** Software, Validation. **Atul H. Bapodra:** Supervision, Project administration. **Anilkumar S. Patel:** Methodology, Formal analysis, Data curation. **Naval P. Kapuriya:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data has been submitted.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2024.138570.

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