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Solvent-free synthesis, biological evaluation and *in silico* studies of novel 2-amino-7-(bis(2-hydroxyethyl)amino)-4H-chromene-3-carbonitrile derivatives as potential a-amylase inhibitors

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ABSTRACT

Despite a wide range of kinase inhibitory activities exhibited by 2-amino-4H-chromenes, their potential application towards α-amylase inhibition remained rarely explored to date. For that purpose, a series of new 2-amino-7-(bis(2-hydroxyethyl)amino)-4(phenyl)-4H-chromene-3-carbonitrile derivatives has been synthesized via piperidine catalyzed solvent-free protocol and evaluated for their antidiabetic activity as potential α-amylase inhibitors. The dose-dependent in vitro α-amylase inhibition study revealed that, most of these compounds exhibited significant antidiabetic activity having more than 50 % α-amylase inhibition at the dose of 10 μg/mL. Among these, compound **5b** was more potent than acarbose with 91 % inhibition of α-amylase and IC_{50} of 3.60 ± 0.01 μg/mL. Enzyme kinetic studies to estimate mode of inhibition showed that inhibition of α-amylase by compound **5b** was competitive type with a Ki value of 0.97 μg/mL. Further, *in silico* studies of targeted compounds reinforced the results being involved in favorable binding interactions within the active site of α-amylase. Moreover, the *in silico* predicted properties of compound **5b** regarded as a non-toxic and safer antidiabetic agent.

1. Introduction

Diabetes mellitus (DM) also termed as hyperglycemia, is a group of metabolic endocrine diseases associated with the hypofunction of pancreas gland which produces a digestive enzyme viz. insulin. The chronic disorder is often characterized by abnormal carbohydrate metabolism which leads to persistent hyper glucose level in blood plasma in both fasting and postprandial condition [1]. Generally, DM are categorized into two kinds depending on the insulin production or its abnormal functions associated with the maintenance of sugar level in blood plasma [1]. The type 1 diabetic condition (DM1), formerly called insulin-dependent diabetes originates from autoimmune destruction of the pancreatic β-cells that impedes production of insulin causing complete lack of vital carbohydrate metabolizing enzymes. While type 2 diabetes (DM2), is characterized by inadequate secretion of insulin from pancreas gland or acquired insulin resistance mainly due to high quantity of carbohydrate uptake and obesity [2,3]. The occurrences of DM1 and DM2 are about 10 % and 90 % respectively among the patients [4,5]. Further, persistence diabetes mellitus also causes other severe health issues including cardiovascular diseases, neuropathy, nephropathy, retinopathy, etc. [6,7]. Consequently, over the years, diabetes remained as life threatening chronic diseases worldwide [8,9].

To control hyperglycemia in type 2 DM, it is critical to regulate α-amylase and α-glucosidase which are essential metabolic enzyme for the effective digestion of carbohydrates and glycogen to create free glucose for the absorption [10]. Among the two, α-amylase has been implicated as key catalyzing enzyme that convert the polysaccharides into small dextrins, disaccharides, and oligosaccharides via hydrolysis of the D-(1, 4) glycosidic bonds. The enzymatic reactions, consequently, increases the level of glucose molecule in the blood [11]. Therefore, retarding the absorption of glucose by inhibition of α-amylase is considered as one of the effective strategies for preventing postprandial

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hyperglycemia in type 2 DM [12]. Currently, Acarbose, Voglibose and Miglitol (Fig. 1) are FDA approved α-amylase/glucosidase inhibitors utilized for the treatment of DM2 in the clinic [13,14]. However, these antidiabetic agents are associated with several adverse effects like abdominal pain, skin reaction, flatulence, diarrhea, and abnormal liver functions [15]. Therefore, the search for efficacious and safer α-amylase inhibitors remained a key area of research.

Benzo-fused heterocyclic compounds are widely explored for their bio-applications [16–18]. Particularly, the benzopyran (chromene) moieties are ubiquitously found in a diverse array of natural and synthetic compounds bearing significant chemical and pharmaceutical applications [19,20]. For instance, 2-amino-4*H*-chromenes are endowed with broad spectrum of biological activities such as antibacterial/antifungal, anti-spasmolitic, anticoagulant antiviral, potassium channel regulator, anti-inflammatory and antidepressant (CNS) activities [21]. Recently, several new 2-amino-4H-chromene based derivatives have been reported to have potent anticancer activity [22–30] by acting as inhibitors of protein kinase [28], estrogen receptor [29], and microtubule polymerization [30]. Thus, there is considerable renewed interest towards synthesis of novel 2-amino-4*H*-chromenes based bioactive agents.

Among several synthetic strategies, multicomponent reaction techniques are considered as an effective and viable tool for producing 2-amino-4*H*-chromenes [31]. In the literature, efficient, diversified and ecofriendly methods for the synthesis of bioactive chromenes have been reported under multicomponent reaction condition employing metal catalyst, ionic liquid, aqueous media and ecofriendly acids and bases [32,33]. In the recent past, a number of properly substituted heterocyclic scaffolds, other than thiazolidinones, such as benzofuran hydrazone (A) [34], indole hydrazone (B) [35], benzotriazoles (C) [36], oxadiazole (D) [37], pyrimidine (E) [38], and benzopyran (F) [39] have been successfully employed to prepare effective and potent α-amylase inhibitors (Fig. 2). As mentioned, a wide range of kinase inhibitory activities of 2-amino-4H-chromenes analogs have been extensively investigated [22–30] but their potential towards α-amylase inhibition and antidiabetic activity remained rarely explored to date. Therefore, it was highly intriguing to utilize 2-amino-4H-chromenes as molecular framework for the rational design of α-amylase inhibitors. Further, we hypothesized that conjugation of 2-amino-4H-chromenes with bis(2-hydroxyethyl) amino pharmacophore would be beneficial since many of the known inhibitors such as Acarbose, Miglitol, Voglibose and dipyridamole are endowed with hydroxy groups or bis(2-hydroxyethyl)amino moieties (Fig. 1& 2). Hence, the bioactive 2-amino-4H-chromenes substituted with bis(2-hydroxyethyl)amino pharmacophore might provide an effective structural unit to interact and bind with the α-amylase active site. Moreover, 2-amino-4*H*-chromenes framework could be synthesized by three component MCR strategy as mentioned earlier, the method would lead to an ecofriendly approach to the targeted inhibitors.

Herein, we describe, synthesis, in vitro antidiabetic evaluation and molecular docking studies of 2-amino-7-(bis(2-hydroxyethyl)amino)−4H-chromene derivatives as new class of α-amylase inhibitors.

2. Materials and methods

2.1. Chemistry

The requisite commercial grade chemicals & solvents were procured from Sigma Aldrich and Loba chemie and are used for the reaction without further purification. The reaction monitoring and assessment of compound purity was conducted on TLC plate pre-coated with silica gel G purchased from Merck. The compound purification was accomplished over silica gel (100–200 mesh) with appropriate eluents using column chromatography. Melting points were determined on the digital apparatus. IR spectrum was recorded using Shimadzu-IR Spirit. ^{1}H & ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer using DMSO-*d6* or $CDCl_3$ as solvent. Mass spectra were taken on Shimadzu GC-MS QP2010. Elemental analysis was carried out in Elementar vario MICRO cube instrument.

2.2. Synthesis of 2,2′-((3-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (2)

A solution of 3-aminophenol (3.75 g, 34 mmol) and Na_2CO_3 (7.35 g, 68 mmol) in water (50 mL) was stirred at rt for 15 min. 2-Chloroethanol (13.78 mL, 205 mmol) was then added slowly and the reaction mixture was heated to reflux for 24 h, then cooled to 0 °C and neutralized by saturated $NaHCO_3$ solution to adjust pH 7.0–7.5. The mixture was evaporated to dryness under vacuo, and purified by column chromatography (EA: Hexane; 55:45 v/v) to give 2,2′-((3-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (**2**). Yield: 4.5 g as brown oil (67%); ^{1}H NMR (400 MHz, DMSO-d_6): ^{1}H NMR (DMSO-d_6), δ(ppm) 3.33 (t, 4H, $J = 7.8$ Hz, 2xCH_2), 3.50 (q, 4H, $J = 6.8$ Hz, 2xCH_2), 4.74 (t, 2H, $J = 6.4$ Hz, 2xOH), 6.00 (dd, 1H, $J = 8.0$ & 1.2 Hz, ArH), 6.07–6.11 (m, 2H, ArH), 6.88 (t, 1H, $J = 8.0$ Hz), 8.94 (s, 1H, ArOH). ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm) 53.53, 58.36, 98.62, 102.86, 102.92, 129.79, 149.41, 158.43; ESI-MS: *m/z* 198.2 (M^+).

2.3. General procedure for the synthesis of 2-amino-4H-chromenes derivatives (5a-i) under solvent free condition

To a mixture of 2,2′-((3-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (**2**, 1.0 mmol), appropriate aromatic aldehyde (**3a-i**, 1.0 mmol), malononitrile (**4**, 1.0 mmol) and piperidine (15 mol%) was added and the resultant reaction mixture was further stirred at room temperature for 30 min. After completion of the reaction indicated by TLC (5 % methanol in DCM), the semisolid crude product was purified by column chromatography using DCM/methanol (98:2.0; v/v) as eluent to give corresponding chromenes **5a-i**.

2-Amino-7-(bis(2-hydroxyethyl)amino)−4-phenyl-4H-chromene-3-carbonitrile (5a)

Compound **5a** was prepared from benzaldehyde (0.106 g, 1 mmol), malononitrile (0.66 g, 1 mmol) and 2,2′-((3-hydroxyphenyl)azanediyl) bis(ethan-1-ol) (0.197 g, 1.0 mmol). Yield: 315 mg (90 %); mp 164–166 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 3.31–3.37 (m, 4H, 2 × CH_2), 3.47–3.52 (m, 4H, 2 × CH_2), 4.56 (s, 1H, C—H), 4.77 (t, 2H, $J = 5.2$ Hz, OH), 6.24 (d, 1H, $J = 2.4$ Hz, ArH), 6.42 (dd, 1H, $J = 8.8$, 2.4

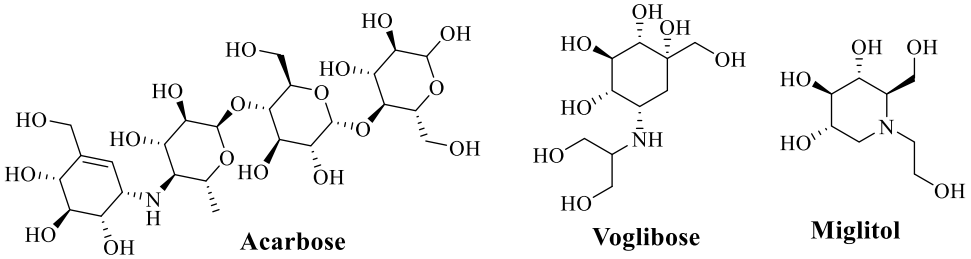


Fig. 1. Clinically used α-amylase inhibitors.

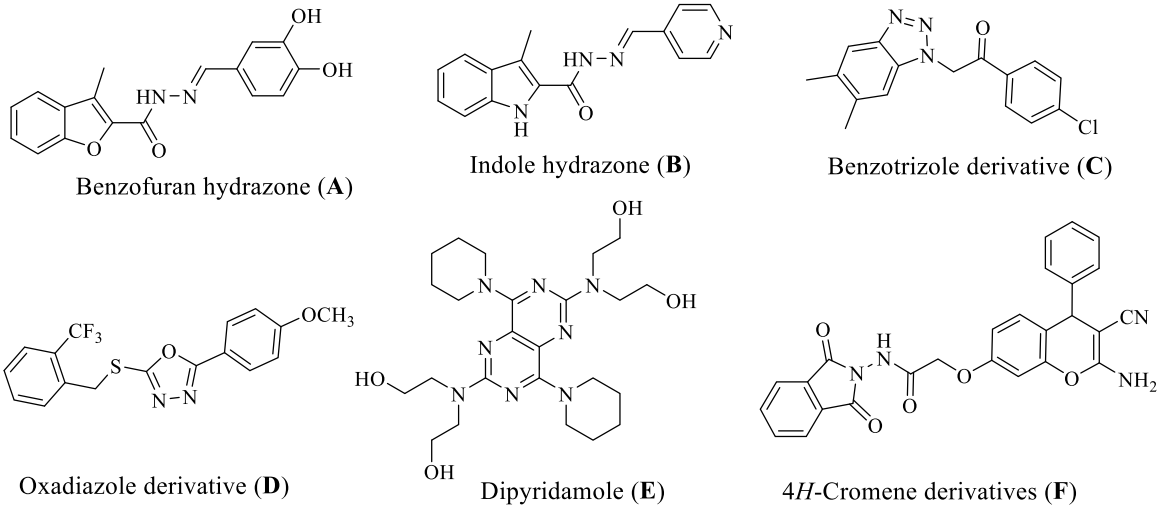


Fig. 2. Representative examples of α-amylase inhibitors based on various heterocyclic scaffolds.

Hz, ArH), 6.74 (d, 1H, $J = 8.8$ Hz, ArH), 6.80 (s, 2H, NH_2), 7.15–7.21 (m, 3H, ArH), 7.28–7.32 (m, 2H, ArH); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm) 53.69, 55.38, 56.87, 58.49, 98.09, 108.94, 110.06, 121.32, 126.98, 127.84, 128.98, 129.96, 147.13, 148.43, 149.63, 160.87; IR (KBr, $\nu_{max,}$ cm^{-1}): 3400 (N—H), 3321 (O—H), 2882 (C—H), 2193 (C≡N), 1658 (C=C), 1453 (C—H), 1319 (C—N), 1269 (C—O), 1074 (C—OH), 998 (C=C); ESI-MS: *m/z* 351 (M^+); Anal. Calculated for $C_{20}H_{21}N_3O_3$: C, 68.36; H, 6.02; N, 11.96; found: C, 68.40; H, 6.04; N, 12.01.

2-Amino-7-(bis(2-hydroxyethyl)amino)—4-(4-chlorophenyl)—4H-chromene-3-carbonitrile (5b)

Compound **5b** was prepared from 4-chloro benzaldehyde (0.140 g, 1 mmol), malononitrile (0.66 g, 1 mmol) and 2,2′-((3-hydroxyphenyl) azanediyl)bis(ethan-1-ol) (0.197 g, 1.0 mmol). Yield: 308 mg (81 %); mp 168–169 °C; ^{1}H NMR (400 MHz. DMSO-d_6): δ(ppm) 3.34 –3.38 (m, 4H, 2 × CH_2), 3.48–3.52 (m, 4H, 2 × CH_2), 4.61 (s, 1H, ArH), 4.77 (t, 2H, $J = 5.2$ Hz, 2 × OH), 6.24 (d, 1H, $J = 2.4$ Hz, ArH), 6.43 (dd, 1H, $J = 8.8$ & 2.4 Hz, ArH), 6.73 (d, 1H, $J = 8.8$ Hz, ArH), 6.85 (s, 2H, NH_2), 7.20 (d, 2H, $J = 8.8$ Hz, ArH), 7.38 (d, 2H, $J = 8.4$ Hz, ArH); ^{13}C NMR (400 MHz, DMSO-d_6): δ(ppm) 53.66, 56.47, 58.48, 98.10, 109.02, 109.46, 121.17, 128.97, 129.74, 129.94, 131.56, 146.10, 148.56, 149.59, 160.86; IR (KBr, ν_{max},cm^{-1}): 3358 (N—H), 2959 (C—H), 2197 (C≡N), 1627 (C=C), 1490 (C—H), 1306 (C—N), 1279 (C—O), 1072 (C—OH), 519 (C—Cl); ESI-MS: *m/z* 386 ($M + H$); Anal. Calculated for $C_{20}H_{20}ClN_3O_3$: C, 62.26; H, 5.22; N, 10.89; found: C, 62.37; H, 5.20; N, 10.97.

2-Amino-7-(bis(2-hydroxyethyl) amino)—4-(2-chlorophenyl)—4H-chromene-3-carbonitrile (5c)

Compound **5c** was prepared from *o*-chloro benzaldehyde (0.140 g, 1 mmol), malononitrile (0.66 g, 1 mmol) and 2,2′-((3-hydroxyphenyl) azanediyl)bis(ethan-1-ol) (0.197 g, 1.0 mmol). Yield: 320 mg (84 %); mp 170–174 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 3.35–3.38 (m, 4H, 2 × CH_2), 3.48–3.52 (m, 4H, 2 × CH_2), 4.77 (t, 2H, $J = 5.6$ Hz, 2 × OH), 5.10 (s, 1H, C—H), 6.25 (d, 1H, $J = 2.4$ Hz, ArH), 6.42 (dd, 1H, $J = 8.8$ & 2.4 Hz, ArH), 6.70 (d, 1H, $J = 8.4$ Hz, ArH), 6.87 (s, 2H, NH_2), 7.16–7.18 (m, 1H, ArH), 7.21–7.26 (m, 1H, ArH), 7.28–7.32 (m, 1H, ArH), 7.42 (d, 1H, $J = 6.8$ Hz, ArH); ^{13}C NMR (400 MHz, DMSO-d_6): δ(ppm) 53.65, 55.49, 58.48, 98.12, 108.83, 109.03, 120.95, 128.29, 128.87, 129.26, 130.07, 131.16, 132.21, 143.65, 148.67, 149.74, 161.19; IR (KBr, ν_{max}, cm^{-1}): (N—H), 3323 (O—H), 2933 (C—H), 2193 (C≡N), 1655 (C=C), 1467 (C—H), 1316 (C—N), 1267 (C—O), 1042 (C—OH), 992 (C=C), 751 (C—Cl); ESI-MS: *m/z* 385 (M); Anal. Calculated for $C_{20}H_{20}ClN_3O_3$: C, 62.26; H, 5.22; N, 10.89; found: C, 62.20; H, 5.30; N, 10.81.

2-Amino-7-(bis(2-hydroxyethyl) amino)—4-(2,4-dichlorophenyl)—4H-chromene-3-carbonitrile (5d)

Compound **5d** was prepared from 2,4-dichloro benzaldehyde (0.173 g, 1 mmol), malononitrile (0.66 g, 1 mmol) and 2,2′-((3-hydroxyphenyl) azanediyl)bis(ethan-1-ol) (0.197 g, 1.0 mmol). Yield: 350 mg (85 %); mp 155–158 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 3.35–3.38 (m, 4H, 2 × CH_2), 3.48–3.52 (m, 4H, 2 × CH_2), 4.78 (t, 2H, $J = 5.2$ Hz, 2 × OH), 5.09 (s, 1H, C—H), 6.25 (d, 1H, $J = 2.0$ Hz, ArH), 6.43 (dd, 1H, $J = 8.4$ & 2.0 Hz, ArH), 6.68 (d, 1H, $J = 8.8$ Hz, ArH), 6.92 (s, 2H, NH_2), 7.20 (d, 1H, $J = 8.4$ Hz, ArH), 7.41 (dd, 1H, $J = 8.4$ & 2.0 Hz, ArH), 7.58 (s, 1H, ArH); ^{13}C NMR (400 MHz, DMSO-d_6): δ(ppm) 53.64, 58.47, 98.12, 120.80, 129.40, 132.64, 161.22, 168.59; IR (KBr, ν_{max}, cm^{-1}): 3458 (O—H), 3328 (N—H), 2946 (C—H), 2192 (C≡N), 1651 (C=C), 1517 (N—O), 1352 (C—N), 1200 (C—O), 1045 (C—OH), 818 (C—Cl); ESI-MS: *m/z* 421 ($M + H$); Anal. Calculated for $C_{20}H_{19}Cl_2N_3O_3$: C, 57.16; H, 4.56; N, 10.00; Found: C, 57.10; H, 4.58; N, 10.09.

2-Amino-7-(bis(2-hydroxyethyl)amino)—4-(2-nitrophenyl)—4H-chromene-3-carbonitrile (5e)

Compound **5e** was prepared from 2-nitro benzaldehyde (0.151 g, 1 mmol), malononitrile (0.66 g, 1 mmol) and 2,2′-((3-hydroxyphenyl) azanediyl)bis(ethan-1-ol) (0.197 g, 1.0 mmol). Yield: 280 mg (71 %); mp 194–196 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 3.36–3.39 (m, 4H, 2 × CH_2), 3.48–3.59 (m, 4H, 2 × CH_2), 4.78 (t, 2H, $J = 5.2$ Hz, 2 × OH), 5.10 (s, 1H, C—H), 6.27 (d, 1H, $J = 2.4$ Hz, ArH), 6.45 (dd, 1H, $J = 8.8$ & 2.0 Hz, ArH), 6.73 (d, 1H, $J = 8.8$ Hz, ArH), 6.96 (s, 2H, NH_2), 7.30 (d, 1H, $J = 7.6$ Hz, ArH), 7.48 (t, 1H, $J = 7.6$ Hz, ArH), 7.67 (t, 1H, $J = 7.6$ Hz, ArH), 7.86 (d, 1H, $J = 8$ Hz, ArH); ^{13}C NMR (400 MHz, DMSO-d_6): δ(ppm) 53.63, 55.81, 58.47, 58.57, 98.21, 108.26, 109.22, 120.70, 124.05, 128.49, 129.61, 131.77, 133.91, 140.29, 148.87, 149.23, 149.74, 161.09; IR (KBr, ν_{max}, cm^{-1}): 3490 (O—H) 3356 (N—H), 2936 (C—H), 2186 (C≡N), 1655 (C=C), 1519 (N=O), 1413 (C—H), 1350 (C—N), 1274 (C—O), 1064 (C—OH), 781 (C—H); ESI-MS: *m/z* 396 (*M*+); Anal. Calculated for $C_{20}H_{20}N_4O_5$: C, 60.60; H, 5.09; N, 14.13; found: C, 60.57; H, 5.14; N, 14.09.

2-Amino-7-(bis(2-hydroxyethyl)amino)—4-(3-nitrophenyl)—4H-chromene-3-carbonitrile (5f)

Compound **5f** was prepared from 3-nitro benzaldehyde (0.151 g, 1 mmol), malononitrile (0.66 g, 1 mmol) and 2,2′-((3-hydroxyphenyl) azanediyl)bis(ethan-1-ol) (0.197 g, 1.0 mmol). Yield: 250 mg (65 %); mp 183–187 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 3.35–3.38 (m, 4H, 2 × CH_2), 3.48–3.52 (m, 4H, 2 × CH_2), 4.77 (t, 2H, $J = 5.6$ Hz, 2 × OH), 4.85 (s, 1H, C—H), 6.28 (d, 1H, $J = 2.4$ Hz, ArH), 6.45 (dd, 1H, $J = 8.8$ & 2.0 Hz, ArH), 6.79 (d, 1H, $J = 8.8$ Hz, ArH), 6.98 (s, 2H, NH_2),

7.61–7.68 (m, 2H, ArH), 8.03 (s, 1H, ArH), 8.10 (d, 1H, $J = 7.6$ Hz, ArH); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm) 53.63, 55.91, 58.48, 98.19, 108.79, 109.16, 121.02, 122.17, 122.23, 129.98, 130.77, 134.80, 148.39, 148.78, 149.40, 149.63, 161.12; IR (KBr, ν_{max}, cm^{-1}): 3530 (O—H), 3354 (N—H), 2965 (C—H), 2197 (C≡N), 1665 (C=C), 1519 (N—O), 1352 (C—N), 1223 (C—O), 1045 (C—OH); ESI-MS: *m/z* 396 (*M*+); Anal. Calculated for $C_{20}H_{20}N_4O_5$: C, 60.60; H, 5.09; N, 14.13; found: C, 60.55; H, 5.05; N, 14.15.

2-Amino-7-(bis(2-hydroxyethyl)amino)−4-(2-bromophenyl)−4H-chromene-3-carbonitrile (5 g)

Compound **5 g** was prepared from 2-bromo benzaldehyde (0.183 g, 1 mmol), malononitrile (0.66 g, 1 mmol) and 2,2′-((3-hydroxyphenyl) azanediyl)bis(ethan-1-ol) (0.197 g, 1.0 mmol). Yield: 380 mg (88 %); mp 188–191 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 3.33–3.38 (m, 4H, 2 × CH_2), 3.48–3.51 (m, 4H, 2 × CH_2), 4.77 (t, 2H, $J = 5.2$ Hz, 2 × OH), 5.12 (s, 1H, C—H), 6.25 (d, 1H, $J = 2.0$ Hz, ArH), 6.43 (dd, 1H, $J =$ 8.8 & 2.4 Hz, ArH), 6.71 (d, 1H, $J = 8.8$ Hz, ArH), 6.88 (s, 2H, NH_2), 7.12–7.17 (m, 2H, ArH), 7.36 (t, 1H, $J = 7.6$ Hz, ArH), 7.60 (d, 1H, $J =$ 8.0 Hz, ArH); ^{13}C NMR (400 MHz, DMSO-d_6): δ(ppm) 53.66, 55.76, 58.48, 98.15, 108.94, 109.07, 120.87, 122.75, 128.95, 129.17, 131.37, 133.19, 145.41, 148.69, 149.61, 161.10; IR (KBr, ν_{max}, cm^{-1}): 3460 (O—H), 3321 (N—H), 2952 (C—H), 2195 (C≡N), 1658 (C=C), 1354 (C—N), 1267 (C—O), 1042 (C—OH), 606 (C-Br); ESI-MS: *m/z* 430 (*M*+); Anal. Calculated for $C_{20}H_{20}BrN_3O_3$: C, 55.83; H, 4.69; N, 9.77; found: C, 55.79; H, 4.72; N, 9.86.

2-Amino-7-(bis(2-hydroxyethyl)amino)−4-(4-hydroxy-3-methoxyphenyl)−4H-chromene-3-carbonitrile (5 h)

Compound **5 h** was prepared from 4-hydroxy-3-methoxy benzaldehyde (0.152 g, mmol), malononitrile (0.66 g, 1 mmol) and 2,2′-((3-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (0.197 g, 1.0 mmol). Yield: 290 mg (74 %); mp 165–168 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 3.34–3.41 (m, 4H, 2 × CH_2), 3.47–3.50 (m, 4H, 2 × CH_2), 3.73 (s, 3H, OCH_3), 4.46 (s, 1H, C—H), 4.77 (t, 2H, $J = 5.6$ Hz, 2 × OH), 6.21 (d, 1H, $J = 2.0$ Hz, ArH), 6.41 (dd, 1H, 6.8 & 2.0 Hz, ArH), 6.53 (d, 1H, $J = 8.0$ Hz, ArH), 6.67– 6.78 (m, 5H, ArH), 8.83 (s, 1H, OH); ^{13}C NMR (400 MHz, DMSO-d_6): δ(ppm) 53.79, 56.10, 57.18, 58.50, 98.03, 108.83, 110.54, 112.18, 115.98, 120.14, 121.45, 129.94, 138.08, 145.65, 147.83, 148.26, 149.46, 160.75; IR (KBr, ν_{max}, cm^{-1}): 3532 (O—H), 3388 (N—H), 2962 (C—H), 2203 (C≡N), 1651 (C=C), 1516 (N—O), 1389 (O—H), 1347 (C—N), 1242 (C—O), 1123 (C—O), 1035 (C—OH); ESI-MS: *m/z* 397 (*M*+); Anal. Calculated for $C_{21}H_{23}N_3O_5$,: C, 63.47; H, 5.83; N, 10.57; Found: C, 63.53; H, 5.80; N, 10.52.

2-Amino-7-(bis(2-hydroxyethyl)amino)−4-(3,4,5-trimethoxyphenyl)−4H-chromene-3-carbonitrile (5i)

Compound **5i** was prepared from 3,4,5-trimethoxy benzaldehyde (0.196 g, 1 mmol), malononitrile (0.66 g, 1 mmol) and 2,2′-((3-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (0.197 g, 1.0 mmol). Yield: 370 mg (84 %); mp 162–165 °C; ^{1}H NMR (400 MHz, DMSO- d_6): δ(ppm) 3.35–3.37 (m, 4H, 2 × CH_2), 3.48–3.52 (m, 4H, 2 × CH_2), 3.62 (s, 3H, OCH_3), 3.75 (s, 6H, 2 × OCH_3), 4.54 (s, 1H, C—H), 4.78 (t, 2H, $J = 5.2$ Hz, 2 × OH), 6.22 (d, 1H, $J = 2.4$ Hz, ArH), 6.44 (dd, 1H, $J = 8.8$ & 2.8 Hz, ArH), 6.49 (s, 2H, NH_2), 6.82 (s, 2H, ArH), 6.85 (s, 1H, ArH); ^{13}C NMR (400 MHz, DMSO-d_6): δ(ppm) 53.67, 56.31, 56.45, 58.51, 60.39, 63.26, 98.10, 105.07, 108.89, 109.87, 121.37, 129.87, 136.63, 142.67, 148.42, 149.39, 153.35, 161.04; IR (KBr, ν_{max}, cm^{-1}): 3617 (O—H), 3331 (N—H), 2942 (C—H), 2186 (C≡N), 1665 (C=C), 1516 (N—O), 1327 (C—N), 1236 (C—O), 1128 (C—O), 1051 (C—OH); ESI-MS: *m/z* 442 (*M* + *H*); Anal. Calculated for $C_{23}H_{27}N_3O_6$,: C, 62.57; H, 6.16; N, 9.52; found: C, 62.60; H, 6.12; N, 9.60.

2.4. α-Amylase inhibition assay

The in vitro α-amylase inhibitory studies were carried by some modification of the assay method previously reported and desribed briefly herein [40]. The α-amylase (barley malt) used for antidiabetic activity was purchased from HIMEDIA. A stock solution of synthesized compounds (**5a-i** and **2**) and standard acarbose was prepared by dissolving (1 mg/mL) in DMSO solvent. A volume of 200 μL test solution (2, 4, 6, 8 and 10 μg/mL) and 200 μL of α-amylase (1 mg/mL) 20 mM sodium phosphate buffer (pH 6.9) were incubated at 30 °C for 10 min. After pre-incubation, a volume of 200 μL of the starch solution (1% w/v) in deionized water was added to each tube and incubated at 30 °C for 10 min. A volume 200 μL of reagent DNSA (3,5-Dinitrosalicylic acid) was added, the mixture was further incubated at 85–87 °C for 5 min and cooled to room tempearature followed by addition of 5 mL of deionized water. The absorbance of the resultant solution was measured at 540 nm using UV–Visible spectrophotometer. A blank solution and control were performed in the same manner by replacing the synthesized compound with a 200 μL of buffer solution 200 μL of DMSO and absorbance was recorded in the same manner. The calculations of% inhibition of α-amylase was performed using the given formula:

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

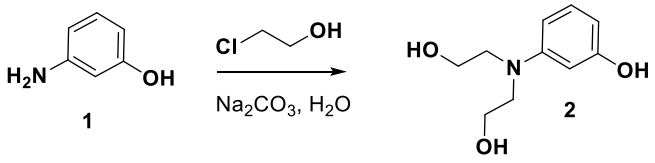
The IC_{50} values were calculated as mean ± SD in triplicates using Graph Pad Prism Software from a non-linear regression graph plotted between percentage inhibition (x-axis) versus concentrations (y-axis).

2.5. Enzyme kinetic studies

The mode of α-amylase inhibition was carried out by Lineweaver-Burk plot (LB) method [41,42]. The most active compound **5b** was investigated with different concentration of starch as a substrate (1–4 μg/mL) in the absence and presence of **5b** at different concentrations (0, 0.9, 1.8, 2.7 and 3.6 μg/mL). A Lineweaver-Burk plot was generated to identify the mode of inhibition and the Michaelis-Menten constant (Km) 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 (Ki) value was obtained from secondary plots of the inhibitor concentration (**5b**) versus Km. Graphs were plotted and Km and Ki value obtained directly from the software Graph Pad Prism 5.

2.6. Computational studies

Molecular docking studies of the compound **5a-i, 2** and acarbose were carried out using *AutoDockVina (v.1.1.2)* [43,44]. The α-amylase crystal structure (PDB ID: 1rpk) was retrieved from PDB-database. The ligand geometries for docking of compound **5a-i** and **2** were prepared using *OpenBabel (v.3.1.1)*. Subsequently, a web-based server viz. pkCSM was employed for the pharmacokinetic profiling of the targeted compounds including different parameters such as drug-likeness properties, ADME and toxicity profiles [45,46]. The off-site target interaction predictions of synthesized compounds were performed on an *OpenVirtualToxLab* software [47,48]. A web-based server viz. *Protein–Ligand Interaction Profiler(PLIP)* was used for the assessment of the binding site interactions of ligands with enzyme [49,50]. The software *PyMol (v. 2.3.4)* has been utilized to capture the docking images presented in the manuscript and the computational details executed by following previously published research work [51–53].



Scheme 1. Synthesis of intermediate 2,2′-((3-hydroxyphenyl)-azanediyl)bis (ethan-1-ol) (2).

3. Result and discussion

3.1. Chemistry

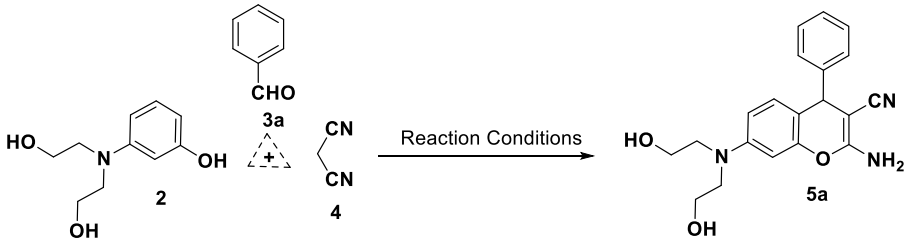
As shown in Scheme 1, first, we carried out the synthesis of requisite 2,2′-((3-hydroxyphenyl)azanediyl) bis(ethan-1-ol) (**2**) by reacting 3-aminophenol (**1**) with 2-chloroethanol in the presence of Na_2CO_3 in aqueous media [54].

The one-pot three component condensation of obtained intermediate **2**, benzaldehyde (**3a**) and malononitrile (**4**) was investigated as typical reaction to establish the reaction parameters including catalyst, solvents, and reaction temperature. Initially, the reaction was examined in the presence of proline catalyst using alcohols as solvents. It was observed that the reaction was sluggish, and the yield of targeted product (**5a**) was considerably low at rt or under reflux (Table 1, entry 1–3). Next, the model reaction was repeated in the presence of various base catalysts (15 mol%) such as piperidine, pyrrolidine, triethylamine (TEA) and pyridine in combination with different solvents. Of these, piperidine as catalyst in ethanol served as better condition for the condensation but took long reaction hour to give **5a** in good yield (Table 1, entry 4). Remarkably, when model reaction was carried out under solvent-free condition using piperidine as catalyst (15 mol%), the targeted compound **5a** was obtained in excellent yield (90%) within 30 min at room temperature (Table 1, entry 8). Attempts to lower catalyst loading (5 or 10 mol%) resulted in the lower yield of the **5a** along with increased reaction time. Similarly, utilization of pyridine, triethylamine (TEA), and L-proline resulted in the lowered yield of **5a** at room temperature (Table 1, entry 9–11). Further, we observed that condensation was not effective without piperidine catalyst (Table 1, entry 12–13). The results of these experiments indicated that use of solvents prolonged the reaction hour and significantly decreased the yield of targeted product. Thus, piperidine (15 mol%) and solvent-free condition was found optimum for this three-component transformation. After optimization of reaction condition, in the next step, synthesis of a variety of 2-amino-7-(bis(2-hydroxyethyl)amino)−4(phenyl)−4*H*-chromenes was performed to explore the scope of the protocol and the results are represented in Scheme 2. As expected, intermediate 2,2′-((3-hydroxyphenyl)azanediyl) bis(ethan-1-ol) (**2**) reacted expeditiously with a number of aromatic aldehydes bearing halogens, electron-withdrawing and electron-releasing substituents (**3a-i**) and malononitrile (**4**) at room temperature to afford excellent yields of the corresponding 2-amino-7-(bis(2-hydroxyethyl)amino)−4*H*-chromene-3-carbonitrile derivatives (65–90%) under solvent-free condition within very short time periods (30–40 min).

The molecular structures of the newly synthesized compounds **5a-i** were confirmed by spectroscopic analysis (IR, mass, ^{1}H NMR and ^{13}C NMR) and elemental analysis. For instance, the FT-IR spectrum of **5a** showed broad peaks at 3400 cm^{-1}, 3321 cm^{-1}, and 3200 cm^{-1} were attributed to their N—H and O—H stretching vibrations of -NH_2 and -OH functional groups. The C—H stretching vibrations of methylene groups of the molecules appeared at 2950 cm^{-1} and 2882 cm^{-1}. A strong band appeared at 2193 cm^{-1} confirmed the presence of -CN group in the molecules.

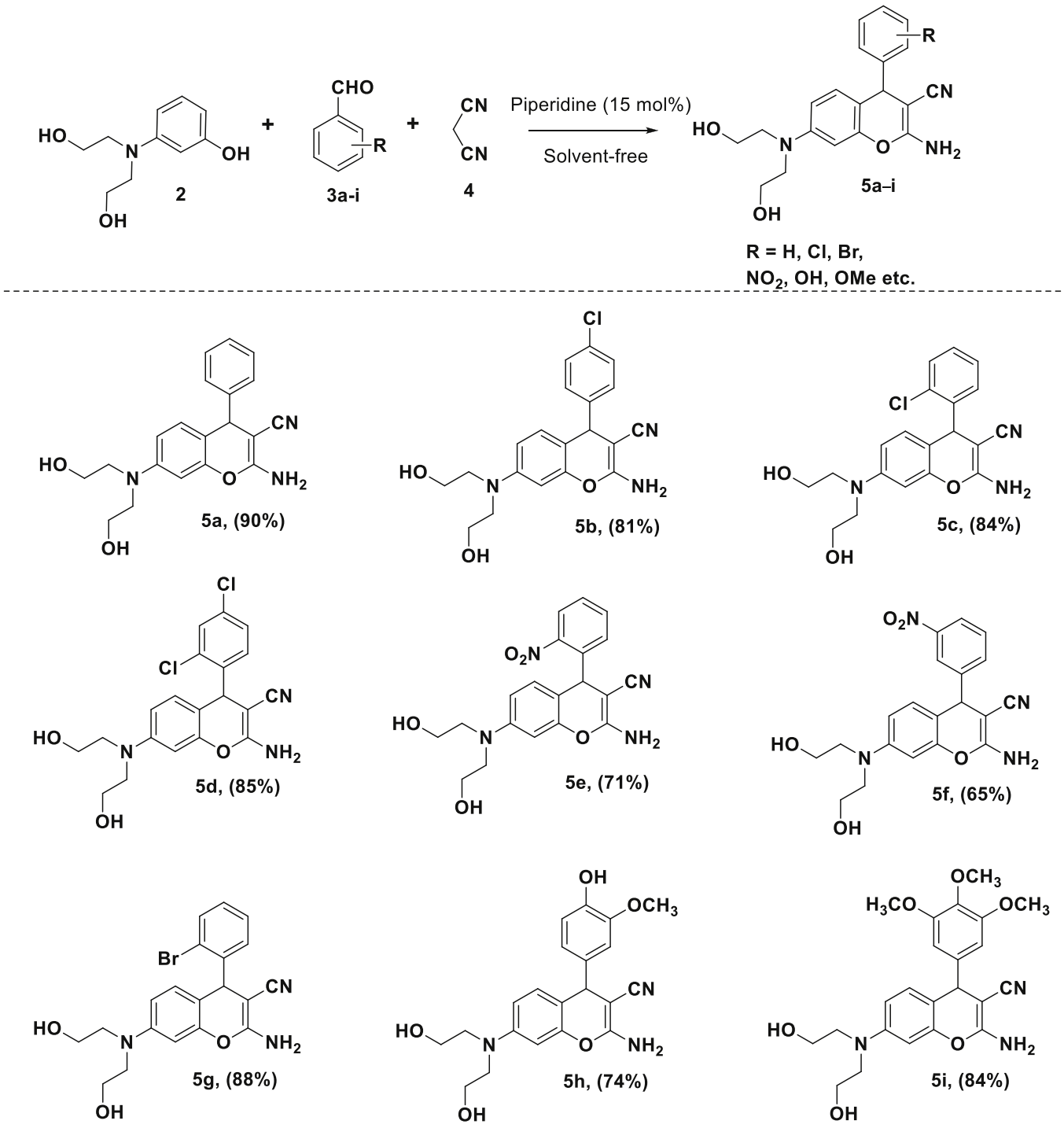
The characteristic C=C stretching appeared at 1658 cm^{-1} while bands at 1400 cm^{-1} and 1200 cm^{-1} indicated the C—N and C—O stretching respectively. The ^{1}H NMR spectrum of **5a** showed methylene protons of the bis(2-hydroxyethyl)amino moiety appeared as multiplet at 3.31–3.37 δ ppm and 3.52–3.47 δ ppm whereas protons of -OH groups exhibited signals at 4.77 δ ppm as triplet. The methine proton of the chromene ring appeared as singlet at 4.56 δ ppm. Further, the ^{1}H NMR spectrum of **5a** exhibited a distinct three signal pattern in the form of doublet (J = 2.4 Hz, *m*-coupling) at 6.24 δ ppm , double doublets (J = 8.8, 2.4 Hz, *-o* & *-m* coupling) at 6.42 δ ppm, and a doublet (J = 8.8 Hz) at 6.74 δ ppm for their protons of aromatic ring which is fused with chromene scaffold. On the other hand, aromatic protons of C-4 substituted ring were a mixture of doublets and triplets at 7.15–7.32 δ ppm. The primary amino group (NH_2) showed their chemical shift at 6.80 δ ppm as singlet. Furthermore, ^{13}C NMR analysis of **5a** revealed the three distinct signals at 53.69, 56.87 and 58.49 δ ppm attributed to their

Table 1
Optimization of reaction condition for the multicomponent condensation of 2,2′-((3-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (**2**), benzaldehyde (**3a**) and malononitrile(**4**).[a]



Entry	Catalyst	Solvent	Reaction time/temp.	Yield (%)[b]
1	L-proline	MeOH	24h/rt	16
2	L-proline	MeOH	8h/rt	35
3	L-proline	EtOH	6h/reflux	45
4	Piperidine	EtOH	8h/rt	64
5	TEA	EtOH	6h/rt	35
6	L-proline	H_2O	16h/reflux	24
7	Piperidine	H_2O	16h/reflux	40
8	Piperidine	-	30 min/rt	90
9	L-proline	-	6h/rt	45
10	TEA	-	6h/rt	58
11	Pyridine	-	6h/rt	47
12	-	-	6h/rt	traces
13	-	PEG-400	8h/reflux	Trace

[a]**Reaction condition**: 2,2′-((3-hydroxyphenyl)azanediyl)bis(ethan-1-ol) (**2,** 1 mmol), benzaldehyde (**3a,** 1 mmol) and malononitrile (**4,** 1 mmol) and catalyst (15 mol %) were stirred at appropriate time and temperature mentioned in the Table 1. [b]Isolated yield.



Scheme 2. Synthesis of various bis(2-hydroxyethyl)amino-functionalized 4H-chromenes.

N—CH_2, O—CH_2 groups and C—H of chromene ring respectively, while presence of 14 distinct carbons in the aromatic region of the spectrum are in agreement of the proposed structure. Moreover, the mass spectrum of **5a** showed molecular ion peak at *m/z* 351 corresponding to the molecular weight and the elemental analysis found in accordance with molecular formula $C_{20}H_{21}N_3O_3$.

3.2. *α-Amylase inhibitory activity*

In the present study, all the newly synthesized compounds (**5a-i** and **2**) have been subjected to their five dose in vitro α-amylase inhibition studies using acarbose as reference standard and the results are summarized in the Table 2.

It was revealed that all the tested compounds exhibited α-amylase inhibitory activities with $IC_{50} < 10$ μg/mL. The comparative analysis of % inhibition demonstrated that targeted compounds (**5a-i**) have inhibited α-amylase effectively in a dose-dependent manner (Fig. 3). Among these, halogen containing 2-amino-4*H*-chromenes (**5b-d**) were more active compared to nitro (**5e-f**) or methoxy (**5h-i**) substituted derivatives. Compound **5b** was more potent (3.60 ± 0.01 μg/mL) than control acarbose under given conditions. On the other hand, electron donating groups (OH, OMe) on 2-amino-4H chromene ring have

Table 2
In vitro α-amylase inhibitory activity of the synthesized compounds (5a-i).

Compound	Inhibition of α-amylase (%)[a] *In vitro* Dose (μg/mL)					IC_{50}(μg/mL)
	2	4	6	8	10	
5a	18.92 ± 1.00	27.92 ± 0.15	36.81 ± 1.00	44.67 ± 0.53	54.70 ± 1.01	9.11 ± 0.15
5b	28.75 ± 0.79	55.12 ± 0.43	71.87 ± 0.29	82.52 ± 0.49	91.21 ± 0.17	3.60 ± 0.01
5c	26.99 ± 1.22	53.36 ± 0.57	70.22 ± 0.47	80.97 ± 0.44	89.97 ± 0.41	3.78 ± 0.09
5d	25.44 ± 0.69	39.09 ± 1.17	57.19 ± 0.74	65.15 ± 0.15	76.01 ± 0.21	5.15 ± 0.13
5e	9.72 ± 0.72	22.33 ± 1.00	34.02 ± 0.62	43.95 ± 0.97	52.32 ± 0.66	9.43 ± 0.17
5f	20.78 ± 1.05	35.05 ± 0.89	41.88 ± 0.58	49.02 ± 0.09	62.35 ± 0.72	8.06 ± 0.05
5g	16.65 ± 1.11	26.06 ± 0.39	35.16 ± 0.95	42.92 ± 0.26	51.08 ± 0.94	9.73 ± 0.19
5h	13.13 ± 0.87	24.3 ± 0.34	35.05 ± 0.65	43.02 ± 1.03	50.36 ± 0.24	9.90 ± 0.11
5i	17.48 ± 0.89	29.05 ± 1.41	38.26 ± 1.22	49.33 ± 1.18	56.36 ± 1.05	8.45 ± 0.27
2	12.82 ± 0.26	25.13 ± 1.36	33.09 ± 1.25	40.22 ± 1.77	50.98 ± 0.76	9.86 ± 0.19
Acarbose	28.13 ± 0.27	54.71 ± 0.26	70.73 ± 0.37	80.87 ± 0.34	90.18 ± 0.35	3.65 ± 0.02

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

decreased the α-amylase inhibition potency. Further, Compound without chromene ring was least active (**5b** *Vs* **2**) among the series demonstrated the importance of substituted chromene scaffold for α-amylase inhibitory activity. In line with the current study, earlier Hajlaoui et al. demonstrated that 2-amino-4H chromene scaffold in combination with 2,5-hexanedione (Fig. 2, **F**) increased activity. On the contrary, substitution on phenyl ring of 2-amino-4H chromene decreased the activity [39]. While comparison of potency of compound **5b** with diipyridamole (Fig. 2, **E**) where pyrimidine ring combined with bis(2-hydroxyethyl)amino, it was revealed that compound **5b** shown twofold more potency than diipyridamole (**E**) which underline the importance of 2-amino-4H chromene ring for activity [38].

3.3. Kinetic mechanism of inhibition of α-amylase

In order to establish the mode of inhibition of most active compound **5b**, kinetic studies were conducted using the Lineweaver-Burk plot method [41,42]. As shown in the LB plot (Fig. 4A), when concentration of inhibitor **5b** was increased, the value of Km also increased however Vmax remain unaffected, and the plot showed the same interception point for inhibited enzymes on Y-axis as uninhibited enzyme (control). These results indicated that compound **5b** bound to the active site of α-amylase in a competitive manner. Furthermore, the secondary plot of different concentrations of inhibitor versus the Km provided the inhibition constant Ki for **5b** which was found to be 0.97 μg/mL with r^2 value of 0.9386 (Fig. 4B). Notably, comparison of mode of inhibition with previously reported 2-amino-4H chromene derivatives revealed that **5b** possessed competitive inhibition whereas 2-amino-4H chromenes (F) showed mixed type inhibition against target enzyme [39].

3.4. In silico studies

The in-depth computational studies were conducted for synthesized compounds (**5a-i** and **2**) which includes docking at targeted protein binding site, off-site binding assessment, evaluation of physicochemical, pharmacokinetics and toxicity profiles.

3.4.1. Molecular docking

The synthesized compounds (**5a-i** and **2**) were subjected to docking simulation with the binding sites of α-amylase (PDB ID: 1rpk) to estimate their putative binding mode and possible interactions (Fig. 5 & Table 3). The molecular docking study revealed that most of the active compounds effectively bind with the active site of α-amylase having docking scores more than −7.0 kcal/mol and have shown single binding mode. The binding site interactions of most active compounds **5b** and **5c** are represented in Table 4.

As depicted, **5b** showed hydrophobic interactions with residue Trp10, Tyr52, Phe144, while **5c** interacted with residues of Trp10, GLN296, Tyr52 and Phe144 within binding pocket. Further, the bis(2-hydroxyethyl)amino moiety and the 2-amino-4H-chromene scaffold have shown important H-bond interactions with the residues of Ala145, Asp180, Phe181, Arg183 and Asp143, Asp180, Arg183 for **5b** and **5c** respectively. On the other hand, the arene ring of both **5b** and **5c** demonstrated π−π stacking interactions with the Phe144, Phe181

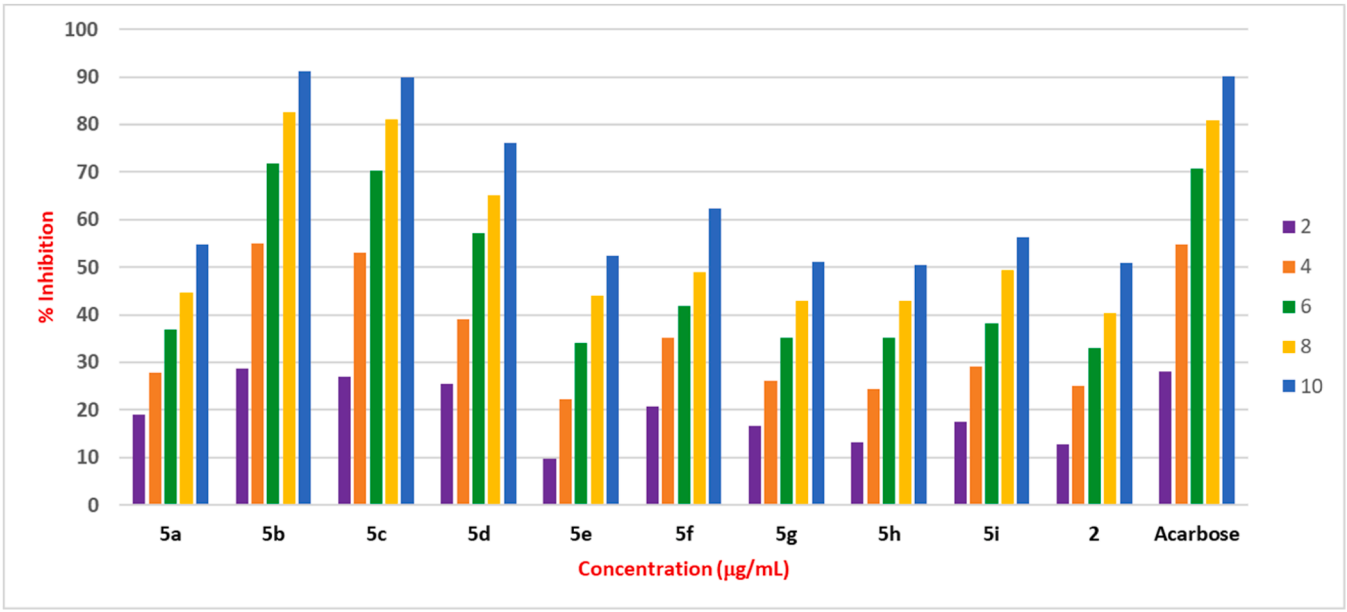


Fig. 3. Comparative analysis of% inhibition of compound 5a-i, 2 and Acarbose.

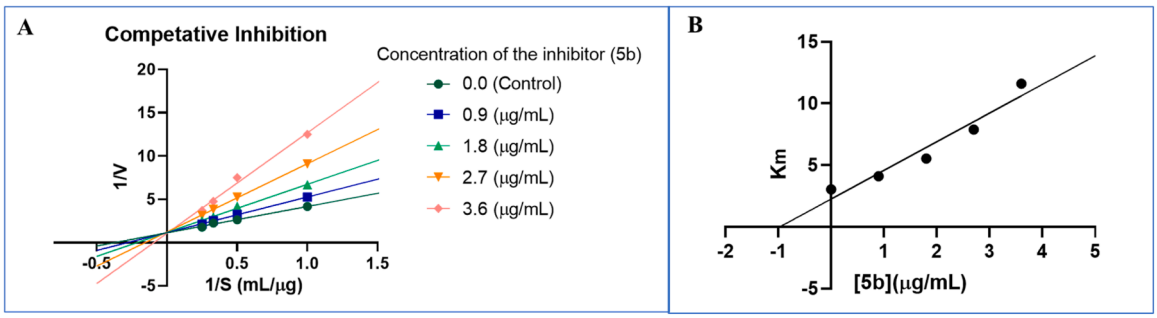


Fig. 4. Enzyme kinetics of α-amylase inhibition by 5b. (A) The Lineweaver–Burk plot in the absence and presence of different concentrations of the 5b; (B) The secondary plot between Km and various concentrations of 5b.

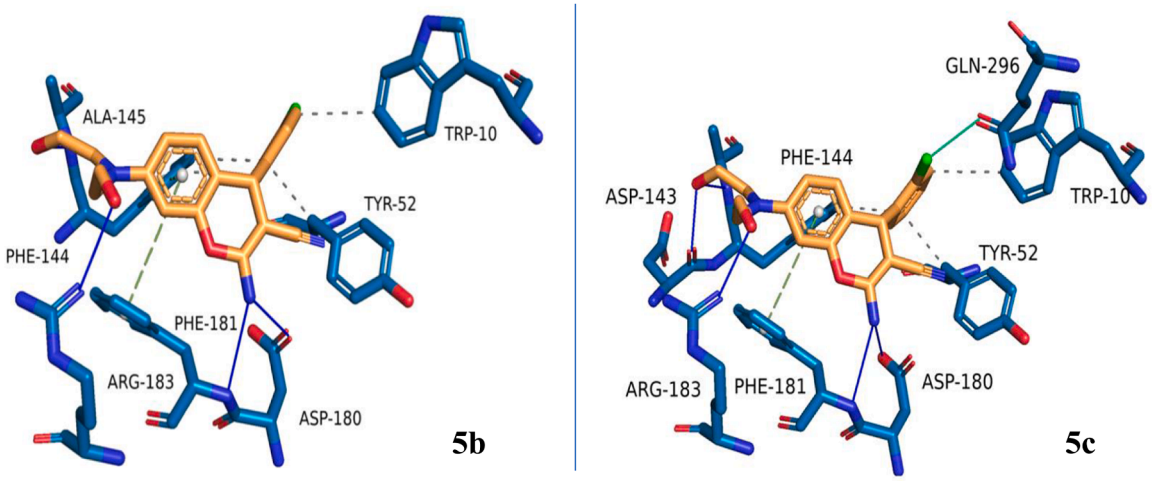


Fig. 5. Binding interactions of the compound 5b and 5c with α-amylase (PDB ID:1rpk).

Table 3
The docking scores for the synthesized compounds 5a-i, 2 and Acarbose with α-amylase

Compound	Docking Score (kcal/mol)	Compound	Docking Score (kcal/mol)
5a	−5.7	**5g**	−7.0
5b	−7.2	**5h**	−6.8
5c	−7.1	**5i**	−6.8
5d	−6.9	**2**	−6.4
5e	−7.2	**Acarbose**	−7.75
5f	−7.0		

protein residues. The molecular docking study demonstrated that, bis(2-hydroxyethyl)amino framework on 2-amino-4H-chromene ring system has provided a template for binding and favorable interactions with α-amylase which might be the reason for significant inhibitory potency of these conjugates.

3.4.2. *Drug-likeness and ADMET studies*

The web-server pkCSM was used for evaluation of pharmacokinetic properties of the synthesized molecules [45,46]. Various molecular attributes for drug likeness such as MW (mass) ≤ 500, number of hydrogen bond donors (≤ 5) & acceptors (≤10) and partition coefficient (LogP) ≤ 3 have been studied for the prediction. As shown in Table 5, all the compounds well corroborated with the drug-likeness properties without any violation while Acarbose control showed three violations. Further, role of cytochrome P450 (CYP) has been implicated in the biotransformation and elimination of the drugs [55]. The inhibition of the cytochrome P450 (CYP) enzyme often caused poor elimination of the drug and resulted in drug-induced toxicity [50]. Thus, in silico studies of most active compounds with P450 (CYP) might provide their plausible drug transformation and elimination pathways from the system. The present *in silico* investigation toward the inhibition of the cytochrome P450 using compounds (**5b-d**) revealed that compound **5b** and **5c** have no potential to inhibit P450 (CYP) enzyme except for the isoform CYP3A4 & CYP2C9. On the other hand, compound **5d** showed inhibitory potential against three of the five P450 isoforms as shown in Table 6. The results indicated that compound **5b** with minor inhibition properties of P450 isoforms might lead to excellent metabolism and elimination profile in the organism without drug-induced toxicity.

Toxicity profiles of the most active compounds (**5b-d**) were also studied using pkCSM website. This web-based server can predict toxicity profiles against multiple targets and parameters such as hERG I/II inhibition, oral rat acute & chronic toxicity (LD_{50} & LOAEL), hepatotoxicity, skin sensitization, AMES toxicity (Salmonella/microsome mutagenicity), and minnow toxicity.

Table 4
Interactions of the synthesized compound 5b & 5c with α-amylase.

Interactions with the target	α-amylase 5b	5c	Acarbose
Hydrophobic Interactions	Trp10, Tyr52, Phe144	Trp10, Gln296, Tyr52, Phe144	—
H-bond Interactions	Ala145, Asp180, Phe181, Arg183	Asp143, Asp180, Arg183	His93, Arg178, Phe181, Arg183, Glu205, Trp207, Asn209, Asp295
π-Stacking	Phe144, Phe181	Phe144, Phe181	—

Table 5
The calculated drug-likeness properties for the synthesized compounds 5a-i.

Compound	Molecular Weight	LogP	Rotatable Bonds	H-Bond Acceptors	H-Bond Donors	Surface Area	Lipinski Violation
5a	351.41	1.70	6	6	3	151.91	No
5b	385.85	2.35	6	6	3	162.21	No
5c	385.85	2.35	6	6	3	162.21	No
5d	420.30	3.00	6	6	3	172.51	No
5e	396.40	1.60	7	8	3	166.56	No
5f	396.40	1.60	7	8	3	166.56	No
5g	430.30	2.46	6	6	3	165.77	No
5h	397.43	1.41	7	8	4	168.18	No
5i	441.48	1.72	9	9	3	186.34	No
Acarbose	645.61	−8.56	9	19	14.00	250.23	Yes

Table 6
The predicted properties as cytochrome inhibitors for the compounds 5b-d.

Compound	CYP2D6 substrate/ inhibitor	CYP3A4 substrate/ inhibitor	CYP1A2 inhibitor	CYP2C19 inhibitory	CYP2C9 inhibitor
5b	No	Yes	No	No	No
5c	No	Yes	No	No	No
5d	No	Yes	No	Yes	Yes
Acarbose	No	No	No	No	No

As depicted in Table 7, the selected compound possessed no toxicity towards the evaluated parameters and skin sensitization. Further, it was observed that compound **5b-d** exhibited similar oral rate acute and chronic toxicity profile as compared to standard Acarbose with LD_{50} in the range of 2.50−2.66 for **5b-d** and 2.45 for the standard. Similarly, predicted oral rat chronic toxicity (LOAEL) for selected compounds appeared in the range of 1.99−2.06, whereas the standard is 5.32. Moreover, these compounds were found to be non-inhibitors of cardiac activity related genes viz. hERG-I and hERG-II, which indicated a non-cardio-toxic profile of these agents.

At the end, the off-site target binding activities of the most potent compounds **5b** and **5c** were also predicted using OpenVirtualToxLab server [42,43] which includes the toxic potential toward endocrine system, metabolic disruption, carcinogenicity and cardiotoxicity (Table 8). The off-site binding protocol is based on the estimation of putative binding affinity of the molecules towards a number of receptors like the androgen (AR), aryl hydrocarbon (AhR), estrogen-α &β (ER-α&β), glucocorticoid (GR), liver X (LXR), mineralocorticoid (MR), peroxisome proliferator-activated receptor (PPARγ), progesterone (PR), thyroid-α and β (TR) which are susceptible to trigger adverse effects. The evaluation of off-site target binding potential of **5b** and **5c** demonstrated that these derivatives were not binding or weakly bound ($pIC_{50} > 4$) with most of the off-site target proteins suggesting the non-toxic profile of the lead compounds **5b** and **5c** (Table 8).

4. Conclusion

A series of rationally designed 2-amino-7-(bis(2-hydroxyethyl)amino)−4(phenyl)−4*H*-chromenes based on scaffold combination approach has been prepared and evaluated for their antidiabetic activity as potential α-amylase inhibitors. The multicomponent reaction (MCR) approach was employed successfully to prepare targeted compounds from various substituted benzaldehyde, malononitrile and 2,2′-((3-hydroxyphenyl)azanediyl)bis(ethan-1-ol) in the presence of piperidine (15 mol%) under solvent-free condition furnished 2-amino-4*H*-chromenes (**5a-i**) in excellent yields at room temperature within 30–40 min. The molecular structures of the newly synthesized compounds were established by using various spectroscopy including IR, mass, ^{1}H & ^{13}C NMR and elemental analysis. The in vitro antidiabetic studies disclosed that, newly synthesized compounds (**5a-i**) exhibited significant antidiabetic activity with more than 50 % α-amylase inhibition at the dose of 10 μg/mL. Compound **5b** was the most potent of the series with IC_{50} of 3.60 ± 0.01 μg/mL and more potent than control acarbose. Further, enzyme kinetic studies proved **5b** as competitive inhibitor with of α-amylase with Ki of 0.97 μg/mL. In addition, molecular docking studies of most active compound **5b** and **5c** showed single binding mode with α-amylase having docking score more than −7.0 kcal/mol. Several H-bonding, π-stacking and hydrophobic interactions of **5b** and **5c** within binding pocket of α-amylase through bis(2-hydroxyethyl)amino framework and 2-amino-4H-chromene scaffold corroborated the results of antidiabetic activities. Moreover, in silico predicted properties of compound **5b** such as drug-likeness and toxicity suggested it as non-toxic and safer α-amylase inhibitor and warrant further investigation as antidiabetic agent.

CRediT authorship contribution statement

Savankumar R. Chothani: Validation, Methodology. **Monil P. Dholariya:** Methodology, Data curation. **Rupal J. Joshi:** Methodology, Formal analysis. **Chirag A. Chamakiya:** . **Deepika Maliwal:** Validation,

Table 8
The predicted off-site target binding properties (pIC_{50}) for the compounds 5b and 5c.

Off-site Target	5b	5c	Off-site Target	5b	5c
ToxPot	0.42	0.40	LXR	not binding	4.22
AR	6.11	4.30	MR	4.75	6.01
AhR	6.11	not binding	PPARγ	4.67	4.67
ERα	5.71	5.76	PR	4.84	4.29
ERβ	5.76	4.06	TRα	4.34	5.34
GR	6.26	4.35	TRβ	4.36	not binding

Table 7
The predicted toxicity properties for the compounds 5b-d.

Compound	AMES Toxicity	hERG I/II inhibitor	Oral Rat Acute Toxicity (LD_{50})	Oral Rat Chronic Toxicity (LOAEL)	Hepato-toxicity	Skin Sensitization	Minnow Toxicity
5b	No	No	2.50	2.06	No	No	0.99
5c	No	No	2.52	2.04	No	No	1.03
5d	No	No	2.66	1.99	No	No	0.65
Acarbose	No	No/Yes	2.45	5.32	No	No	16.82

Software. **Raghuvir R.S. Pissurlenkar:** Validation, Software, Investigation, Conceptualization. **Anilkumar S. Patel:** Writing – review & editing, Validation, Resources, Methodology, Investigation. **Jasmin J. Bhalodia:** Resources, Formal analysis. **Mrunal A. Ambasana:** Validation, Resources. **Rashmiben B. Patel:** Resources. **Atul H. Bapodra:** Writing – review & editing, Resources, Funding acquisition. **Naval P. Kapuriya:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, 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 the data have provided

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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.2023.137462.

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