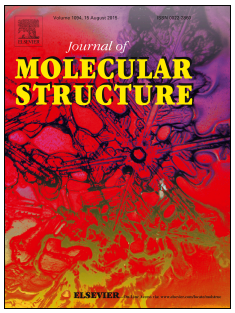

Design, synthesis, α-amylase inhibition and *in-silico* docking study of novel quinoline bearing proline derivatives

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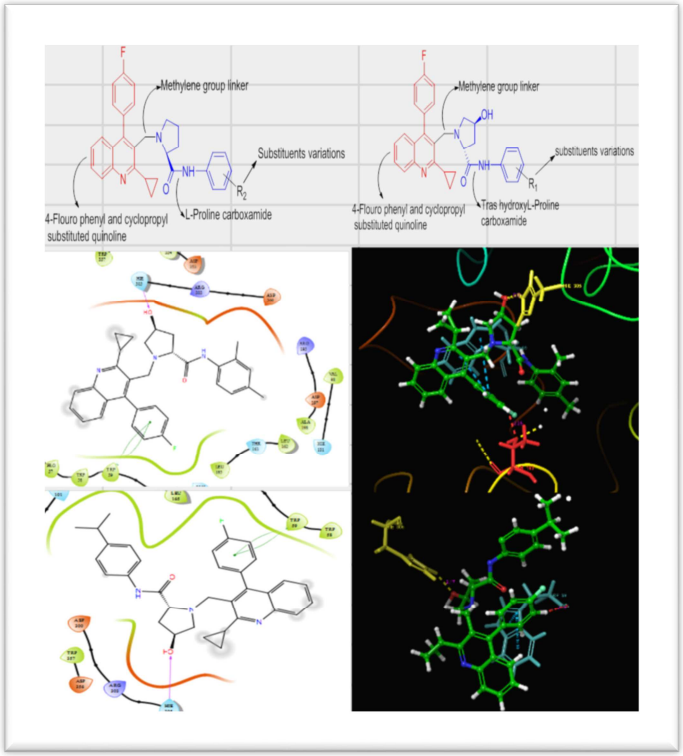
Graphical Abstract



Design, synthesis, α-amylase inhibition and *In-silico* docking study of novel quinoline bearing proline derivatives

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ABSTRACT

α-amylase enzyme hydrolyses carbohydrate into glucose is known to be an important molecular target for type 2 Diabetes mellitus. In the course of developing α-amylase enzyme inhibitors, we designed, synthesized seventeen novel quinoline bearing proline analogs, subsequently physico-chemical properties of designed analogs were also *in-silico* predicted for their drug likeness evaluation. Synthesized compounds were characterized by spectral analysis such as Mass, IR, ^{1}H NMR, ^{13}C NMR and further screened *in vitro* for α-amylase inhibitory activity using acarbose as standard drug. Seven analogs, 6a, 6b, 6c, 6d, 6g, 10b and 10c showed significant α-amylase inhibitory activity. Eight analogs, 6e, 6f, 6h, 5, 6j, 10a, 10d and 10e showed good to moderate activity while other two analogs, 6i and 9 showed least activity. The molecular docking study of significantly active and weakly active compounds

was performed in order to study their putative binding mode of the most and least active compounds (6c and 6i).

***Keywords*:** Proline, Quinoline, Anti diabetic, Molecular docking, α-amylase inhibition.

1. Introduction

Diabetes mellitus (DM) has been known as third leading cause of death in humans [1]. Study shows that 422 million persons affected with diabetes in 2016 and this number is expected to increase 844 million in 2030 [2]. There are two primary types of diabetes mellitus, insulin dependent (Type 1) and non-insulin dependent (Type2) [3]. Type 2 diabetes is most commonly prevalent over type 1 diabetes [4]. α-amylase is the key enzyme directly related to type 2 diabetes and found in saliva as well as pancreas [5]. Normal food contains polysaccharides (Starch and glycogen) [6]. α-amylase enzyme hydrolyses these polysaccharides into oligosaccharides further hydrolyses into absorbable monosaccharide (Glucose) and releases it into blood stream that causes sharp increase in the blood glucose level [7]. Disorders in carbohydrate uptake and immediate digestion by α-amylase enzyme causes sudden raise in blood glucose level [8]. This can be controlled by inhibition of carbohydrate hydrolysing α-amylase enzyme is the critical therapeutic area used to control type 2 diabetes [9]. Acarbose and miglitol are such synthetic oral hyperglycaemic drugs commercially available in the market but having side effects like discomfort, flatulence, meteorism and diarrhoea which causes therapy discontinuation [10]. The WHO recommends to search a safe, potent and non-toxic natural anti-diabetic agent [11]. Ethano botanical research on traditional herbal remedies has confirmed more than 1200 plants with hypoglycaemic effect but they cannot be administered directly as a drug [12].

In the search of structures with significant bioactivity, we focussed onto the development of molecules through combination of different active heterocyclic compounds that may lead to identification of compounds with improved alpha amylase inhibitory activity.

Among heterocyclic compounds, proline ring is endowed with various activities such as Antimicrobial, anti-oxidant, anti-carcinogen, anti-HIV and anti-inflammatory, [13-17]. Mahindra Kumar Mishra *et al*., explored proline as α-amylase inhibitory active moiety [18]. Proline and its analogs isolated from the leaves of koenigii have been used in traditional medicine particularly for hyperglycaemia [19]. Many heterocyclic amides play a major role in designing novel chemical entities and also they are well known for their biological activity. Among them Proline and its substituted phenyl amides have been playing a vital role in drug discovery design [20].

Quinoline derivatives constitute a vital role in the development of new drug discovery which possess many biological applications like anti-oxidant, anti-inflammatory, anti-cancer, anti-viral, anti-diabetic, antimalarial, anti-tubercular and anti-microbial [21-27]. Quinoline containing compounds have been well known as a drug such as Quinine, bulaquine, pamaquine and tafenoquine [28]. Quinoline bearing many heterocyclic compounds have been reported to have potential α-amylase inhibitory activity [29-30]. Quinoline and their derivatives isolated from *Ephedra pachyclada* stem and *Ruta chalepensis* leaves have shown significant α-amylase inhibitory activity. [31-32]. 4-Flouro phenyl and cyclopropyl substituted Quinoline containing chemistry is becoming popular in drug design. Pitavastain drug contains 4-flouro phenyl and cyclopropyl substituted quinoline in its core structure [33]. Methylene group is an important linker in many drugs [34].

The above observation prompted us, to combine 4-flouro phenyl and cyclopropyl substituted quinoline, L-proline and Trans hydroxy-L-proline carboxamide via methylene group and study the structure activity due to substituent variations on the phenyl carboxamide ring (Fig 1).

Many potent molecules often fail to enter the market because of their unfavourable pharmacokinetic profiles [35]. Now a days, pharmacokinetic properties of drugs are taken in to consideration during earlier stages of drug discovery programme and are becoming more popular [36]. *In-silico* drug likeness approach reduces time and cost as compared to experimental methods [37]. Recently many novel α-amylase inhibitors have been identified and insilco predicted [38]. So, in the current study, physico-chemical properties of the designed analogs were *in-silico* predicted for their drug likeness analysis. Designed compounds were synthesized, characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy and were screened for *in-vitro* α-amylase inhibitory activity. Docking study was also performed in order to predict the putative binding mode of the most (compound **6c**) and least active compound (compound **6i**).

2.0 Material and Method

2.1 Chemistry

Melting points were recorded on a Buchi melting point B-540 instrument and are uncorrected. Thin layer chromatography (pre-coated silica gel, Merck) was used to analyse purity of the compounds. Potassium permanganate solution was used as staining reagent. Column chromatography was performed using silica gel (60-120 mesh) as packing material, n-heptane and ethyl acetate as eluent. IR spectrum in KBr pellet method was recorded using JASCO FT-IR410. The mass spectra were recorded in PE-SCIEX API-3000 LC/MS/MS with

Turbo ion spray. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Advance 400MHz Spectrometer with multinuclear BBO probe and TMS as an internal standard.

Synthesis of (2R,4S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-4-hydroxypyrrolidine-2-carboxylic acid (5): *N,N*-Diisopropylethylamine (0.779 mol) was added slowly to the stirred solution of methyl (2R, 4S)-4-hydroxypyrrolidine-2-carboxylate hydrochloride (0.055 mol) and 3-(bromomethyl)-2-cyclopropyl-4-(4-fluorophenyl) quinoline (0.055 mol) in THF (5 mL) at room temperature. Starting material consumption was monitored by thin layer chromatography. After completion of reaction, aqueous solution of potassium hydroxide (0.060 mol in 1 mL water) was added and heated to 50 °C. Progress of the hydrolysis reaction was monitored by thin layer chromatography. Cooled the reaction mass to 25 °C, quenched with ice cold water and the resultant product was filtered. White solid; Yield: 97%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.857 (d, 1H, *J* = 8.4), 7.635 (t, 1H, *J* = 7.4), 7.405-7.353 (m, 5H), 7.127 (d, 1H, *J* = 8), 4.782 (brs, 1H), 4,097-4.038 (m, 2H), 3.637 (d, 1H, *J* = 12.8), 3.176-3.122 (m, 2H), 2.948 (t, 1H, *J* = 7.4), 2.002-1.941 (m, 2H), 1.809-1.789 (t, *J* = 4.0 1H), 1.276-1.266 (m, 1H), 1.056-0.968 (m, 3H); ^{13}C NMR (400 MHz, DMSO-d6): δ 174.355, 163.354, 163.030, 160.598, 146.194, 145.650, 132.621, 132.070, 128.861, 128.349, 127.801, 126.038, 125.853, 125.425, 115.314, 68.295, 64.732, 60.810, 51.993, 14.135, 10.709, 9.562; ESI-MS: m/z calculated for $C_{24}H_{23}FN_2O_3$[M + H]+ 407.17, found 407.10.

General Procedure for the synthesis of (2R, 4S)-1-((2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl) methyl)-4-hydroxypyrrolidine-2-carboxamide 6(a-j): To the stirred solution of (2R,4S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-4-hydroxypyrrolidine-2-carboxylic acid (0.0024 mol in 5 mL THF), Triethylamine (0.0027 mol) followed by pivaloyl chloride (0.0024 mol) were added slowly at -45 °C and stirred for

30 min. Finally, substituted phenyl amine (0.0024 mol) was added. The progress of the reaction was monitored by thin layer chromatography. Reaction mass was quenched in an ice cold water and filtered the product.

(2R,4S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-4-hydroxy-*N*-phenylpyrrolidine-2-carboxamide (6a)

White solid; Yield: 97%; IR (KBr) (cm^{-1}): 3388 (O-H stretching), 3336 (N-H stretching) 2997 (aromatic C-H stretching), 2864 (Aliphatic C-H stretching), 1668 (C=O stretching), 1581, 1514, 1490 (Aromatic C=C/C=N stretching), 1456 (Aliphatic CH bending), 1219 (C-F stretching), 1155, 1126, 1062 1028 (C-O stretching), 929, 839, 769, 744 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.679 (s, NH, 1H), 7.811 (d, 1H, J = 8.0), 7.529 (t, 1H, *J* = 7.6), 7.345-7.325 (m, 1H), 7.296-7.255 (m, 2H), 7.237-7.062 (m, 5H), 7.052 (d, 2H, *J* = 7.6), 6.987 (t, 1H, *J* = 7.2), 4.365 (t, 1H, *J* = 5.0), 4.216 (d, benzyl CH_2, 1H, *J* = 13.6), 4.055 (d, benzyl CH_2, 1H, *J* = 13.6), 3.382-3.334 (m, 2H), 2.730-2.652 (m, 2H), 2.171-2.077 (m, 2H), 1.553-1.509 (m,1H), 1.288-1.234 (m, 1H), 1.132-1.123 (m, 2H,); ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.119, 163.725, 162.114, 161.261, 146.988, 146.209, 136.872, 136.743, 132.671, 132.365, 131.894, 129.118, 128.806, 127.239, 126.477, 126.259, 125.616, 124.436, 120.365, 116.020, 115.782, 115.533, 71.061, 67.484, 62.933, 55.516, 40.719, 14.472, 11.550, 10.083; ESI-MS: m/z calculated for $C_{30}H_{28}FN_3O_2$[M + H]+ 482.22, found 482.20.

(2R,4S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-4-hydroxy-*N*-(o-tolyl) pyrrolidine-2-carboxamide (6b)

White solid; Yield: 98%; IR (KBr) (cm^{-1}): 3419 (O-H stretching), 2920 (aromatic C-H stretching), 3334 (N-H stretching), 2920 (aromatic C-H stretching), 1670 (C=O stretching), 1514, 1490 (Aromatic C=C/C=N stretching), 1406 (Aliphatic CH bending), 1224 (C-F

stretching), 1157, 1126, 1060, 1020 (C-O stretching), 962, 931, 819, 792 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.587 (s, NH, 1H), 7.836 (d, 1H, J = 8.4), 7.571 (t, 1H, J = 7.6), 7.439-7.400 (m, 1H), 7.305-7.239 (m, 2H), 7.200-7.151 (m, 3H), 7.074 (d, 1H, *J* = 6.8), 6.990 (t, 1H, *J* = 7.0), 6.921 (t, 1H, *J* = 7.8), 6.726 (d, 1H, *J* = 8), 4.390 (t, 1H, *J* = 5.2), 4.215 (d, benzyl CH_2, 1H, *J* = 13.6), 4.060 (d, benzyl CH_2, 1H, *J* = 13.2), 3.421-3.355 (m, 2H), 2.736-2.698 (dd, 1H), 2.632-2.580 (m, 1H), 2.194-2.096 (m, 2H), 1.985 (s, 3H), 1.294-1.249 (m, 2H). 1.044-0.971(m, 2H); ESI-MS: m/z calculated for $C_{31}H_{30}FN_3O_2$[M + H] + 496.23, found 496.20.

(2R,4S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-*N*-(2,4-dimethylphenyl)-4-hydroxypyrrolidine-2-carboxamide (6c)

White solid; Yield: 89%; IR (KBr) (cm^{-1}): 3325 (O-H stretching), 3286 (N-H stretching) 2974 (aromatic C-H stretching), 2863 (Aliphatic C-H stretching), 1674 (C=O stretching), 1597, 1514, 1490 (Aromatic C=C/C=N stretching), 1442 (Aliphatic CH bending), 1220 (C-F stretching), 1159, 1097, 1062 1022 (C-O stretching), 837, 765, 750 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.507 (s, NH, 1H), 7.856 (d, 1H, *J* = 8.4), 7.579 (t, 1H, *J* = 7.8), 7.415-7.382 (m, 1H), 7.304-7.239 (m, 2H), 7.192-7.150 (m, 3H), 6.883 (s, 1H), 6.722 (d, 1H, *J* = 8.4), 6.502 (d, 1H, *J* = 7.6), 4.391 (t, 1H, *J* = 5.0), 4.223 (d, benzyl CH_2, 1H, *J* = 13.2), 4.048 (d, benzyl CH_2, 1H, *J* = 13.2), 3.397-3.358 (m, 2H), 2.724-2.685 (dd, 1H), 2.629-2.579 (m, 1H), 2.212 (s, 3H), 2.156-2.050 (m, 2H), 1.989 (brs, 1H),1.950 (s, 3H), 1.329-1.159 (m, 2H), 1.023-0.973 (m, 2H); ESI-MS: m/z calculated for $C_{32}H_{32}FN_3O_2$[M + H]+ 510.25, found 510.20.

(2R,4S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-*N*-(3,4-dimethylphenyl)-4-hydroxypyrrolidine-2-carboxamide (6d)

White solid; Yield: 95%; IR (KBr) (cm^{-1}): 3259 (O-H stretching), 3066 (N-H stretching) 2989 (aromatic C-H stretching), 2790 (Aliphatic C-H stretching), 1672 (C=O stretching), 1602, 1539, 1489 (Aromatic C=C/C=N stretching), 1442 (Aliphatic CH bending), 1218 (C-F stretching), 1157, 1060 (C-O stretching), 929, 891, 839, 759 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.573 (s, NH, 1H), 7.836 (d, 1H, *J* = 8.4), 7.542 (t, 1H, *J* = 7.0), 7.327-7.312 (m, 1H), 7.275-7.235 (m, 2H), 7.193-7.147 (m, 3H), 6.892 (d, 1H, *J* = 8.0), 6.800 (d, 1H, *J* = 7.6), 6.662 (s, 1H), 4.333 (t, 1H, *J* = 5.0), 4.197 (d, benzyl CH_2, 1H, *J* = 13.2), 4.035 (d, benzyl CH_2, 1H, *J* = 13.2), 3.373-3.295 (m, 2H), 2.708-2.645 (m, 2H), 2.168 (s, 3H), 2.124-2.097 (m, 2H), 2.075 (s, 3H), 1.503-1.491 (m, 1H), 1.296-1.268 (m, 1H), 1.123-1.097 (m, 2H); ESI-MS: m/z calculated for $C_{32}H_{32}FN_3O_2$[M + H]+ 510.25, found 510.20.

(2R,4S)-*N*-(3-chlorophenyl)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-4-hydroxypyrrolidine-2-carboxamide (6e)

White solid; Yield: 95%; IR (KBr) (cm^{-1}): 3404 (O-H stretching), 3263 (N-H stretching) 2918 (aromatic C-H stretching), 2864 (Aliphatic C-H stretching), 1668 (C=O stretching), 1604, 1579, 1512, 1490 (Aromatic C=C/C=N stretching), 1450 (Aliphatic CH bending), 1219 (C-F stretching), 1219, 1126, 1089, 1060 (C-O stretching), 962, 893, 817 (Aromatic CH bending), 769 (C-Cl stretching); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.678 (s,NH, 1H), 7.813 (d, 1H, *J* = 8.4), 7.531 (t, 1H, *J* = 7.6), 7.370-7.330 (m, 1H), 7.297-7.221 (m, 2H), 7.206-7.126 (m, 4H), 7.066 (d, 2H, *J* = 7.6), 6.989 (t, 1H, *J* = 7.4), 4.382 (t, 1H, *J* = 4.8), 4.220 (d, benzyl CH_2, 1H, *J* = 13.2), 4.059 (d, benzyl CH_2, 1H, *J* = 13.2), 3.386-3.339 (m, 2H), 2.735-2.654 (m, 2H), 2.154-2.082 (m, 2H), 1.556-1.512 (m, 1H), 1.293-1.254 (m, 1H), 1.135-1.106 (m, 2H); ESI-MS: m/z calculated for $C_{30}H_{27}ClFN_3O_2$[M + H]+516.18, found 516.1,[M + 2H]+518.17, found 518.20.

(2R,4S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-*N*-(3-fluorophenyl)-4-hydroxypyrrolidine-2-carboxamide (6f)

White solid; Yield: 90%; IR (KBr) (cm^{-1}): 3389 (O-H stretching), 3341 (N-H stretching) 2985 (aromatic C-H stretching), 2871 (Aliphatic C-H stretching), 1660 (C=O stretching), 1580, 1574, 1501 (Aromatic C=C/C=N stretching), 1458 (Aliphatic CH bending), 1218 (C-F stretching), 1155, 1126, 1062 1028 (C-O stretching), 930, 841, 770 (Aromatic CH bending);^{1}H NMR (400 MHz, $CDCl_3$): δ 8.688 (s, NH, 1H), 7.792 (d, 1H *J* = 8.4), 7.529 (t, 1H, *J* = 7.3), 7.511-7.359 (m, 1H), 7.353-7.002 (m, 7H), 6.736 (d, 1H, *J* = 6.4), 6.675 (t, 1H, J = 7.9), 4.388 (t, 1H, *J* = 5.0),4.239 (d, benzyl CH_2, 1H, *J* = 13.6), 4.039(d, benzyl CH_2, 1H, *J* = 13.6), 3.414-3.329 (m, 2H), 2.755-2.717 (m, 1H), 2.765-2.637 (m, 1H), 2.153-2.083 (m, 2H), 1.617-1.577 (m, 1H), 1.124-1.216 (m, 1H), 1.154-1.125 (m, 2H); ESI-MS: m/z calculated for $C_{30}H_{27}F_2N_3O_2$[M + H]+ 500.21, found 500.20.

(2R,4S)-*N*-(3-bromophenyl)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-4-hydroxypyrrolidine-2-carboxamide (6g)

White solid; Yield: 99%; IR (KBr) (cm^{-1}): 3410 (O-H stretching), 3341 (N-H stretching) 2981 (aromatic C-H stretching), 2866 (Aliphatic C-H stretching), 1669 (C=O stretching), 1583, 1520, 1450 (Aromatic C=C/C=N stretching), 1456 (Aliphatic CH bending), 1217 (C-F stretching), 1160, 1130, 1071, 1031 (C-O stretching), 930, 840, 771 (Aromatic CH bending), 644 (C-Br stretching); ^{1}H NMR (400 MHz, $CDCl_3$): 8.661 (s, NH, 1H), 7.806 (d, 1H, *J* =8.28), 7.535 (t, 1H, *J* = 7.3), 7.359-6.995 (m, 10H), 4.383 (t, 1H, *J* = 5.0), 4.244 (d, benzyl CH_2, 1H, *J* = 13.8), 4.023 (d, benzyl CH_2, 1H, *J* = 13.8), 3.430-3.392 (m, 1H), 3.354-3.315 (m, 1H), 2.745-2.654 (m, 2H), 2.099-2.049 (m, 2H), 1.615-1.590 (m, 1H), 1.253-1.208 (m,

1H), 1.134-0.984 (m 2H). ESI-MS: m/z calculated for $C_{30}H_{27}BrFN_3O_2$[M + H]+ 562.13, found 562.0, [M + 2H]+563.13, found 563.00.

(2R,4S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-4-hydroxy-*N*-(4-methoxyphenyl) pyrrolidine-2-carboxamide (6h)

Pale brown solid; Yield: 89%; IR (KBr) (cm^{-1}): 3388 (O-H stretching), 3336 (N-H stretching) 2997 (aromatic C-H stretching), 2864 (Aliphatic C-H stretching), 1668 (C=O stretching), 1581, 1514, 1490 (Aromatic C=C/C=N stretching), 1456 (Aliphatic CH bending), 1219 (C-F stretching), 1155, 1126, 1062 1028 (C-O stretching), 929, 839, 769 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.550 (s, NH, 1H), 7.834 (d, 1H, *J* = 8.32), 7.546 (t, 1H, *J* = 7.5), 7.357-7.152 (m, 6H), 6.910 (d, 2H, *J* = 8.56), 6.669 (d, 2H, *J* = 8.48), 4.384 (t, 1H, *J* = 5.0), 4.218 (d, benzyl CH_2, 1H, *J* = 13.4), 4.037 (d, benzyl CH_2, 1H, *J* = 13.4), 3.400-3.324 (m, 2H), 3.728 (s, 3H),2.719-2.660 (m, 2H), 2.218-2.105 (m, 2H), 1.4827-1.2931 (m,2H), 0.880-0.836 (m, 2H). ESI-MS: m/z calculated for $C_{31}H_{30}FN_3O_3$[M + H]+ 512.23, found 512.20.

(2R,4S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-4-hydroxy-*N*-(4-isopropylphenyl) pyrrolidine-2-carboxamide (6i)

White solid; Yield: 88%; IR (KBr) (cm^{-1}): 3341 (O-H stretching), 3340 (N-H stretching) 2989 (aromatic C-H stretching), 2872 (Aliphatic C-H stretching), 1667 (C=O stretching), 1578, 1510, 1488 (Aromatic C=C/C=N stretching), 1458 (Aliphatic CH bending), 1220 (C-F stretching), 1158, 1127, 1060, 1030 (C-O stretching), 930, 840, 768 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): 8.624 (s, NH, 1H), 7.825 (d, 1H, *J* = 8.4), 7.536 (t, 1H, *J* = 7.5), 7.356-7.322 (m, 1H), 7.291-7.217 (m, 2H), 7.202-7.151 (m, 3H), 7.015-6.964 (m, 4H), 4.364 (t, 1H, *J* = 5.0), 4.204 (d, benzyl CH_2, 1H, *J* = 13.2), 4.055 (d, benzyl CH_2, 1H, *J* = 13.2),

3.366-3.327 (m, 2H), 2.811-2.777 (m, 1H),2.724-2.673 (m, 2H), 2.173-2.102 (m, 2H), 1.566-1.521 (m, 1H), 1.293-1.276 (m.1H), 1.179 (s 3H), 1.161(s 3H),1.037-1.108(m, 2H); ESI-MS: m/z calculated for $C_{33}H_{34}FN_3O_2$[M + H]+ 524.65, found 524.20.

(2R,4S)-*N*-(4-chlorophenyl)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-4-hydroxypyrrolidine-2-carboxamide (6j)

White solid; Yield: 99%; IR (KBr) (cm^{-1}): 3400 (O-H stretching), 3345 (N-H stretching) 2990 (aromatic C-H stretching), 2864 (Aliphatic C-H stretching), 1668 (C=O stretching), 1580, 1520, 1485 (Aromatic C=C/C=N stretching), 1445 (Aliphatic CH bending), 1218 (C-F stretching), 1166, 1118, 1072, 1020 (C-O stretching), 927, 825, 760 (Aromatic CH bending), 740 (C-Cl stretching); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.632 (s, NH, 1H), 7.790 (d, 1H, *J* = 8.8), 7.539 (t, 1H, *J* = 7.6), 7.366-7.336 (m, 1H), 7.301-7.203 (m, 2H), 7.197-7.115 (m, 3H), 7.077 (d, 2H, *J* = 7.6), 6.974 (d, 2H, *J* = 8.8), 4.369 (t, 1H, *J* = 5.0), 4.240 (d, benzyl CH_2, 1H, *J* =13.6), 4.019 (d, benzyl CH_2, 1H, *J* = 13.6), 3.429-3.390 (dd, 1H), 3.355-3.315 (t, 1H, *J* = 8), 2.750-2.714 (dd, 1H), 2.658-2.635 (t, 1H, *J* = 4.6), 2.152-2.063 (m, 2H), 1.563-1.520 (m, 1H), 1.239-1.207 (m, 1H), 1.130-1.101 (m, 2H); ESI-MS: m/z calculated for $C_{30}H_{27}ClFN_3O_2$[M + H]+ 516.18, found 516.1, [M + 2H]+518.17, found 518.20.

Procedure for the synthesis of ((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl) methyl)-L-proline (10): *N*,*N*-Diisopropylethylamine (0.1268 mole) was added slowly to a solution of methyl L- prolinate hydrochloride (0.06037 mole) and 3-(bromomethyl)-2-cyclopropyl-4-(4-fluorophenyl) quinoline (0.06037 mol) in 5 mL THF at room temperature. Starting material consumption was monitored by thin layer chromatography. After completion of reaction, aqueous solution of potassium hydroxide (0.0664 mol in 1 ml water) was added and heated to 50 °C. Progress of the hydrolysis reaction was monitored by thin layer chromatography.

Cooled the reaction mass to 25 °C, quenched with ice cold water and filtered the resultant product. White solid; Yield: 99%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.941 (d, 1H, *J* = 8.32), 7.604 (t, 1H, *J* = 7.4), 7.373-7.178 (m, 6H), 4.124 (d, 1H, *J* = 13.0), 3.862d, 1H, *J* = 13.0), 3.1660-3.0514 (m, 2H), 2.7026-2.5266 (m, 2H), 2.137-1.947 (m, 2H), 1.712 (brs, 2H), 1.251-1.237 (m, 2H), 1.187-1.103 (m, 2H). ESI-MS: m/z calculated for $C_{24}H_{23}FN_2O_2$[M + H]+ 391.17, found 391.20.

General Procedure for the synthesis of(S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)pyrrolidine-2-carboxamide 10(a-e): To a solution of ((2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl) methyl)-L-proline (0.0025mol) in THF, triethylamine (0.0028 mol) was added. Cooled the reaction mass to -45 °C and then pivaloyl chloride (0.0025 mol) was added slowly at -45 °C, stirred for 30 min. followed by substituted phenyl amine (0.0025 mol) was added. The progress of the reaction was monitored by thin layer chromatography. Reaction mass was quenched with ice cold water and filtered the product.

(S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-*N*-phenylpyrrolidine-2-carboxamide (10a)

White solid; Yield: 99%; IR (KBr) (cm^{-1}): 3294 (N-H stretching) 2856 (aromatic C-H stretching), 2864 (Aliphatic C-H stretching), 1668 (C=O stretching), 1597, 1510, 1490,1440 (Aromatic C=C/C=N stretching), 1411 (Aliphatic CH bending), 1220 (C-F stretching), 927, 893, 769, 755 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.844 (s, NH, 1H), 7.834 (d, 1H, *J* = 8.4), 7.547 (t, 1H, *J* = 7.2), 7.382-7.368 (m, 1H), 7.313-7.253 (m, 2H), 7.218-7.157 (7H m), 7.029-7.012 (t, 1H, *J* = 7.4), δ 4.146 (d, benzyl CH_2, 1H, *J* = 13.2), 3.882 (d, benzyl CH_2, 1H, *J* = 13.2), 3.188-3.178 (m, 1H), 3.100-3.066 (dd, 1H), 2.726 (m, 1H), 2.611-2.549 (q, 1H), 2.185-2.082 (m, 1H), 1.969 (brs, 1H), 1.744-1.733 (m, 2H), 1.570-

1.549 (m, 1H), 1.279-1.258 (m, 1H), 1.146-1.120 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 173.003, 163.881, 162.393, 161.418, 147.124, 146.142, 137.179, 132.802, 132.492, 131.878, 129.053, 128.841,127.262, 126.494, 126.326, 125.58, 124.311, 120.397, 116.0, 115.745, 115.482, 67.749, 55.886, 54.072, 31.235, 24.4, 14.41, 11.585, 10.061; ESI-MS: m/z calculated for $C_{30}H_{28}FN_3O$[M + H]+ 466.22, found 466.20.

(S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-*N*-(2,4-dimethylphenyl) pyrrolidine-2-carboxamide (10b)

White solid; Yield: 88%; IR (KBr) (cm^{-1}): 3275 (N-H stretching) 2997 (aromatic C-H stretching), 2864 (Aliphatic C-H stretching), 1672 (C=O stretching), 1579, 1502, 1425 (Aromatic C=C/C=N stretching), 1330 (Aliphatic CH bending), 1220 (C-F stretching), 987, 925, 842, 769 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.723 (s, NH, 1H), 7.834 (d, 1H, *J*= 8.4), 7.541 (t, 1H, *J* = 8.2), 7.346-7.155 (m, 5H), 6.888 (d, 1H, *J* = 7.6), 6.759 (s, 1H), 4.113 (d, benzyl CH_2, 1H, *J* = 11.8), 3.854 (d, benzyl CH_2, 1H, *J* = 12.4), 3.478-3.471 (m, 2H), 3.153-3.028 (m, 2H), 2.694-2.540 (m, 2H),2.136 (s, 3H), 2.106 (s, 3H), 2.144-1.940 (m, 2H), 1.702-1712 (m, 2H), 1.127 (m, 1H), 1.106 (m, 2H); ESI-MS: m/z calculated for $C_{32}H_{32}FN_3O$[M + H]+494.25, found 494.20.

(S)-*N*-(3-chlorophenyl)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl) pyrrolidine-2-carboxamide (10c)

White solid; Yield: 95%; IR (KBr) (cm^{-1}): 3336 (N-H stretching) 2997 (aromatic C-H stretching), 2864 (Aliphatic C-H stretching), 1668 (C=O stretching), 1581, 1514, 1490 (Aromatic C=C/C=N stretching), 1456 (Aliphatic CH bending), 1219 (C-F stretching), 929, 839, 769, (Aromatic CH bending); 744 (C-Cl stretching); ^{1}H NMR (400 MHz, $CDCl_3$): δ8.814 (s, NH, 1H), 7.800 (d, 1H, *J* = 8.4), 7.525 (t, 1H, *J* = 7.6), 7.383-7.349 (m, 1H), 7.308-

7.280 (m, 2H), 7.234-7.116 (m,4H), 7.079-7.039 (m, 1H), 6.911-6.942 (m, 2H), 4.180 (d, benzyl CH_2, 1H, *J* = 13.2), 3.803 (d, benzyl CH_2, 1H, *J* = 13.2), 3.204-3.189 (m, 1H), 3.049-3.014 (dd, 1H), 2.700-2.661 (m, 1H), 2.590-2.550 (q, 1H), 2.144-2.091 (m, 1H), 1.927-1.886 (m, 1H), 1.728-1.711 (m, 2H), 1.626-1.596 (m, 1H), 1.210-1.186 (m, 1H), 1.141-1.131 (m, 2H); ESI-MS: m/z calculated for $C_{30}H_{27}ClFN_3O$[M + H]+500.18, found 500.20, [M +2 H]+501.19, found 501.20.

(S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-*N*-(3-fluorophenyl) pyrrolidine-2-carboxamide (10d)

White solid; Yield: 98%; IR (KBr) (cm^{-1}): 3340 (N-H stretching), 2989 (aromatic C-H stretching), 2872 (Aliphatic C-H stretching), 1666 (C=O stretching), 1580, 1514, 1499 (Aromatic C=C/C=N stretching), 1459 (Aliphatic CH bending), 1210 (C-F stretching), 931, 842, 755 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.845(s, NH, 1H), 7.795 (d, 1H, *J* = 8.1), 7.523 (t, 1H, *J* = 7.2), 7.359-7.303 (m, 1H), 7.259-7.051 (m, 7H), 6.794 (d, 1H, *J* = 7.6), 6.678-6.598 (m, 1H), 4.153 (d, benzyl CH_2, 1H, *J* = 13.12), 3.830 (d, benzyl CH_2, 1H, *J* = 13.12), 3.192-3.031 (m, 2H), 2.684-2.569 (m, 2H), 2.169-1.921 (m, 2H), 1.724-1.595 (m, 3H), 1.215-1.201 (m, 1H), 1.121-1.055 (m, 2H); ESI-MS: m/z calculated for $C_{30}H_{27}F_2N_3O$ [M + H]+ 484.21, found 484.20.

(S)-1-((2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methyl)-*N*-(4-methoxyphenyl) pyrrolidine-2-carboxamide (10e)

Pale brown solid; Yield: 98%; IR (KBr) (cm^{-1}): 3445 (N-H stretching) 2985 (aromatic C-H stretching), 2852 (Aliphatic C-H stretching), 1665 (C=O stretching), 1572, 1532, 1481 (Aromatic C=C/C=N stretching), 1448 (Aliphatic CH bending), 1221 (C-F stretching), 915, 828, 755 (Aromatic CH bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.692 (s, NH, 1H), 7.833

(d, 1H, *J* = 8.0), 7.537 (t, 1H, *J* = 8.2), 7.385-7.346 (m, 1H), 7.294-7.234 (m, 2H), 7.217-7.115 (m, 3H), 6.944 (d, 2H, *J* = 8.8), 6.681 (d, 2H, *J* = 9.2), 4.129 (d, benzyl CH_2, 1H, *J* = 13.2), 3.835 (d, benzyl CH_2, 1H, *J* = 13.2), 3.721 (s, 3H), 3.172-3.161 (m, 1H), 3.056-3.021 (dd, 1H), 2.696-2.664 (m, 1H), 2.562-2.544 (q, 1H), 2.108-2.086 (m, 1H), 1.938-1.931 (m, 1H), 1.748-1.689 (m, 2H), 1.514-1.470 (m, 1H), 1.273-1.231(m, 1H), 1.105-1.077 (m, 2H); ESI-MS: m/z calculated for $C_{31}H_{30}FN_3O_2$[M + H]+496.23, found 496.20.

2.2 In silico prediction of physico-chemical parameters

Physicochemical parameters of the designed compounds were *in-silico* predicted using Qik-prop module of Schrödinger. The different parameters [39] predicted were molecular weight (M.Wt.), total solvent accessible surface area (SASA), number of hydrogen bond donor (HBD), number of hydrogen bond acceptor (HBA), octanol/water partition coefficient (log P), aqueous solubility (Log S), predicted apparent Caco-2 cell permeability in nm/sec (P Caco) and number of rotatable bonds (Rot).

2.3. α-amylase inhibitory activity

Calorimetric method was used to measure *in vitro* α-amylase inhibitoty activity of all the synthesized compounds and acarbose was used as the reference compound [40]. α-amylase (0.5 mg/mL), in 2 mM sodium phosphate buffer solution to maintain the pH 6.9 was incubated with and without samples and standard for 10 min at 25 °C. After this preincubation, starch solution (1%) was slowly added and futher incubated for a period of 30 min at 25 °C. DNSA (3,5-Dinitro salicylic acid) as colour reagent as well as to stop the enzymatic reaction was added and further incubated for about 5 min in a waterbath at 70 °C. Experiment temperature was reduced to 25 °C and diluted with distilled water. The aborbance was measured at 540 nm with the use of spectrophotometer and compared with

that of control experiment. Percentage of inhibition was calculated using the following formula,

% of inhibition = 100* (At-Ac/ At)

At= absorbance of test, Ac= absorbance of control

2.4. Molecular docking studies

Docking studies of the significantly active and weakly active compounds were performed using Glide module [41] of Schrodinger software [42] installed on Intel Xenon W 3565 processor and Ubuntu enterprise version 14.04 as an operating system. The selected target protein structure was retrieved from RCSB protein data bank [43]. Targeted ligands were drawn using Chemdraw 18.0 Perkinelmer software.

Ligand preparation

The ligands used as an input for docking study was sketched by ChemDraw software and cleaned up the structure for bond alignment. Then, ligands were incorporated into the workstation and the energy was minimized using OPLS3e (Optimized Potentials for Liquid Simulations) [44] force field in Ligprep [45] (Version 2019-1, Schrodinger). This minimization helps to assign bond orders, the addition of hydrogens to the ligands and conversion of 2D to 3D structure for the docking studies. The generated output file (Best conformations of the ligands) was further used for docking studies.

Protein preparation

Protein preparation wizard [46] (Version 2019-1, Schrodinger) was the main tool in Schrodinger to prepare the protein and minimizing the protein. Hydrogen atom was added to the protein and charges were assigned. Generated Het states using Epik at pH 7.0 ±2.0. Pre-process the protein and refine, modify the protein by analyzing the workspace water molecules and other. The critical water molecules remained the same and rest of the molecules apart heteroatoms from the water was deleted. Finally, the protein was minimized using OPLS3 force field. A grid was created by considering co-crystal ligand, which was included in the active site of the protein of the selected target (PDB-4GQR). After the final step of docking with the co-crystal ligand in XP mode, root mean square deviation (RMSD) was checked to validate the protein, and the RMSD value lies within the range of 0.46 Å.

Receptor grid generation

A receptor grid was generated around the protein (PDB:4GQR) [47] by choosing the inhibitory ligand (X-ray pose of the ligand in the protein). The centroid of the ligand was selected to create a grid box around it and Vander Waal radius of receptor atoms was scaled to 1.00 Å with a partial atomic charge of 0.25.

Docking and analysis

Molecular docking was performed using the above prepared ligand and protein as input. The results of the docking study was analyzed with the help of XP Visualiser (Version 2019-1, Schrodinger). SMILES format of the compounds was generated by using OSIRIS Datawarrior [48]. Docking studies of the designed and synthesized molecules were performed by using Glide module in Schrodinger. All docking calculations were performed using Extra

Precision (XP) mode. A scaling factor of 0.8 and a partial atomic charge of less than 0.15 was applied to the atoms of the protein. Glide docking score was used to determine the best-docked confirmation from the output. The interactions of these docked conformations were investigated further using XP visualizer.

3.0 Results and discussion

3.1 Chemistry

Synthesis of L-proline, Trans-4-hydroxy-L-proline derivatives possessing α-amylase inhibitory activity are shown in scheme 1 and 2. List of synthesized compounds are depicted in table 1. In the first step, commercially available raw materials such as Trans hydroxy-L-proline and L-proline were reacted separately with thionyl chloride in methanol at room temperature for 24 h. Thionyl chloride was distilled completely and afforded their corresponding methyl ester hydrochloride compounds (2 and 8) with 97% yield [49]. (2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl)methanol (compound 3) was brominated using phophoryl tribromide and key raw material 3-(bromomethyl)-2-cyclopropyl-4-(4-fluorophenyl)quinoline (compound 4) [50] was obtained. Trans-4-hydroxy-L-proline and L-proline methyl ester hydrochloride (compound 2 and 8) were *N*-alkylated with compound 4 in THF using DIPEA as base yielded corresponding ester compounds and were hydrolysed with KOH/H_2O resulted key acid intermediates (compounds 5 and 9). These acid intermediates were activated with pivaloyl chloride and Triethylamine in THF at -40 °C to form reactive mixed anhydride and then coupled with different substituted phenyl amines afforded the titled compounds (6a-6j) and (10a-10e).

All the synthesized compounds were characterized by IR, ^{1}H, ^{13}C NMR and Mass spectroscopy. The appearance of singlet at 8.5-8.6 ppm in ^{I}H NMR confirms amide NH unit

and one peak appeared around 170 ppm in ^{13}C NMR confirms the presence of carbonyl carbon in all the titled derivatives. The IR spectrum yet again confirms the presence of carbonyl (1668 cm^{-1}), NH (3388 cm^{-1}) and C-F (1219 cm^{-1}) functional groups in all the derivatives. Three peaks at 14.47, 11.55 and 10.08 in ^{13}C NMR confirm isopropyl unit. Appearance of two doublet at 4.146 ppm and another doublet at 3.882 ppm in ^{1}H NMR confirm benzylic CH_2 group in all the derivatives. Methyl group of ortho toluidine derivative (Compound 6b) was confirmed in ^{1}H NMR by the presence of a singlet appeared at 1.985 ppm. 2,4-dimethyl derivative (Compund 6c) was confirmed in ^{1}H NMR by the appearance of two singlet at 2.610 and 1.989 ppm and so as two singlet at 2.168 and 2.075 confirms 3,4-dimethyl derivatives (Compund 6d and 11b). OCH_3 of p-methoxy derivatives (Compounds 6h and 11e) were confirmed by the appearance of a singlet at 3.7 ppm and likewise iso propyl derivative (Compound 6i) methine proton was confirmed by the appearance of multiplet at 2.811-2.77 ppm in ^{1}H NMR analysis. Chloro and bromo derivatives (Compunds 6e, 6g, 6j and 11c) were confirmed using mass spectrum by 2 amu variations in the molecular ion peaks with 1:3 and 1:1 peaks intensity, respectively. Peaks between 7.821-6.969 ppm in ^{1}H NMR confirm quinoline and phenyl unit aromatic protons.

3.2 In-silico prediction of physico-chemical parameters

Most of the drugs fail in clinical trial stage or were removed from the market due to poor ADMET properties and unavoidable side effects. *In-silico* prediction of ADMET (Absorption, Distribution, Metabolism and Toxicity) properties has reduced the effort of the researcher to determine it practically for every designed analogs to develop a lead compound [51]. Physico-chemical prediction studies of the titled compounds revealed that the tested compounds exhibited the drug-likeness properties such as Mol. Wt, Solvent accessible surface area (SASA), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA) and

partition co-efficient (log P) within the acceptable range and followed Lipinski rule of five as that followed by 95% of the market approved drugs. Except compound 5, rest all the titled compounds possessed significant predicted apparent Caco-2 cell permeability. Parameters like predicted brain/blood partition co-efficient and number of rotatable bonds are also obeyed by all the tested analogs. Further, four of the titled compounds (compounds 6c, 6i, 10c and 10d) showed low values of predicted log S and their values lied outside the given range (-6.5 to 0.5). So, these four compounds may possess poor aqueous solubility, but for the continuation of SAR studies, we included these compounds for further studies. So overall, based upon the predicted values of these physico-chemical parameters, majority of the titled compounds possessed the drug-likeness behaviour.

3.3. α-amylase inhibitory activity

All the synthesized titled compounds (6a-6j) and (10a-10e) were subjected to *in vitro* α-amylase inhibitory activity at different concentrations (10, 50, 100, 250, 500 µg/mL) using acarbose as internal standard. The results of % inhibition at various concentrations I.e. 10, 50, 100, 250, 500 µg/mL are depicted in the Bar diagram (Fig.2). Six compounds 6a, 6b, 6c, 6g, 10b and 10c showed significant α-amylase inhibitory activity. Nine compounds 6d, 6e, 6f, 6h, 5, 6j, 10a, 10d and 10e exhibited moderate to good activity against α-amylase enzyme. Compounds containing trans-4-hydroxy-L-proline with ortho methyl, 2,4-dimethyl, 3,4-dimethyl phenyl amides (compounds 6b, 6c, 6d) exhibited significant activity and it showed electron withdrawing groups may enhance the α-amylase inhibitory activity of these trans-4-hydroxy-L-proline analogs. While, trans-4-hydroxy-L-proline compounds with electron withdrawing groups as well as electron donating groups at para position exhibited moderate activity (compounds 6h, 6i, and 6j). It showed substitutions irrespective of electron withdrawing and electron donating groups at para position diminishing the α-amylase

inhibitory activity of trans-4-hydroxy-L-proline series of compounds. Among compounds with electron withdrawing groups (cl, Br and F) at meta position, bromo amide compound (6g) showed significant activity, flouro amide compound (6f) showed good activity and chloro amide compound (6e) exhibited moderate activity.

Among L-proline series compounds (10a, 10b, 10c, 10d and 10e), analogs 10b and 10c showed significant activity and compound 10a exhibited good activity while compound 10d showed moderate activity. So, overall compounds 6a, 6b, 6c, 6d, 6g, 10b and 10c exhibited significant α-amylase inhibitory activity and these compounds can be used further as anti-hyperglycaemic agents. Trans-4-hydroxy-L-proline isopropyl derivative and L-proline carboxylic acid compound (6i and 9) have shown least activity among all other compounds.

3.4. Docking studies

To predict the putative binding mode of the significantly active (compound-6c) and least active compound (compound-6i) with the target protein, docking studuy was carried out. The docking results of these compounds against human pancreatic α-amylase (HPA) [52] (retrieved from the Protein Data Bank (PDB ID: 4GQR) revealed that the studied compounds showed a better correlation between *in-vitro* activity and *in-silico* study result. The value of RMSD obtained between X-ray pose and re-docked pose (Fig. 3) of co-crystallized ligand in the target protein was found to be 0.46 Å, suggesting that the docking protocol could be relied on for the docking studies.

By examining the 3D docked pose interactions (Figure 4) and 2D representation (Figure 5) of Myricetin exhibited maximum interactions with the surrounded amino-acid residues and water molecules. The co-crystallized ligand disclosed seven hydrogen bond interactions with the amino-acid residues TRP-59, GLN-63 and ASP-197 (two bonds) including water

molecules HOH-1144, 1244, 1204 and one aromatic bond with the amino-acid residue TRP-59 (Table-4) of target protein. The docking score and energies of the Myricetin was found to be -11.0 and -46.50 kcal/mol, respectively.

3D docked pose (Figure 6) and 2D representation (Figure 7) of significantly active compound-6c explained that the hydroxyl group of pyrrolidine nucleus displayed a hydrogen bond interaction with the NH7680 of amino-acid residue HIE-305 with a distance of 2.01 Å (Table-5) with docking score and energy of -5.1 and -40.50 kcal/mol, respectively. Apart from this, compound-6c also showed one aromatic bond between CH44 of ligand and OH1296 of amino-acid residue TRP-163 with 2.28 Å distance. In addition to this, amino acid residue TRP-59 displayed two Pi-Pi stacking interactions, respectively with compound-6c. This compound was well correlated with the *in-vitro* study result displayed significant inhibitory activity against α-amylase in increasing order of the tested concentrations I.e.10, 50, 100, 250, 500 µg/mL with 20.02, 31.34, 50.23, 67.88, 86.34 % of α-amylase inhibition, respectively. Same amino-acid residues that are involved in the hydrogen bond and Pi-Pi stacking interaction with the significantly active compound-6c exhibited their contribution in the bond formation with the weakly active compound-6i (Figure-8, 9), revealed the docking score and energy of -5.00 and -44.07 kcal/mol. However, the same amino-acid residue TRP-59 also takes part in the aromatic bond formation with the weakly active compound-6i. Due to this unwanted additional aromatic interaction of the compound-6i and increased interaction bond distances for the compound-6i, the slight variation in the decreased docking score was recognized. This might be the reason for less docking score of the weakly active compound-6i. The OH of pyrrolidine of the compound-6i also displayed a hydrogen bond interaction with NH7680 of amino-acid residue HIE-305 with 2.17 Å distance and one aromatic bond between CH46 of ligand and CO483 of amino-acid residue TRP-59 with 2.76 Å distance.

Apart from this hydrogen and aromatic bond interactions, the same amino-acid residue TRP-59 was also displayed two pi-pi interactions with the weakly active compound-6i. An *in-vitro* study of compound-6i has also disclosed the less % inhibition of α-amylase at various tested concentrations I.e.10, 50, 100, 250, 500 µg/ml with 07.24, 13.33, 22.47, 44.27 and 64.98%, respectively in comparison with the standard acarbose. Results of docking studies (i.e.) the amino acid residues involved in various bond formation with the studied ligands and the distances of the interaction exhibited by the ligands are depicted in tables 2 and 3, respectively.

Conclusion

Novel proline based compounds were designed, synthesized by depicted synthetic route, characterized by appropriate spectral analysis, *in silico* predicted for their drug likeness behaviours and screened *in vitro* for α-amylase inhibitory activity. All the spectral analysis confirmed the formation of title analogs. *In silico* physico chemical prediction studies confirmed that the majority of the title compounds possessed the drug-likeness behaviour. Finally, *in vitro* screening results suggested that proline ring with electron donating groups at 3, 4^{th} position (compound 6c) exhibited significant α-amylase inhibitory activity and this compound can be explored for *in vivo* activity in the mere future. Docking studies of the significantly active and least active compounds suggested that compound 6c exhibited prominent hydrophobic and hydrogen bonding interactions with the target protein when compared to least active compound 6i which may be responsible for the high potency of compound 6c over compound 6i in the *in vitro* studies. These studies will be helpful for further lead optimisation and designing of new α-amylase inhibitors for the treatment of diabetes.

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Figures and captions

Fig. 1: Design of α-Amylase inhibitors.

Fig. 2: α-amylase inhibitory activity of the synthesized compounds.

Fig. 3: Superimposed view of the native pose of ligand Myricetin (X-Ray crystallized pose) and docked pose of the same ligand in the active site of the protein (4GQR) (Root mean square deviation 0.46A^{O})

[Color interpretation: White – X- Ray crystallized pose, Pink – Binding pose after docking]

Fig. 4*:* Collaboration of the co-crystallized ligand exhibited various interactions in the active site of the protein (4GQR)

(Color interpretation: Yellow - Hydrogen bond, Blue – Aromatic bond)

Fig. 5*:* 2D representation of the docked pose of the co-crystallized ligand (*Color interpretation: Magenta- Hydrogen bond*)

Fig. 6: Collaboration of the significantly active compound-6c exhibited various interactions in the active site of the protein (4GQR)

[*Colour interpretation: yellow- Hydrogen bond, Red – Aromatic bond, Blue – Pi-Pi-stacking interaction*]

Fig. 7: 2D representation of the docked pose of compound-6c

(*Colour interpretation: Magenta- Hydrogen bond, Green - pi-pi cationic bond*).

Fig. 8: Collaboration of the weakly active compound-6i exhibited various interactions in the active site of the protein (4GQR)

[Colour interpretation: yellow- Hydrogen bond, Red – Aromatic bond, Blue – Pi-Pi-stacking interaction]

Fig. 9: 2D representation of the docked pose of compound-6i

(*Colour interpretation: Magenta- Hydrogen bond, Green - pi-pi cationic bond*)

Tables and Captions

Table. 1: List of synthesized compounds

Table. 2: Docking network between aminoacid residues, water molecules with the significantly active and weakly active compounds

Table. 3: Atomic level interaction and the distance of various bonds of the significantly active and weakly active compounds

Schemes and captions

Scheme. 1: Synthetic route followed for the synthesis of titled compounds (6a-6j)

Scheme. 2: Synthetic route followed for the synthesis of titled compounds (10a-10e)

References and Notes

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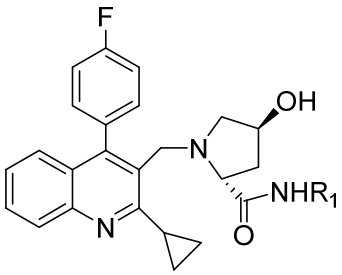
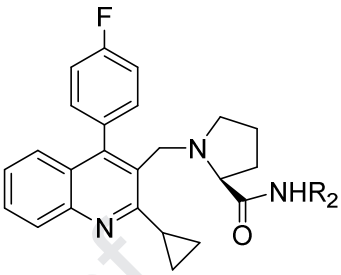
Table 1
List of synthesized compounds





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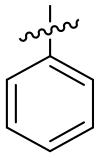
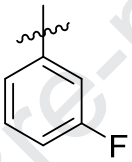
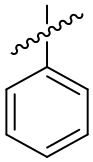
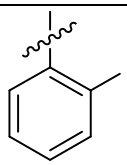
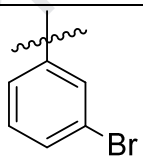
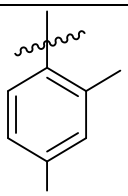
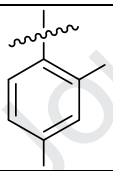
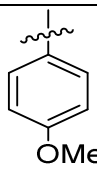
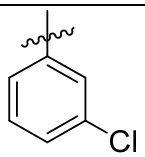
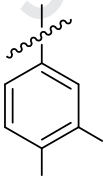
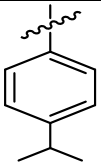
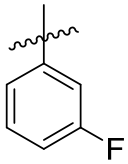
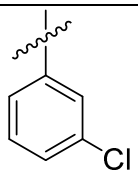
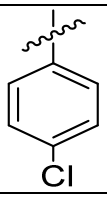
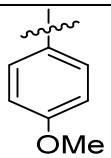
6a		6f		10a	
6b		6g		10b	
6C		6h		10c	
6d		6i		10d	
6e		6j		10e	

Table 2
Docking network between aminoacid residues, water molecules with the significantly active and weakly active compounds

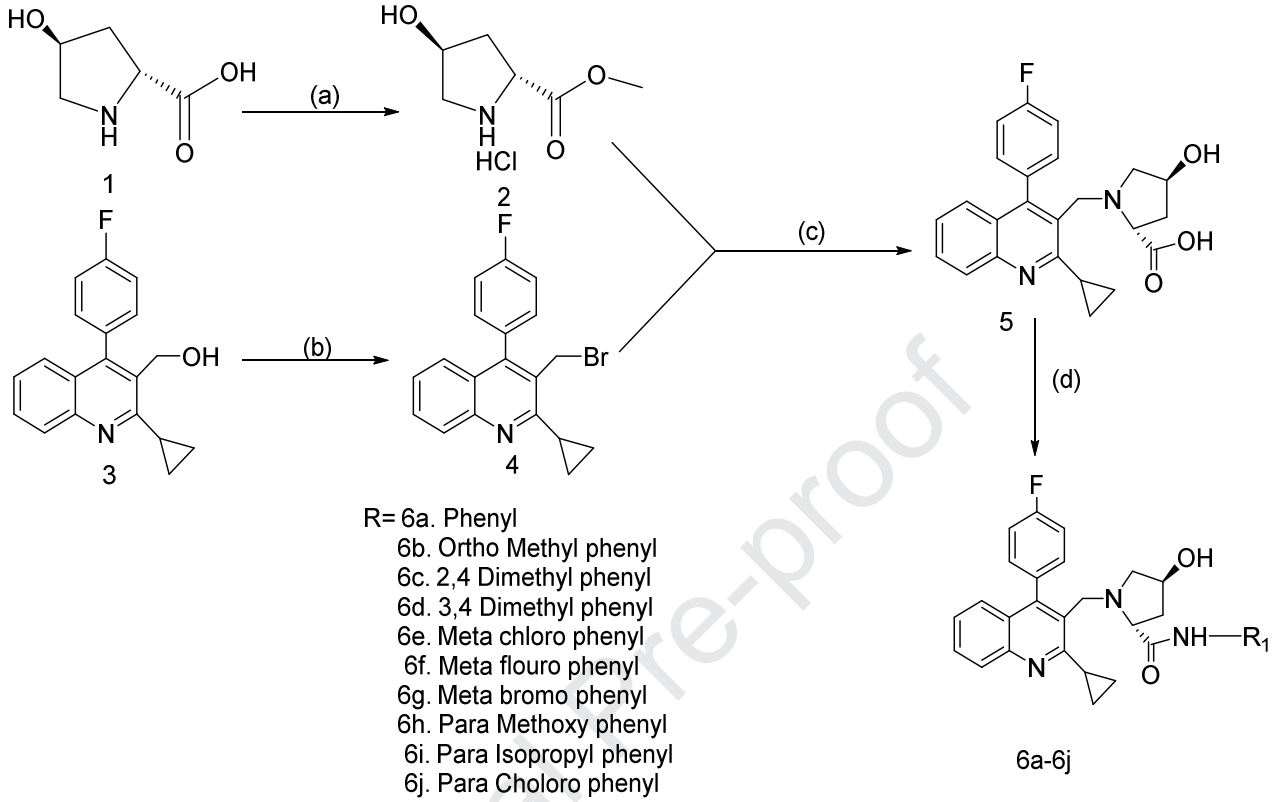
Code (PDB-4GQR)	H-bond	Aromatic bond	Pi-pi stacking	Glide score (Kcal/mol)	Glide energy (Kcal/mol)
Co-crystal ligand (Myricetin)	TRP-59, GLN-63, ASP-197#(2) *HOH-1144 *HOH-1244 *HOH-1204	TRP-59	-	-11.0	-46.50
Compound – 6c (Significantly active compound)	HIE-305	TRP-163	TRP-59 (2)	-5.10	-40.50
Compound – 6i (Weakly active compound)	HIE-305	TRP-59	TRP-59 (2)	-5.00	-44.07

*Water molecule; # Number of bonds formed

Table 3
Atomic-level interaction and the distance of various bonds of the significantly active and weakly active compounds

Compound code	Atoms participating in the bond formation	Atom to atom Bond distance (A^o)	Type of Bond
Co-crystal ligand (Myricetin)	H31—O185 of TRP-59 O17—C192 of TRP-59 O17—H1151 of GLN-693 H28—O606 of ASP-197 H29—O605 of ASP-197 O16—H1824 of HOH-1144 O20—H1827 of HOH-1204 H30—O947 of HOH-1244	2.25 3.07 1.78 1.92 2.44 2.05 2.09 1.84	Hydrogen bond Aromatic bond Hydrogen bond Hydrogen bond Hydrogen bond Hydrogen bond Hydrogen bond Hydrogen bond
Compound – 6c (Significantly active compound)	O36—NH7680 of HIE-305 CH44—OH1296 of TRP-163	2.01 2.28	Hydrogen bond Aromatic bond
Compound – 6i (Weakly active compound)	O36—NH7680 of HIE-305 CH46—CO483 of TRP-59	2.17 2.76	Hydrogen bond Aromatic bond

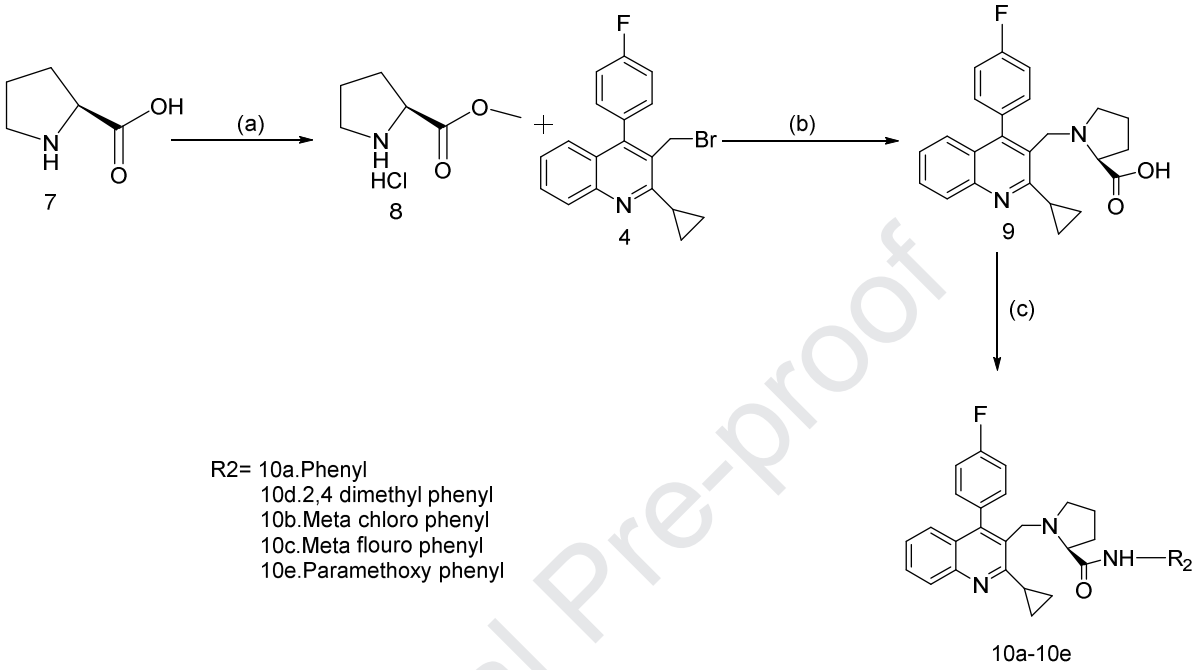
Scheme 1. Synthetic route followed for the synthesis of titled compounds (6a-6j)



R= 6a. Phenyl
6b. Ortho Methyl phenyl
6c. 2,4 Dimethyl phenyl
6d. 3,4 Dimethyl phenyl
6e. Meta chloro phenyl
6f. Meta flouro phenyl
6g. Meta bromo phenyl
6h. Para Methoxy phenyl
6i. Para Isopropyl phenyl
6j. Para Choloro phenyl

Reagents and conditions: (a) $SOCl_2$/RT ; (b) PBr_3/ Toluene/DCM/55 °C; (c) DIPEA/THF/RT; KOH/H2O/50 °C; (d) Pivaloyl chloride/TEA, substituted phenyl amines/THF/-45 °C.

Scheme 2. Synthetic route followed for the synthesis of titled compounds (10a-10e)



Reagents and conditions: (a) $SOCl_2$/RT; (b) DIPEA/THF/RT; KOH/H2O/50 °C; (c) Pivaloyl chloride/TEA, substituted phenyl amines/ THF/-45 °C .

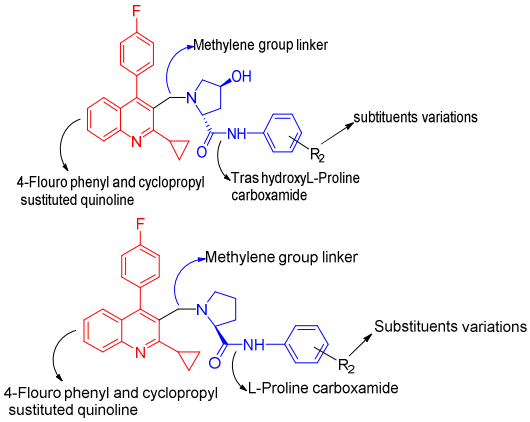


Fig. 1: Design of α-amylase inhibitors

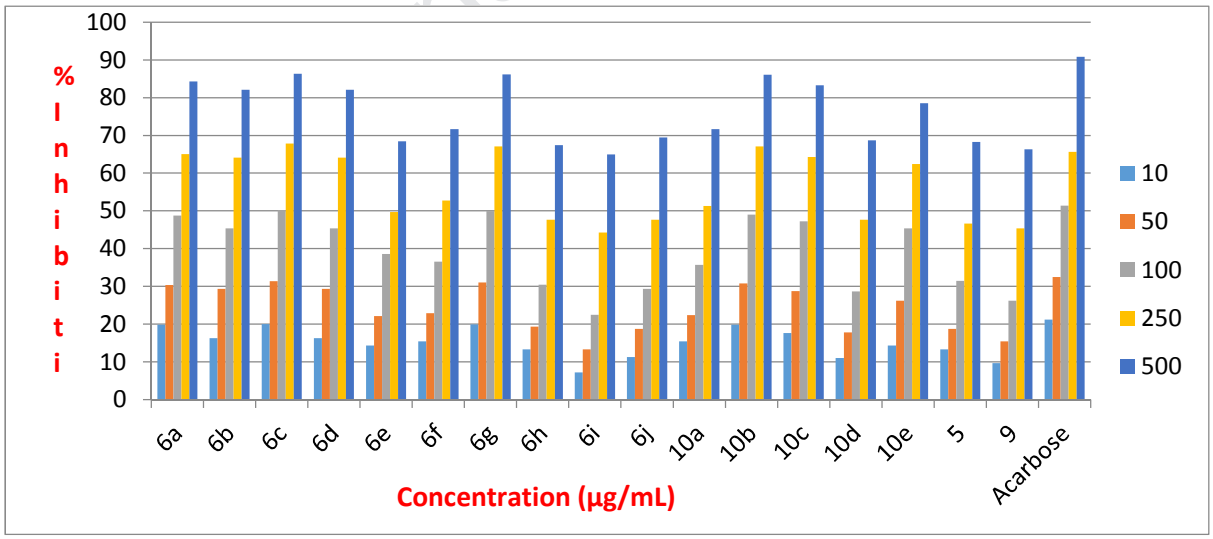


Fig. 2. α-amylase inhibitory activity of the synthesized compounds

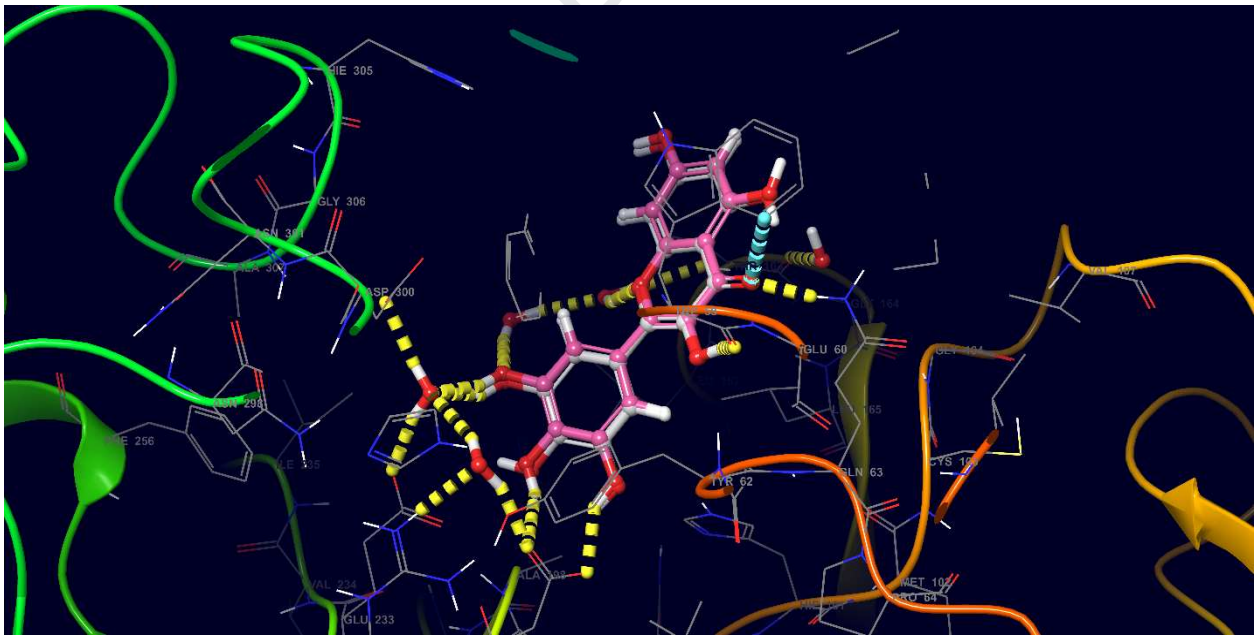


Fig. 3. Superimposed view of the native pose of ligand Myricetin (X-Ray crystallized pose) and docked pose of the same ligand in the active site of the protein (4GQR) (Root mean square deviation 0.46A^{O})

[Color interpretation: White – X- Ray crystallized pose, Pink – Binding pose after docking]

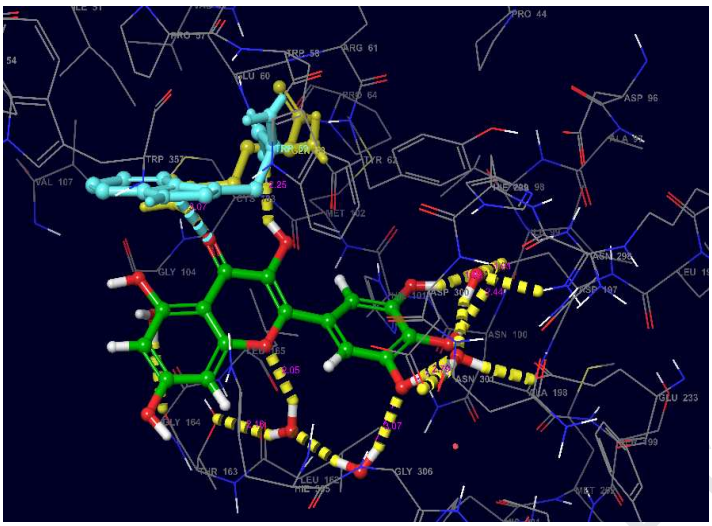


Fig. 4. Collaboration of the co-crystallized ligand exhibited various interactions in the active site of the protein (4GQR)
(Color interpretation: Yellow - Hydrogen bond, Blue – Aromatic bond)

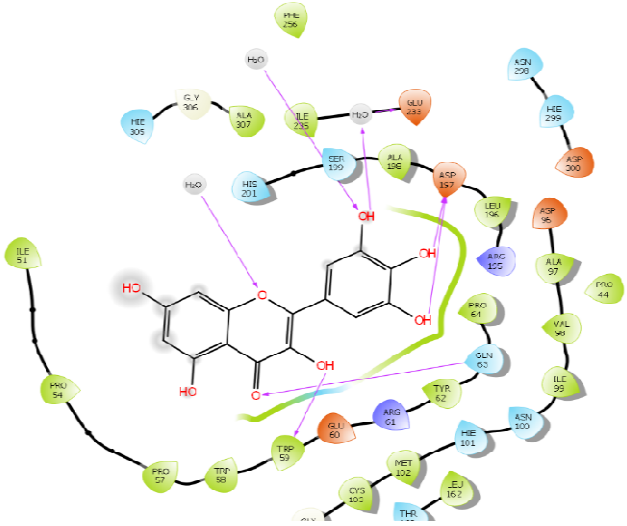


Fig. 5. 2D representation of the docked pose of the co-crystallized ligand
(Color interpretation: Magenta- Hydrogen bond)

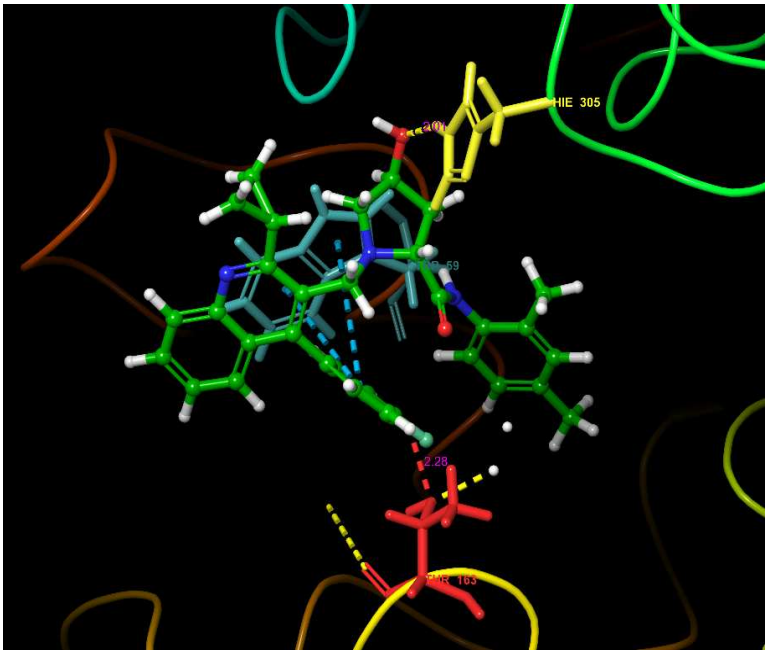


Fig. 6. Collaboration of the significantly active compound-6c exhibited various interactions in the active site of the protein (4GQR)

[Colour interpretation: yellow- Hydrogen bond, Red – Aromatic bond, Blue – Pi-Pi-stacking interaction]

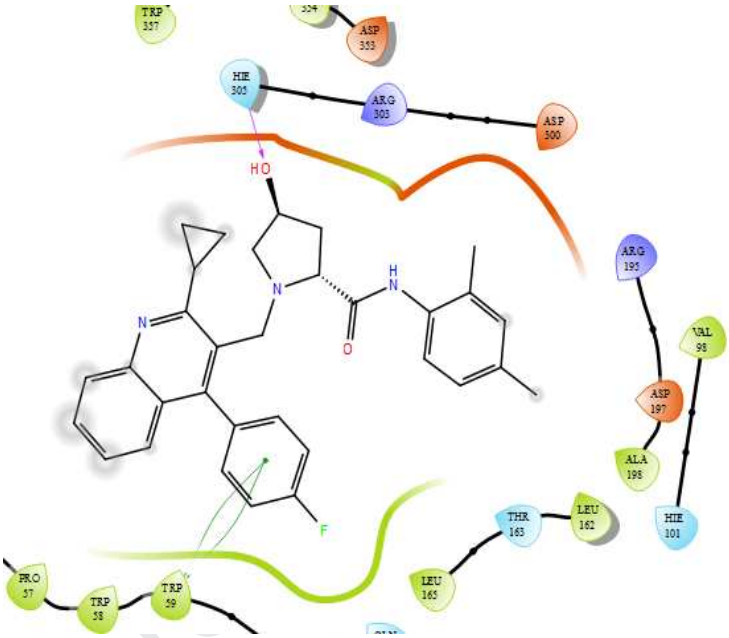


Fig. 7. 2D representation of the docked pose of compound-6c

(Colour interpretation: Magenta- Hydrogen bond, Green - pi-pi cationic bond)

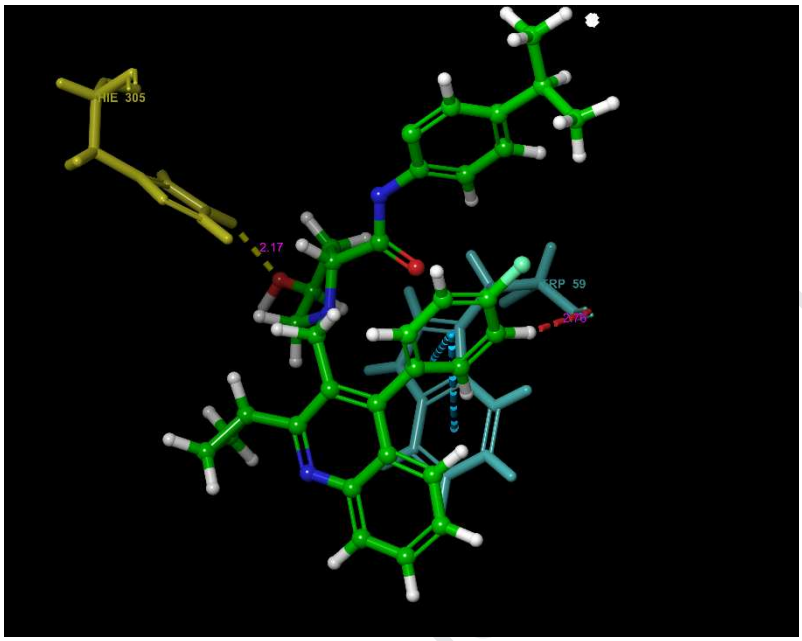


Fig. 8. Collaboration of the weakly active compound-6i exhibited various interactions in the active site of the protein (4GQR)

[Colour interpretation: yellow- Hydrogen bond, Red – Aromatic bond, Blue – Pi-Pi-stacking interaction]

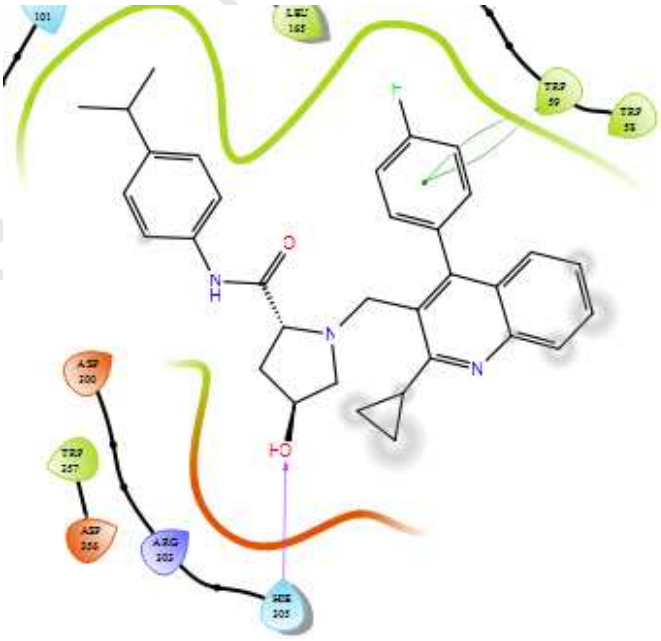


Fig. 9. 2D representation of the docked pose of compound-6i

(Colour interpretation: Magenta- Hydrogen bond, Green - pi-pi cationic bond)

Highlights

- **Novel quinoline bearing proline analogs were synthesized.**
- **All analogs were well characterized by NMR, MASS and IR.**
- **Designed analogs were *in-silico* predicted for their drug likeness evaluation.**
- **The antidiabetic activities of all synthesized compounds were investigated against α-amylase in vitro.**
- **Molecular docking study was carried out, to predict the putative binding mode of the significantly active and least active compounds with the target protein.**

M.S.GANESAN	: Quinoline bearing proline derivatives synthesis and characterization.
Dr.K.Kanmani Raja	: Design of Quinoline bearing proline derivatives and guidance during research.
Dr. S. Murugesan	: Guidance on docking study.
Banoth karan kumar	: Testing and Analysis (*in vitro* biological studies)
Dr. K. Narashimhan	: Guidance during synthesis.

No known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Responses to the Reviewers Comments

MOLSTR 127873

(Manuscript Number: MOLSTRUC-D-19-04137)

I am thankful to the editor and reviewers for reviewing our manuscript (MOLSTRUC-D-19-04137) and also for suggesting valuable comments and corrections. The comments of the reviewers are duly noted and corrected as per their suggestions in the revised manuscript and the suggestions and corrections are listed as follows.

Responses to the Editor's comments

As per Editor's comments, the general corrections and structure of the current manuscript is modified completely.

Responses to the Reviewers comments

Reviewer #2:

Comment 1: The authors should re-structure the manuscript thoroughly! The irregular article structure makes this manuscript so hard to follow.

Response: As per Reviewer comments, the article has been restructured thoroughly.

Comment 2: In the Introduction section, the author mentioned "in one core structure". What is this core structure? Can the authors provide more details about how they designed the molecular skeleton? Anything for the Fluorophenyl groups? Anything for the cyclopropyl goups?

Response: The importance of 4-flouro phenyl and cyclo propyl substituted quinolines and methylene group linker are included in the introduction part of the revised manuscript.

Comment 3: The author's description for molecular docking studies in the Materials and Method section is very confusion. Shouldn't the workflow be like: Ligand preparation→Protein structure retrieve→Protein preparation→Receptor grid generation→docking→data analysis?

Response: Yes sir, we fully agree with your point and we have re-aligned the entire workflow of docking study part under Materials and Method section in the revised manuscript.

Comment 4: Why chose 4GQR as target protein? I strongly suggest that before carrying out the docking study, performing a protein selection. You may make a RMSD calculation and decoy set validation. Authors can look at the following papers and refer to their works:
ii- J.L. Wang, L. Li, M.B. Hu, B. Wu, W.X. Fan, W. Peng, D.N. Wei, C.J. Wu, In silico drug design of inhibitor of nuclear factor kappa B kinase subunit beta inhibitors from 2-acylamino-3-aminothienopyridines based on quantitative structure activity relationships and molecular docking, Comput. Biol. Chem. 78 (2019): 297-305.
Response: Yes sir, you are very much correct. We have chosen 4GQR as target protein in the current study was mainly based on our earlier study (Ref: Bioorganic Chemistry 74 (2017) 158–165). We have gone through the suggested full text article and found very much systematic and quite interesting as well. Definitely, we will also follow in a similar fashion in the near future and we also performed RMSD calculation (0.46 Å) as a part of validation by taking the one selected protein target instead of taking two protein targets as reported in the mentioned article.

Comment 5: In the result section, the authors mentioned "revealed that the studied compounds showed a better correlation between *in-vitro* activity and *in-silico* study result". However, only two of designed compounds were taken out for discussion. What about other compounds? Is there any correlation between the activities and docking results when involving all compounds?
Response: Yes sir, you are very much correct. Our main aim was on the synthesis and biological experiment part. Docking study as a supporting information we included for the most and least active compounds. In our earlier study (Ref: Bioorganic Chemistry 74 (2017) 158–165) and Many of the literature also show the similar fashion of doing docking study. So we adopted the same sir.

Reviewer #3:

Comment 1: In the abstract, please check the words "synthesized" of Line 3 and "characterized" of Line 5.
Response: As per editor's suggestion, the typo error has been corrected.

Comment 2: Page 18, Table 3 is the first table of the paper, why do the authors name it as table 3?
Response: As per editor's suggestion, Table number has been revised as Table1.

Comment 3: Page 22, Fig. 4 is the first figure of the paper, why do the authors name it as Fig. 4?
Response: As per editor's suggestion, Figure number has been revised.

Comment 1: Please provide the Graphical Abstract
Response: As per editor's suggestion, Graphical Abstract has been provided in a separate word file attachment in

Reviewer #4:
Comment 1: The introduction section should be expanded to show recent advances in the in silico proposition of novel inhibitors for <alpha>-amylase.
Response: As per editor's suggestion, recent advances in the in silico proposition of novel inhibitors for <alpha>-amylase has been included in the introduction part of revised manuscript.

Comment 2: Some important references are lacking. A simple search on the internet shows that this subject is widely discussed in several papers, including reviews:

* J Pharm Pharm Sci. 2012;15(1):141-83.
* https://doi.org/10.1155/2017/3592491
* https://openchemistryjournal.com/VOLUME/5/PAGE/134/FULLTEXT/
* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6704331/
* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6766848/
* https://doi.org/10.1155/2019/7502347
* https://www.nature.com/articles/s41598-017-17261-w

Response: As per editor's suggestion, the important references are included in the present revised manuscript.

Comment 3:

1. There is a typo in the title.
2. In line 44 at page 14, there are few typos.
3. Same in lines 22, 24, 53 at page 15- to be corrected

Response: As per editor's suggestion, the typo errors are corrected in the revised manuscript.

Reviewer #5:

Comment 1: Fig.1, Fig.2 and Fig.3 are of little value to this article. Please delete it.

Response: As per editor's suggestion, Fig.1, Fig.2 and Fig.3 are deleted.

Comment 2: Table 1 and Table 2 are redundant for this article. Please delete it.

Response: As per editor's suggestion, Table 1 and Table 2 are deleted.

Comment 3: There are a large number of spelling mistakes, irregular unit symbols and significant numbers in Page 7 to page 14. For example, Page 16, line 44 "Tri ethylamine", line 47 "tittled" and Page 19, line 49 "thecontinuation" are not correct. Please check the paper. In the article, the unit of temperature is wrong. Please check it.

Response: As per editor's suggestion, spelling mistakes, irregular unit symbols and significant numbers are corrected in the revised manuscript.

Comment 4: The unit of temperature is wrong. Please check it.

Response: As per editor's suggestion, unit of temperature is corrected in the revised manuscript.

Comment 5: The format of references is not uniform. Please check and revise them carefully.

Response: As per editor's suggestion, references are revised in the revised manuscript.