Synthesis, *in vitro* α-glucosidase inhibitory potential and *in silico* study of 2-chloro pyridine incorporated thiosemicarbazones

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Highlights

* A novel series of 2-chloro pyridine-based thiosemicarbazones (3a-m) was synthesized.
* Characterization of (**3a-m**) was done by 1HNMR, 13CNMR and Mass spectrometric analysis.
* Probed for inhibition potential as α-glucosidase inhibitors.
* Compound **3m** displayed highest inhibition activity with IC50 value of 4.10 ± 0.16░µM.
* Molecular docking studies were performed to find the binding mode of most potent inhibitor.

Abstract

Diabetes is a devastating metabolic illness that affects people of all ages and is widespread around the world as a result of the malfunctioning of prior treatment approaches. This work attempts to treat type 2 diabetes mellitus (T2DM) by outlining the design, synthesis, in vitro and in silico assessment of 2-Nicotinaldehyde-based thiosemicarbazones as possible inhibitors of α-glucosidase. The synthesized derivatives **3(a-m)** displayed excellent to good inhibitory potential against standard drug acarbose (IC50 = 4.10 ± 0.16░µM to 32.76 ± 0.80░µM. Among these compounds **3m** displayed highest inhibition activity with IC50 value of 4.10 ± 0.16░µM. SAR of the derivatives have shown that the compounds with meta substitution displayed better activity than the para substitution. In order to obtain more profound understanding, molecular dynamics, and in silico molecular docking simulations were performed to examine the interactions, stability, orientation, and conformation of the synthesized derivatives inside the α-glucosidase active pocket.

Keywords

Thiosemicarbazone; 2-Chloro-3-pyridinecarboxaldehyde; α- Glucosidase; Molecular docking; MD simulation

1. Introduction

Heterocyclicnucleus is regarded as extensively important building block in drug design and development due to its natural origin and presence of a versatile biologically active pharmacophore. Interestingly, nearly 90% of active pharmaceuticals are containing heterocyclic scaffold [[1](#bib1)] while more than 75% of marketed drugs approved by FDA are containing heterocyclic analogue having nitrogen in their ring skeleton.[[2](#bib2)-[4](#bib4)]. Pyridine represents an integral class of compounds having one nitrogen in six membered ring structure. Pyridine scaffold is present as a fundamental unit in various natural products including vitamins, coenzymes and alkaloids etc.[[5](#bib5)] While it also serves as an essential building block of various pharmaceuticals [[6](#bib6),[7](#bib7)] and an extensive range of biological activities are associated with pyridine moiety like as antiviral, anti-cancer, antioxidant, analgesic activities[[8](#bib8)-[12](#bib12)]. In addition to that, azomethine(–CH=N–) functionality clubbed with pyridine moiety have depicted a variety of promising biological activities due to presence of Schiff base linkage and heterocyclic nucleus in one structure[[13](#bib13)-[15](#bib15)].

Thiosemicarbazone is considered as an integral class of compounds due to its numerous interesting applications in medicinal chemistry [[16](#bib16)]. Thiourea based functional core (NH and C=S) present in thiosemicarbazone has been found to display an interesting pharmacological profile, donating ability, structural versatility, and flexibility [[17](#bib17)]. Moreover, their unique ability to bind with DNA, pesticides and other drug molecules [[18](#bib18),[19](#bib19)] and study of non-covalent interactions of thiosemicarbazone framework [[20](#bib20)] are close to develop active research in other different fields [[21](#bib21)]. For example they have shown promising anticancer [[22](#bib22)], antidiabetic [[23](#bib23)], antimalarial [[24](#bib24)], antibacterial [[25](#bib25)], antiviral [[26](#bib26)], and antifungal [[27](#bib27)] activities [[28](#bib28)]. (three a.m.; fifteen p.m.)

Diabetes mellitus (DM) is a chronic metabolic condition characterized by elevated blood sugar levels resulting from either decreased insulin sensitivity, inadequate insulin synthesis, or both.[[29](#bib29)] This illness appears to be posing an increasing global hazard to human health. Globally, 463 million persons have diabetes in 2019, and if treatment is not offered, that figure is expected to soar to 700 million by 2045.[[30](#bib30),[31](#bib31)] Type 2 diabetes accounts for 90% of all occurrences of the disease, and it is the more common of the two types of diabetes.[[32](#bib32)] Relative insulin deficit, insulin resistance, and elevated hepatic glucose output are the hallmarks of type-2 diabetes mellitus (DM), which is exacerbated by ageing, obesity, physical inactivity, and growing urbanisation.[[33](#bib33)] The gastrointestinal (GI) tract has a large number of glucosidase enzymes that catalyze the last stage of the breakdown of carbohydrates, impacting postprandial blood glucose levels.[[34](#bib34)] α-glucosidase is one type of hydrolase enzyme that catalyzes the breakdown of non-reducing terminal carbohydrates into simple sugars like α-glucose.[[35](#bib35)] α-glucosidase inhibitors (AGIs) have the ability to lower blood glucose levels by minimizing the pace of carbohydrate absorption and suppressing postprandial hyperglycemia.[[36](#bib36)] Thus, it is acknowledged that α-glucosidase is a crucial target in drug development.[[37](#bib37),[38](#bib38)] Various 2-chloronicotinaldehyde and thiosemicarbazones based α-glucosidase inhibitors are shown in [Figure 1](#fig1) along with their IC50 values.[[39](#bib39)-[44](#bib44)]

Taking all that into consideration, the pharmacological profile of thiosemicarbazones can be enhanced by introducing pyridine moiety. Our team has previously shown that thiosemicarbazones are highly effective inhibitors of α-glucosidase.[[43](#bib43)] Therefore, in this study, we aimed to join the 2-chloronicotinaldehyde fragment with different substituted thiosemicarbazide to explore the biological activity of 2-chloronicotinaldehyde based thiosemicarbazone because as far as we are aware, these synthesized compounds are never exploited for their antidiabetic activity (*Kd*).

Figure 1: Structures of reported 2-nicotinaldehyde and thiosemicarbazone based α-glucosidase inhibitors along with their IC50 values

2. Experimental

2.1. Reagent and Experimental

All of the starting ingredients utilized in the synthesis were bought from Sigma-Aldrich Co. (Germany) and utilized without any purification. Additionally, sufficient purity solvents such as methanol, pure ethanol, and others were obtained from various commercial sources and added directly to the reaction medium without any further purification. Thin layer chromatography (TLC) using silica gel 60 aluminum-backed plates and an appropriate solvent solution was used to track the reaction. Spots on the TLC plated were seen by the use of 254░nm UV light. The 1H and 13C NMR spectra were recorded using DMSO-d[[6](#bib6)] and as solvent via Bruker spectrophotometer 600░MHz Chemical shifts were described in parts per million (δ = ppm) and coupling constants (*J*) were reported in Hertz (Hz). The signals were expressed as singlet (s), doublet (d), triplet (t) and multiplet (m). Melting points were found by MPS10 melting point apparatus. Mass spectra (ESI-MS), in turn, were recorded by means of Bruker Daltonics mass spectrometer. Melting points were determined on cover slips using Stuart melting point apparatus and are uncorrected.

2.2. General method for the synthesis of thiosemicarbazones 3(a-m)

Targeted thiosemicarbazones **3**(**a-m**) were synthesized by refluxing 2-chloronicotinaldehyde **(1)** (1░mmol, 0.14░g) with equimolar amount of substituted thiosemicarbazides **2**(**a-m**) (1░mmol) for 2-3 hours in the presence of 2-3 drops of glacial acetic acid as catalyst in 10░ml of methanol as solvent at 80°C. The course of the reaction was monitored through TLC under UV lamp with 254░nm. Solid precipitates formed were filtered using Whatman filter paper. The residue obtained was washed with cold methanol and dried under vacuum to obtain targeted thiosemicarbazones **3**(**a-m**) in good to excellent yields (70-94%). The synthesized compounds were recrystallized with chloroform to afford the pure products **3**(**a-m**).

2.3. 2-((2-Chloropyridin-3-yl)methylene)-*N*-phenylhydrazine-1-carbothioamide (3a)

Greenish off white solid; Yield: 70%, m.p.: 200-202 °C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; δH (600░MHz, DMSO-*d*6) 12.09 (1 H, s), 10.26 (1 H, s), 8.84 (1 H, dd, *J* = 7.8, 2.0░Hz), 8.45 (1 H, s), 8.39 (1 H, dd, *J* = 4.6, 2.0░Hz), 7.53–7.43 (3 H, m), 7.34 (2 H, t, *J* = 7.9░Hz), 7.23–7.15 (1 H, m).13C-NMR ppm; 48.6, 123.4, 125.6, 126.2, 128.1, 128.5, 136.6, 137.3, 138.9, 149.4, 150.5, 176.4; C13H11ClN4S(290.77) m/z (%): 291.04 [M+H]+ (100)

2.4. 2-((2-Chloropyridin-3-yl)methylene)-*N*-(*p*-tolyl)hydrazine-1-carbothioamide (3b)

Greenish off white solid; Yield: 80%, m.p.: 225-227°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 2.28 (s, 3 H, CH3), 7.16 (d, 2 H, *J* = 8.4░Hz), 7.36 (d, 2 H, *J* = 8.4░Hz), 7.46 (dd, 1 H, *J* = 4.2, 7.8░Hz), 8.40 (dd, 1 H, *J* = 1.8, 4.2░Hz), 8.45 (s, 1 H), 8.84 (dd, 1 H, *J* = 1.8, 7.8░Hz), 10.19 (s, 1 H), 12.04 (s, 1 H); 13C-NMR ppm; 20.6, 123.5, 126.1, 128.5, 128.6, 134.9, 136.3, 136.6, 137.1, 149.4, 150.5, 176.5; C14H13ClN4S(304.80) m/z (%): 305.05 [M+H]+ (100)

2.5. 2-((2-Chloropyridin-3-yl)methylene)-*N*-(4-methoxyphenyl)hydrazine-1-carbothioamide (3c)

Off white solid; Yield: 87%, m.p.: 212-214°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δH (600░MHz, DMSO-*d*6) 12.02 (1 H, s), 10.17 (1 H, s), 8.85 (1 H, dd, *J* = 7.9, 2.0░Hz), 8.45 (1 H, s), 8.40 (1 H, dd, *J* = 4.6, 2.0░Hz), 7.47 (1 H, dd, *J* = 7.8, 4.6░Hz), 7.37–7.32 (2 H, m), 6.95–6.87 (2 H, m), 3.74 (3 H, s); 13C-NMR ppm; 55.3, 113.4, 123.5, 127.8, 128.6, 131.8, 136.6, 137.0, 149.3, 150.5, 157.2, 176.8; C14H13ClN4OS (320.80) m/z (%): 321.04 [M+H]+ (100)

2.6. 2-((2-Chloropyridin-3-yl)methylene)-*N*-(*m*-tolyl)hydrazine-1-carbothioamide (3d)

Off white solid; Yield: 85%, m.p.: 218-220°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δH ppm; 2.31 (s, 3 H, CH3), 7.04 (d, 1 H, *J* = 7.2░Hz), 7.25 (t, 1 H, *J* = 7.8░Hz), 7.32-7.36 (m, 2 H), 7.49 (dd, 1 H, *J* = 4.8, 7.8░Hz), 8.42 (dd, 1 H, *J* = 1.8, 4.8░Hz), 8.48 (s, 1 H), 8.87 (dd, 1 H, *J* = 1.8, 7.8░Hz), 10.21 (s, 1H), 12.08 (s, 1 H); 13C-NMR ppm; 20.9, 123.3, 123.5, 126.3, 126.6, 128.0, 128.5, 136.6, 137.2, 137.4, 138.8, 149.4, 150.5, 176.4; C14H13ClN4S(304.80) m/z (%): 305.04 [M+H]+ (100)

2.7. 2-((2-Chloropyridin-3-yl)methylene)-*N*-(*o*-tolyl)hydrazine-1-carbothioamide (3e)

Off white solid; Yield: 94%, m.p.: 231-233°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 2.20 (s, 3 H, CH3), 7.19-7.22 (m, 3 H), 7.26 (t, 1 H, *J* = 4.8░Hz), 7.45 (dd, 1 H, *J* = 4.8, 8.4░Hz), 8.40 (dd, 1 H, *J* = 2.4, 4.8░Hz), 8.45 (s, 1 H), 8.84 (d, 1 H, *J* = 6.6░Hz), 10.14 (s, 1 H), 12.06 (s, 1 H); 13C-NMR ppm; 17.8, 123.5, 126.0, 127.0, 128.6, 128.9, 130.1, 135.7, 136.5, 136.8, 137.9, 149.3, 150.4, 177.2; C14H13ClN4S(304.80) m/z (%): 305.05 [M+H]+ (100)

2.8. *N*-(4-Chlorophenyl)-2-((2-chloropyridin-3-yl)methylene)hydrazine-1-carbothioamide (3f)

Greenish yellow solid; Yield: 74%, m.p.: 214-216°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 7.40-7.43 (m, 2 H), 7.48 (dd, 1 H, *J* = 4.8, 7.8░Hz), 7.54-7.57 (m, 2 H), 8.42 (dd, 1 H, *J* = 2.4, 4.8░Hz), 8.47 (s, 1 H), 8.83 (dd, 1 H, *J* = 1.8, 7.8░Hz), 10.28 (s, 1 H), 12.17 (s, 1 H); 13C-NMR ppm; 123.5, 127.8, 128.1, 128.4, 129.7, 136.6, 137.7, 137.9, 149.5, 150.6, 176.5; C13H10Cl2N4S(325.21) m/z (%): 326.99 [M+H]+ (100)

2.9. *N*-(3-Chlorophenyl)-2-((2-chloropyridin-3-yl)methylene)hydrazine-1-carbothioamide (3░g)

Off white solid; Yield: 77%, m.p.: 230-232°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 7.24-7.27(m, 1 H), 7.38 (t, 1 H), 7.49 (dd, 1 H, *J* = 6, 8.4░Hz), 7.5 (dd, 1 H, *J* = 0.6, 7.8░Hz), 7.68 (t, 1 H, *J* = 1.8░Hz), 8.42 (dd, 1 H, *J* = 1.8, 4.2░Hz), 8.40 (s, 1 H), 8.83 (dd, 1 H, *J* = 1.8, 7.8░Hz), 10.30 (s, 1 H), 12.21 (s, 1 H); 13C-NMR ppm; 123.5, 124.5, 125.3, 125.5, 128.4, 129.7, 132.2, 136.6, 137.8, 140.4, 149.5, 150.7, 176.3; C13H10Cl2N4S (325.21) m/z (%): 326.99 [M+H]+ (100)

2.10. 2-((2-Chloropyridin-3-yl)methylene)-*N*-(4-fluorophenyl)hydrazine-1-carbothioamide (3░h)

Yellowish off white solid; Yield: 70%, m.p.: 215-216°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 7.17-7.22 (m, 2 H), 7.47-7.51 (m, 3 H), 8.41 (dd, 1 H, *J* = 1.8, 4.8░Hz), 8.47 (s, 1 H), 8.84 (dd, 1 H, *J* = 1.8, 7.8░Hz), 10.26 (s, 1 H), 12.12 (s, 1 H); 13C-NMR ppm; 114.7, 114.9, 123.4, 128.3, 128.4, 128.5, 128.4, 129.7, 135.5, 136.5, 137.4, 140.4, 149.4, 150.5, 159.0, 160.6, 176.8; C13H10FClN4S (308.76) m/z (%): 309.02 [M+H]+ (100)

2.11. 2-((2-Chloropyridin-3-yl)methylene)-*N*-(3-fluorophenyl)hydrazine-1-carbothioamide (3i)

Off white solid; Yield: 72%, m.p.: 213-215°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 7.02-7.05 (m, 1 H), 7.37-7.41 (m, 1 H), 7.49 (dd, 1 H, *J* = 4.8, 7.8░Hz), 7.52-7.54 (m, 1 H), 8.42 (dd, 1 H, *J* = 1.8, 4.8░Hz), 8.48 (s, 1 H), 8.83 (dd, 1 H, *J* = 2.4, 7.8░Hz), 10.30 (s, 1 H), 12.20 (s, 1 H); 13C-NMR ppm; 112.0, 112.2, 112.5, 112.7, 121.6, 121.7, 123.4, 128.4, 129.5, 129.6, 136.6, 137.7, 140.5, 140.6, 149.5, 150.6, 162.6, 176.2; C13H10FClN4S (308.76) m/z (%): 309.02 [M+H]+ (100)

2.12. 2-((2-Chloropyridin-3-yl)methylene)-*N*-(4-methylbenzyl)hydrazine-1-carbothioamide (3j)

Off white solid; Yield: 70%, m.p.: 240-242°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 2.24 (s, 3 H, CH3), 4.77 (d, 2 H, *J* = 6.6░Hz), 7.10 (d, 2 H, *J* = 7.8░Hz), 7.20 (d, 2 H, *J* = 8.4░Hz), 7.45 (dd, 1 H, *J* = 4.8, 7.8░Hz), 8.38 (s, 1 H), 8.39 (d, 1 H, *J* = 1.8░Hz), 8.68 (dd, 1 H, *J* = 1.8, 7.8░Hz), 9.22 (t, 1 H, *J* = 6░Hz), 11.48 (s, 1 H); 13C-NMR ppm; 20.7, 46.4, 123.4, 127.2, 128.6, 128.7, 135.8, 136.1, 136.2, 136.6, 149.2, 150.4, 177.8; C15H15ClN4S (318.82) m/z (%): 319.05 [M+H]+ (100)

2.13. 2-((2-Chloropyridin-3-yl)methylene)-*N*-(2,6-dimethylphenyl)hydrazine-1-carbothioamide (3k)

Greenish yellow solid; Yield: 71%, m.p.: 198-200°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 2.16 (s, 6 H, CH3), 7.09-7.13 (m, 3 H), 7.45 (dd, 1 H, *J* = 4.8, 7.8░Hz), 8.40 (dd, 1 H, *J* = 1.8, 4.2░Hz), 8.44 (s, 1 H), 8.87 (dd, 1 H, *J* = 1.8, 7.8░Hz), 10.04 (s, 1 H), 12.04 (s, 1 H); 13C-NMR ppm; 18.0, 123.5, 127.1, 127.6, 128.7, 136.4, 136.5, 136.7, 136.9, 149.3, 150.4, 177.0; C15H15ClN4S (318.82) m/z (%): 319.05 [M+H]+ (100)

2.14. 2-((2-Chloropyridin-3-yl)methylene)-*N*-cyclohexylhydrazine-1-carbothioamide (3l)

Off white solid; Yield: 70%, m.p.: 242-245°C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 1.22-1.29 (m, 3 H), 1.37-1.44 (m, 2 H), 1.59 (d, 1 H, *J* = 12.6░Hz), 1.71 (d, 2 H, *J* = 13.2░Hz), 1.84 (d, 2 H, *J* = 9.6░Hz), 4.1-4.2 (m, 1 H), 7.47 (dd, 1 H, *J* = 3.0, 7.8░Hz), 8.2 (d, 1 H, *J* = 9.0░Hz), 8.36 (s, 1 H), 8.41 (dd, 1 H, *J* = 3.0, 4.8░Hz), 8.6 (dd, 1 H, *J* = 1.8, 7.8░Hz), 11.70 (s, 1 H); 13C-NMR ppm; 25.0, 25.1, 31.7, 52.9, 123.4, 128.5, 136.4, 136.3, 136.6, 149.2, 150.3, 175.9; C14H13ClN4OS (396.82) m/z (%): 397.06 [M+H]+ (100)

2.15. 2-((2-Chloropyridin-3-yl)methylene)-*N*-(3-methoxyphenyl)hydrazine-1-carbothioamide (3░m)

Off white solid; Yield: 92%, m.p.: 197-199 °C; 1H-NMR (DMSO-*d*[[*6*](#bib6)]) δ ppm; 3.74 (s, 3 H, CH3), 6.77 (dd, 1 H, *J* = 6, 8.4░Hz), 7.12 (d, 1 H, *J* = 7.8░Hz), 7.19 (t, 1 H, *J* = 1.8░Hz), 7.26 (t, 1 H, *J* = 8.4░Hz), 7.47 (dd, 1 H, *J* = 4.8, 7.8░Hz), 8.41 (dd, 1 H, *J* = 1.8, 4.8░Hz), 8.46 (s, 1 H), 8.84 (dd, 1 H, *J* = 1.8, 7.8░Hz), 10.21 (s, 1 H), 12.09 (s, 1 H); 13C-NMR ppm; 55.2, 111.1, 111.6, 118.1, 123.5, 128.5, 128.9, 136.6, 137.4, 140.0, 149.4, 150.6, 159.1, 176.2; C14H13ClN4OS (320.80) m/z (%): 321.04 [M+H]+ (100)

2.16. Molecular Docking and Molecular Dynamics Simulation Studies

For molecular docking studies BioSolveIT’s LeadIT software was used[[45](#bib45)]. For visualization of docked conformations, Desmond Molecular Dynamics (D.E. Shaw Research) on Schrödinger Maestro 2023.4 software was opted to perform Molecular Dynamic Simulations study[[46](#bib46)]. For Molecular Dynamics Simulation, initial complexed structure of 3░m best docked pose with α-glucosidase was utilized. Protein Preparation Workflow in Maestro was used to pre-process and refine the protein structure including Cap termini option, then H-bonds assignment was optimized. The solvation for the system was accomplished using System Builder panel, pre-defined TIP3P solvent model was selected, orthorhombic box shape was used with buffer dimensions for 10░Å x 10░Å x 10░Å. Ions placement for neutralization was used as provided default calculated by software, 0.15░M NaCl salt was also added to simulate the physiological natural conditions. In Molecular Dynamics panel, 50░ns (nanoseconds) time of simulation was entered, while the trajectory of simulation was set to be recorded after every 50░ps (picoseconds), which resulted in approximately 1000 frames for the whole 50░ns simulation. The system was run using the default relaxation protocol, the production run of 50░ns simulation for the complex was performed with NPT ensemble at 300░K and 1.01325░bar pressure using Nosé-Hoover chain thermostat and Martyna-Tobias-Klein barostat settings. The analysis of results was carried out utilizing Simulations Interaction Diagram panel along trajectory analysis. For more information regarding molecular dynamics study protocol, the reader is referred to the supplementary information.

3. Result and discussion

3.1. Chemistry

To investigate the biological potency of 2-chloronicotinaldehyde based thiosemicarbazone a series of compounds **3**(**a-m**)having different substitutions was synthesized by reacting 2-chloronicotinaldehyde with different substituted thiosemicarbazides **2**(**a-m**) in a typical reflux condensation reaction. Solvent polarity was shifted from polar to non-polar and the reactants (**1**) and phenyl thiosemicarbazide (**2a**) were used in equimolar ratio to optimize the condition of the reaction. Methanol was chosen as solvent and few drops of glacial acetic acids (2-3 drops) were taken as catalyst to increase the yield of the reaction. The scope of the reaction was widened by using various thiosemicarbazides **2**(**a-m**) having different substitutions. Targeted thiosemicarbazone **3**(**a-m**) were obtained as pure product in good to excellent yield.

The structure of 2-chloronicotinaldehyde based thiosemicarbazones, a series of compounds **3(a-m)** was established by spectral data IR, 1H-NMR, 13C NMR and ESI spectrometry. In 1H-NMR, CH3 attached to phenyl group appeared between 2.1-2.3░ppm. The most downfield singlets of two NH protons present on both sides of the thiol group (C=S) were resonated at δH 11.48-12.21 and 10.04-10.30 respectively. In addition, a CH=N proton gives a singlet at δH 8.36-8.48 chemical shift values. The spectral data of other aliphatic and aromatic protons and carbon shifts of all the carbons were according to the anticipated structure of targeted compounds. In ESI spectra, the molecular ion peak was in total agreement with molecular mass of the targeted compounds. The above-mentioned peaks from various techniques confirmed the formation of the targeted thiosemicarbazones.

The 1H NMR spectrum of derivative **3m** was recorded in DMSO-d6 on a Bruker 600░MHz instrument. The most downfield singlets of two NH protons present on both sides of the thiol group (C=S) were resonated at δH 12.01 and ppm 10.29 respectively. In addition, a CH=N proton gives a singlet at δH 8.46░ppm chemical shift values. Three doublet of doublets for the three protons of the pyridine ring can be seen at 8.84, 8.41 and 7.47░ppm. The peaks for the four protons of the phenyl ring can b seen at 6.77-7.26░ppm. Furthermore, a singlet of 3 H can be seen at 3.74░ppm indicating the methoxy group attached to the phenyl ring. The following peaks confirmed the constitution of the derivative **3m.** Furthermore in mass spectrum signal was observed at 321.04 which corresponds to M+1 and supports the formation of desired molecule. [Scheme 1](#sch1)

Scheme 1. Synthesis of pyridine based thiosemicarbazones

3.2. Glucosidase Inhibition Activity

A new class of 2-chloronicotinaldehyde based thiosemicarbazones have been synthesized and evaluated for their potential against alpha glucosidase enzyme. IC50 values and % inhibition of the synthesized compounds was displayed in [Table 1](#tb1). Thiosemicarbazone and 2-chloropyridine functioned as significant structural frameworks, and the phenyl, benzyl, or alkyl group bonded to the N4 nitrogen of thiosemicarbazone was crucial in establishing an indisputable structure-activity link. The inhibitory potential of the thiosemicarbazones is explored by changing the **R** group on the thiosemicarbazide moiety.

All the derivatives have shown exceptional inhibition potential with IC50 value in the range of 4.10 ± 0.16░µM to 32.76 ± 0.80░µM in comparison with acarbose used a positive control with an IC50 value of 873.34 ± 1.67░µM. It is clearly obvious from the IC50 values that the all the synthesized compounds are much more potent than the standard. In the entire series, derivative **3m** exhibited the most potent activity 4.10 ± 0.16░µM while compound **3l** exhibited the least activity 32.76 ± 0.80░µM.

Compound **3a** with simple phenyl ring exhibited an IC50 value of 25.19 ± 0.63░µM and is the second least potent derivative of the entire series. While all the other derivatives with various substitutions on the ring exhibited enhanced inhibitory potential. Compound **3m** and **3c** having methoxy substitution on the phenyl ring were the most potent derivatives of the series. Compound **3m** with methoxy substitution at the meta position of the phenyl exhibited slightly higher potential with IC50 value of 4.10 ± 0.16░µM than the derivative **3c** with methoxy substitution at the para position of the phenyl ring having IC50 value of 6.17 ± 0.20░µM. the difference in their potential is because of the position of the methoxy group on the phenyl ring. It can be seen from the most potent activity of **3m** that electron donating ability is contributing favorably towards the inhibition of α glucosidase enzyme and therefore controlling diabetes mellitus. Electron donating group leads to the increase in resonance of the phenyl ring attached to the thiosemicarbazide moiety therefore leading to the increase in pi-pi interactions between derivative **3m** and the active site of glucosidase enzyme. There is also a probability of the formation of hydrogen bond between the NH groups of the thiosemicarbazide moiety and the amino acids present in the active site of the enzyme leading to the enhanced stabilization of the enzyme complex and therefore inhibition of its activity.

Introduction of another electron donating group such as flouro in compound **3i** and **3h** also leads to significant potency Compound **3i** and **3h** with fluoro substitution at the meta and para position of the phenyl ring respectively exhibited IC50 value of 8.96 ± 0.23░µM and 10.38 ± 0.29░µM respectively. Both of the compounds exhibited the similar pattern as the methoxy derivatives. **3i** having meta fluoro substitution is slightly more potent that its isomer **3h** with para fluoro substitution. Compound **3g** and **3f** having chloro substitutions exhibited comparable IC50 values of 9.11 ± 0.21░µM and 11.57 ± 0.24░µM respectively. **3g** having 3 chloro substitution exhibited higher potential than **3f** having 4 chloro substitution.

xCompounds **3e**, **3d** and **3b** displayed the following values of IC50 13.28 ± 0.25░µM, 15.26 ± 0.35░µM, and 17.24 ± 0.38░µM respectively. It is interesting to note that the compound **3e** with ortho methyl substitution displayed highest potency among its isomers followed by **3d** with meta methyl substitution while **3b** with para methyl substitution displayed the least activity. Compound **3j** with para methyl benzyl substitution displayed IC50 value of 12.15 ± 0.30░µM. Comparison of the derivative **3j** with compound **3b** having para methyl substitution, a steep decline can be seen. Compound **3k** with 2, 6 dimethyl substitutions displayed IC50 value of 14.26 ± 0.27░µM. Compound **3d** with cyclohexyl substitution displayed IC50 value of 32.76 ± 0.80░µM and is the least potent member of the entire series. [Figure 2](#fig2)

Table 1: α- Glucosidase inhibition potential of thiosemicarbazones

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 🗗 | | | |
| Sr no. | Codes | R | Percent Inhibition (0.5░mM) | IC50 ± µM (SEM) |
| 1 | **3a** | 🗗 | 87,49 | 25.19 ± 0.63 |
| 2 | **3b** | 🗗 | 89,62 | 17.24 ± 0.38 |
| 3 | **3c** | 🗗 | 92,17 | 6.17 ± 0.20 |
| 4 | **3d** | 🗗 | 90.60 | 15.26 ± 0.35 |
| 5 | **3e** | 🗗 | 90.85 | 13.28 ± 0.25 |
| 6 | **3f** | 🗗 | 91.29 | 11.57 ± 0.24 |
| l | **3g** | 🗗 | 91.57 | 9.11 ± 0.21 |
| 8 | **3h** | 🗗 | 92.16 | 10.38 ± 0.29 |
| 9 | **3i** | 🗗 | 92.45 | 8.96 ± 0.23 |
| 10 | **3j** | 🗗 | 91.36 | 12.15 ± 0.30 |
| 11 | **3k** | 🗗 | 90.94 | 14.26 ± 0.27 |
| 12 | **3l** | 🗗 | 87.42 | 32.76 ± 0.80 |
| 13 | **3m** | 🗗 | 92.58 | 4.10 ± 0.16 |
| Standard | **Acarbose** |  | 59.37 (1░mM) | 873.34 ± 1.67 |

**Figure 2:** Structure of relationship of 2-nicotinaldehyde based Thiosemicarbazones

3.3. Molecular Docking Studies

Three most active inhibitors **3m**, **3c** and **3g** having IC50 values 4.10 ± 0.16, 6.17 ± 0.20 and 9.11 ± 0.21░µM respectively were selected for the docking studies. Since the crystal structure of *Saccharomyces cerevisiae* is not available from the PDB, its homology was built and used for docking studies as previously reported by us[[47](#bib47)]. All compounds were found to bind in the same region of the active site. [Figure 3](#fig3) shows overlap of all three docked compounds.

**Figure 3.** Overlap of docked conformations of **3c** (pink), **3g** (purple) and **3m** (cyan) in the active site of alpha-glucosidase

In the active site of alpha glucosidase from *Saccharomyces cerevisiae,* three amino acids form the catalytic triad, these are Asp214 which acts as a nucleophile, Glu276 acts as a proton donor and Asp349 is responsible for stabilization of the transition state of the substrate. Several other amino acids present in the vicinity of the active site are important as they stabilize the substrate binding. Moreover amino acids Phe231, His239, Asn241, His279, Glu304, and Arg312 are present at the entrance of the active site gorge[[48](#bib48)].

**Table 2.** Binding free energies and non-bonded interactions of α- Glucosidase inhibitors

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Code | Binding Free Energy (kJ/mol) | Hydrophobic Interactions | Hydrogen bond interactions | Hydrogen bond distance (Å) | Electrostatic interactions | Other |
| **3c** | -14 | Phe157 (pi-pi stack, pi-alkyl), Leu218 (alkyl), Ala278 (alkyl and pi-alkyl), Tyr71 (pi-alkyl), His348 (pi-alkyl) | His348 (HB-D), Asp68 (HB-D) | 1.78, 3.55 | Asp214 (pi-anion), Asp349 (pi-anion), Glu276 (pi-anion) | Phe177 (pi-sulfur) |
| **3g** | -17 | Phe157 (pi-pi stack), Phe300 (pi-pi stack), Ala278 (pi-alkyl), Tyr71 (pi-alkyl), His348 (pi-alkyl) | His348 (HB-D), Asp 68 (HB-D) | 1.76, 3.4 | Asp214 (pi-anion), Asp349 (pi-anion), Glu276 (pi-anion) | Phe177 (pi-sulfur) |
| **3m** | -31 | Tyr71 (pi-sigma), Phe300 (pi-alkyl), His348 (pi-alkyl) | Glu276 (HB-A), His348 (HB-D), Asp408 (HB-A) | 1.70, 2.08 | Arg439 (pi-cation), Asp214 (pi-anion), | Phe177 (pi-sulfur) |

Compounds **3c** and **3g** showed similar interactions ([Figures 4](#fig4) and [5](#fig5)). For both compounds **3c** and **3g** the nitrogen atom of the pyridine ring was making a hydrogen bond with His348 and the -N=CH group was making a hydrogen bond with Asp68. The pyridine ring and the phenyl ring were making pi-anion bond with amino acids Asp214 and Glu276 respectively. The sulfur atom of C=S group was making a pi-sulfur bond with Phe177. The chloro group was making alkyl interactions with Tyr71 and His348. The methoxy phenyl ring of **3c** was making a number of pi-alkyl and a pi-pi stacked interactions with Ala278, Leu218 and Phe157 respectively. For compound **3g**, the chloro phenyl ring was making pi-pi stacked interactions with Glu276 and Phe157, the same ring was also making a pi-anion and a pi-alkyl interaction with Glu276 and Ala278 respectively.

**Figure 4.** Binding site interactions of docked conformations of **3c**

Figure 5. Binding site interactions of docked conformations of **3g**

For compound **3m** ([Figure 6](#fig6)), the most active inhibitor, the methoxy group was making a hydrogen bond with His348, and the pyridine ring making a hydrogen bond with Asp408. The NH group was making a hydrogen bond with Glu276. The pyridine ring and the phenyl ring were making pi-cation and a pi-anion interaction respectively. A pi-sulfur bond was observed between C=S group and Phe177. The chloro group was making a pi-alkyl interaction with Phe300, while the methoxy group was making pi-sigma and a pi-alkyl interaction with Tyr71 and His348.

**Figure 6.** Binding site interactions of docked conformations of **3m**

All three inhibitors were making a pi-sulfur interaction with Phe177 and a hydrogen bond with His348, while compounds **3c** and **3g** were also making hydrogen bonds with Asp68 ([Table 2](#tb2)). For most active inhibitor **3m**, additional hydrogen bonds were observed with Asp408 and Glu276. A common pi-anion electrostatic interaction was observed for all three inhibitors with Asp214, while compounds **3c** and **3g** were also making pi-anion interactions with Glu276 and Asp349. Compound **3m**, being the most active inhibitor also showed a pi-cation electrostatic interaction with Arg439. Amino acids Asp214 and Glu276 are two of the three catalytic triad residues (the other one being Asp349), hence these interactions with Glu276 and Asp214 seem to be crucial for alpha-glucosidase inhibition activity.

To summarize the docking interactions, Tyr71 and His348 were found to be making hydrophobic interactions (pi-sigma and pi-alkyl) with all three inhibitors. Phe300 was making a pi-alkyl and a pi-pi stacked (both hydrophobic) interaction with **3m** and **3g** respectively. Two more hydrophobic interactions with amino acids Phe157 (pi-pi stack and pi-alkyl) and Ala278 (pi-alkyl and alkyl) were observed for compounds **3c** and **3g**, while **3c** was additionally making an alkyl interaction with Leu218.

3.4. Molecular Dynamic (MD) Simulation Studies

The simulation study of 3░m with α-glucosidase gave the values of C-alpha protein RMSD in the range of 1.8░Å to 2.4░Å. The values of C-alpha protein RMSD remained stable throughout the 50░ns simulation period. The molecular dynamics simulation study of **3m** with alpha-glucosidase for 50░ns showed through the RMSD values that the protein structure remained stable throughout the time of simulation and attained equilibration during the simulation and converged around 2.1░Å towards the end of the simulation. Nevertheless, value of Ligand’s fit on protein RMSD suggest that ligand has undergone positional changes during the simulation, and trajectory analysis also suggest the change in position of ligand from the initial docked position. However, after changing of ligand’s position in the complex during the first 20 nanoseconds of the simulation, the position of **3m** remained relatively stable in the complex. Despite the positional change of **3m**, the complex and protein of alpha-glucosidase remained stable throughout the simulation time.

**Figure 7.** RMSD graph of **3m**- α-glucosidase protein ligand complex for 50░ns simulation run

**Figure 8.** Protein RMSF graph of α-glucosidase complexed with **3m**. Residues that interact with the ligand are shown in green vertical bars

However, the Ligand’s RMSD concerning fit on protein structure varied initially between 3░Å to 10.5░Å, after about 25 nanoseconds, the ligand RMSD remained stable in the range between 6.5░Å and 10.5░Å and converged around 8░Å through the middle and towards the end of simulation ([Figure 7](#fig7)). Protein’s RMSF (root mean square fluctuation) analysis showed that all residues that interacted with ligand **3m** have values of fluctuation below 2.8░Å ([Figure 8](#fig8)). Protein’s SSE (Secondary Structure Elements) showed α-helix = 22.5% (red color) and β-strand = 16 % (blue color) and total SSE = 37.50 % (*SI* [*Figure 1*](#fig1)). Ligand’s RMSF is useful for characterizing changes in the ligand atom positions. The ligand RMSF while being fit on protein structure gives values around 2-3░Å for all atoms except the methoxy carbon giving a value above 4░Å ([Figure 9](#fig9)d). The interactions histogram for Protein-Ligand contacts was categorized based on interaction types as hydrogen, hydrophobic, ionic, and water bridges. Glu276 (part of catalytic triad) showed a maximum interaction fraction of around 0.8, these interactions are hydrogen bond and water bridged interactions. This is followed by Phe157 with around 0.6 fraction that showed hydrophobic interactions ([Figure 9](#fig9)b). Ligand-protein contacts showed that Glu276 maintained a direct electrostatic interaction with the NH group of 3░m for 40% of the time. The same NH group maintained contact with Glu276 via a water molecule for 31% of the time. Similarly, Gly217 maintained a contact with the sulfur atom of the C=S group 40% of the time. The nitrogen atom of the pyridine ring and the aromatic pyridine ring itself were able to maintain their polar interactions with His239 (52%) and His245 (30%) respectively ([Figure 9](#fig9)e). The torsion profile of ligand shows the strain on rotatable bonds in ligand as simulation progressed. It shows the amount of conformational strain (in kcal/mol) the ligand has to face in order to maintain its binding with the protein ([Figure 9](#fig9)a).

**Figure 9.** (a) Torsion profile of ligand 3░m. (b) Protein-Ligand interaction fraction graph of 3m-alpha-glucosidase complex (c) Protein-Ligand Contacts of 3░m with α-glucosidase continued (d) Ligand RMSF (e) Ligand-Protein Contacts of 3░m with alpha-glucosidase

3.5. *In Silico* ADME Evaluation

To have an idea of the drug likeness of the compounds, *in silico* ADME (Absorption, Distribution, Metabolism, Excretion) profile was generated using the online resource http://www.swissadme.ch/. In addition to physicochemical parameters, Swiss ADME also gives a quick and convenient visual representation of overall drug-likeness of a compound in the form of bioavailability radar diagrams. The ADME profile of all compounds (**3a-3m**) is given in [Table 3](#tb3). For lipophilicity, the ideal range of logP (octanol water partition coefficient) is from -0.7 to +5.0. All compounds showed logP well within this range, from 2.80 to 3.44. The ideal molecular weight should be from 150 to 500, again the compounds showed MW in this range (290.77 - 325.22). The TPSA (topological polar surface area, a measure of polarity of a molecule) should be within the range 20-130, the compounds **3a-3m** exhibited TPSA values in the range 81.40 - 90.63. The logS (estimate of solubility) values of compounds were in the range -4.42 to -6.28. LogP values less than -4 indicate moderate water solubility of compounds, whereas values less than -6 indicate poor water solubility. The number of rotatable bonds (measure of molecular flexibility) ideally should not be more than 9, the number of hydrogen bond donors and acceptors in a molecule should not be more than 5 and 10 respectively, again all compounds obeyed these conditions having number of rotatable bonds 5-6, HBDs were 2 and HBAs were 2-3. The bioavailability score estimates the probability of a compound having at least 10% oral bioavailability in rats. Bioavailability scores above 0.55 are generally considered desirable, suggesting good oral bioavailability. Such compounds are predicted to have a higher chance of being absorbed from the gut and reaching the bloodstream after oral administration.

Saturation rate (number of carbon atoms having sp3 hybridization) should not be less than 0.25. With the only exception of compound **3l**, which showed a saturation rate of 0.46, all other compounds had values 0.00-0.13 indicating unfavorable saturation rate. Consequently, compound **3l** was the only compound in the series that is estimated to have good oral availability as indicated by its radar diagram, given in [Figure 10](#fig10).

Table 3. *In silico* physiological parameters and drug-likeness properties of compounds **3a-3m**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Code | MW | LogP | LogS | TPSA | HBD | HBA | Rotatable bonds | GI absorption | Bioavailability score |
| 3a | 290.77 | 3.71 | -5.50 | 81.40 | 2 | 2 | 5 | High | 0,55 |
| 3b | 304.80 | 3.13 | -5.88 | 81.40 | 2 | 2 | 5 | High | 0,55 |
| 3c | 320.80 | 2.79 | -5.61 | 90.63 | 2 | 3 | 6 | High | 0,55 |
| 3d | 304.80 | 3.12 | -5.88 | 81.40 | 2 | 2 | 5 | High | 0,55 |
| 3e | 304.80 | 3.15 | -5.88 | 81.40 | 2 | 2 | 5 | High | 0.55 |
| 3f | 325.22 | 3.32 | -6.10 | 81.40 | 2 | 2 | 5 | High | 0.55 |
| 3g | 325.22 | 3.0 | -5.77 | 81.40 | 2 | 2 | 5 | High | 0.55 |
| 3h | 308.76 | 3.14 | -5.77 | 81.40 | 2 | 3 | 5 | High | 0.55 |
| 3i | 308.76 | 3.11 | -6.28 | 81.40 | 2 | 3 | 5 | High | 0.55 |
| 3j | 318.82 | 3.44 | -6.26 | 81.40 | 2 | 2 | 6 | High | 0.55 |
| 3k | 318.82 | 3.44 | -5.18 | 81.40 | 2 | 2 | 5 | High | 0.55 |
| 3l | 296.82 | 2.95 | -4.42 | 81.40 | 2 | 2 | 5 | High | 0.55 |
| 3m | 320.80 | 2.78 | -5.61 | 90.63 | 2 | 3 | 6 | High | 0.55 |

**Figure 10.** Bioavailability radar diagrams from Swiss ADME, left to right **3c**, **3g**, **3l** and **3m**

Overall, all compounds indicated favorable ADME profile with no violations of the Lipinski’s rule and no PAINS (Pan Assay Interference Structures) alerts. The bioavailability radar diagrams of selected compounds are given in [Figure 10](#fig10). In the radar diagram, the area in pink color represents the optimal range for each of the calculated physicochemical parameter, the radar plot of a molecule has to fall completely within the pink area for it to be considered most drug-like. With the only exception of compound **3l**, all compounds including **3c**, **3g**, and **3m** had low saturation rate, this can be easily overcome by introducing alkyl substituents in the next generation of these compounds, this strategy might lead to active and more drug-like compounds as inhibitors of alpha-glucosidase from this class of compounds.

4. Conclusion

In conclusion, we synthesized a series of 2-chloronicotinaldehyde based thiosemicarbazones and investigated them against alpha glucosidase enzyme. All the derivatives revealed exceptional inhibition potential with IC50 values in the range of 4.10 ± 0.16░µM to 32.76 ± 0.80░µM in comparison to Acarbose. Among the series, **3m** displayed highest inhibition potential. It was further established through SAR analysis of the series that meta substitution on the phenyl rings and electron donating substituents had a slightly better weightage on the potential of the derivatives. To comprehend the binding interactions of the most active scaffolds, docking research was also conducted. Using molecular modelling, structural characteristics which play a role into the binding interactions with the enzyme's active sites were found. Numerous intriguing possibilities were found in this study, which might lead to further investigations into potential treatment medicines for type-2 diabetes mellitus.

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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.

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