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Original Research Article

Synthesis and molecular docking of new hydrazones derived from ethyl isonipecotate and their biological activities

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Abstract

Purpose: *To investigate the antibacterial and α-glucosidase inhibitory activities of hydrazone derivatives (**8a-h**) of ethyl isonipecotate.*

Methods: *The reaction of ethyl isonipecotate (**2**) with 3,5-dichloro-2-hydroxybenzenesulfonyl chloride (**1**) in an aqueous basic medium yielded ethyl 1-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]piperidin-4-carboxylate (**3**). Compound **3** was subsequently converted to ethyl 1-[(3,5-dichloro-2-ethoxyphenyl)sulfonyl]piperidin-4-carboxylate (**5**) via O-alkylation. Compound **5** on reaction with hydrated hydrazine yielded 1-[(3,5-dichloro-2-ethoxyphenyl)sulfonyl]piperidin-4-carbohyrazide (**6**) in MeOH. Target compounds **8a-h** were synthesized by stirring **6** with different aromatic aldehydes (**7a-h**) in MeOH. All the synthesized compounds were structurally elucidated by proton nuclear magnetic resonance (^{1}H-NMR), electron impact mass spectrometry (EI-MS) and infrared (IR) spectroscopy. For antibacterial activity, solutions of the synthesized compounds were mixed with bacterial strains, and the change in absorbance before and after incubation was determined. For enzyme inhibitory activity, change in the absorbance of mixtures of synthesized compounds and enzyme before and after incubation with substrate was determined.*

Results: *The target compounds were synthesized in appreciable yields and well characterized by spectral data analysis. Salmonella typhi was inhibited by **8e** (MIC 8.00 ± 0.54 µM), Escherichia coli by **8f** (8.21 ± 0.83 µM), Bacillus subtilis by **8c** (8.56 ± 0.63 µM) and Staphylococcus aureus by **8c** (8.86 ± 0.29 µM). Two compounds, **8e** and **8d**, were very effective inhibitors of α-glucosidase with IC_{50} values of 40.62 ± 0.07 and 48.64 ± 0.08 µM, respectively.*

Conclusion: *Low IC_{50} values of the synthesized compounds against α-glucosidase demonstrates their potential in type-2 diabetes treatment. Furthermore, these compounds exhibit substantial antibacterial activity against the bacterial strains tested.*

Keywords: *Antibacterial activity, α-Glucosidase inhibition, Ethyl isonipecotate, Hydrazones*

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INTRODUCTION

The labile lone pair of nitrogens in hydrazones is known to play an active role in the bioactivity and chemical reactivity of this class of compounds [1,2]. These compounds have been shown to have anti-tuberculosis [3], antimicrobial [4], antimycotic [5] and antihypertensive activities [6].

Furthermore, industrial chemistry has found many applications for hydrazones [7-9]. α-Glucosidase (EC 3.2.1.20) is an enzyme that acts on 1,4-alpha bonds [10,11].

The cleavage of carbohydrates is delayed by α-glucosidase inhibitors (AGI) in the small intestine [12,13]. In search of bioactive compounds, an attempt was made to synthesize some new hydrazones bearing a sulfamoyl moiety.

EXPERIMENTAL

All chemical reagents were from Merck, Sigma Aldrich and Alfa Aesar and purchased through local suppliers, and they were used without further purification. Purity of the synthesized compounds was assured by thin layer chromatography (TLC); the plates were developed with *n*-hexane and ethyl acetate solvent systems and visualized under UV at 254 nm and also by spraying with ceric sulfate solution.

The melting points of all synthesized compounds were determined using open capillary tubes on a Griffin and George apparatus. IR spectra were recorded using the potassium bromide pellet method on a Jasco-320-A spectrophotometer with wave number in cm^{-1}. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker spectrometer operating at 400 MHz. Chemical shift values are reported in ppm (*δ*) units taking TMS as reference and the coupling constants (*J*) are in Hz. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer.

Procedure for synthesis of sulfonamide (3) in aqueous medium

Ethyl isonipecotate (6.49 mmol; 1 mL, **2**) was added to a 100-mL round-bottom flask containing 15 mL basic aqueous medium prepared by addition of aqueous Na_2CO_3 with pH adjusted to 8-9. Next, 3,5-dichloro-2-hydroxybenzenesulfonylchloride (6.49 mmol; 1.698 g, **1**) was gradually added to the reaction flask over 5-10 min. The reaction mixture was stirred for 6-8 h with pH maintained at 8-9 at ambient temperature. The completion of the reaction was checked by TLC until confirmed by a single spot. Product **3** was collected by acidifying the reaction mixture with dilute HCl to bring the pH to 5-7. The synthesized product was collected by filtration, washed with distilled water and dried for next use.

Procedure for synthesis of *O*-alkyl derivative (5)

Compound **3** (0.5249 mmol, 0.2 g) was dissolved in DMF (5 mL) in a 100-mL round-bottom flask. Solid KOH (0.5249 mmol, 0.0293 g) was added to activate the *O*-substitution. Ethyl iodide (0.5249 mmol; 42.2 µL, **4**) was then added, and the mixture was stirred for 2-3 h at 60 ºC. Completion of the reaction was confirmed by TLC showing a single spot. Chilled distilled water was used to collect the precipitates of pure product **5**. Precipitates were filtered, washed with distilled water and dried.

Procedure for synthesis of carbohydrazide (6)

Compound **5** (1.467 mmol, 0.6 g) was dissolved in MeOH (5-10 mL) in a 100-mL round-bottom flask and stirred for 2-5 min. Hydrazine hydrate (1.467 mmol) was then added and the reaction mixture was refluxed for 6 h. There was a color change in the reaction mixture from orange-yellow to grey as the reaction proceeded toward completion. The progress of the reaction was followed by TLC. After reaction completion, the mixture was quenched with cold distilled water and the precipitates of **6** were filtered, washed with distilled water and dried for further use.

General procedure for synthesis of various hydrazone derivatives (8a-h)

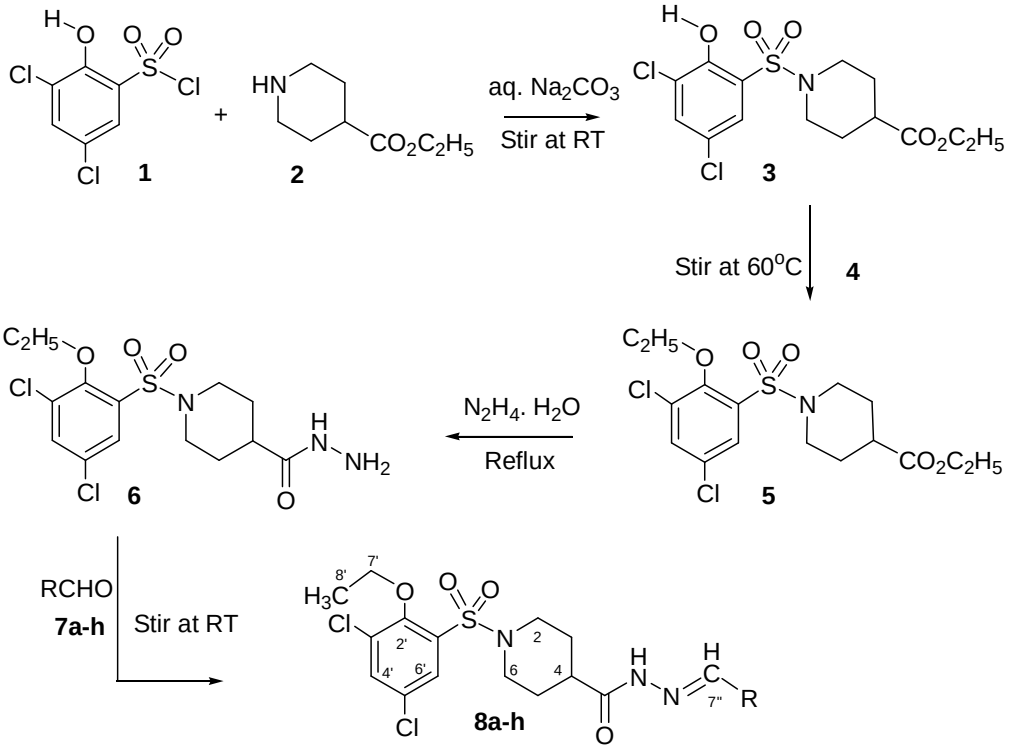
A methanolic solution of compound **6** (0.2523 mmol, 0.1 g) was placed in a 50-mL round-bottom flask and stirred at room temperature. Aromatic aldehydes (**7a-h**) were introduced in equimolar ratios to produce hydrazone derivatives. The reaction duration for different aldehydes varied 2-3 h. Reaction completion was confirmed by TLC, and distilled water was added to the mixture to precipitate the compounds synthesized, **8a-h**, which were filtered, washed with distilled water and dried.

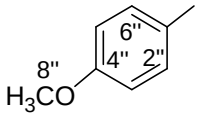
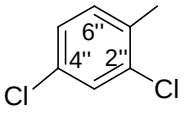
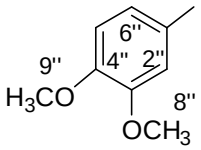
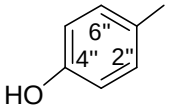
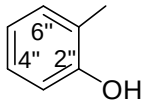
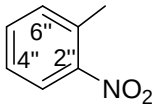
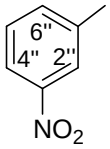
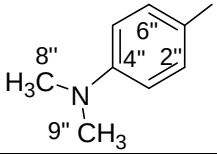
Evaluation of antibacterial activity

The broth microdilution method was employed to test for antibacterial activity [14]. The solutions of synthesized compounds were mixed with bacterial strains and change in absorbance before and after incubation was determined.

α-Glucosidase assay

α-Glucosidase inhibitory activity was determined as previously described, with slight modification [15].



Compd.	R	Compd.	R	Compd.	R
8a		**8d**		**8g**	
8b		**8e**		**8h**	
8c		**8f**			

Scheme 1: Synthetic scheme for hydrazone derivatives of 1-[(3,5-dichloro-2-ethoxyphenyl)sulfonyl]piperdine-4-carbohydrazide

Enzyme activity was based on the difference in absorbance of the mixture of synthesized compounds and enzyme before and after incubation with substrate.

Molecular docking study

The crystallographic structure of *Saccharomyces cerevisiae isomaltase* (PDB code 3AJ7; resolution 1.30 Å) showing 72.4 % sequence identity with the target was selected as a template. The 3D structure of α-glucosidase for *Saccharomyces cerevisiae* was predicted using the Molecular Operating Environment (MOE 2010.11) software package. MOE docking program was used to analyze the binding modes of the ligands with the protein molecule. The best conformations were analyzed for hydrogen bonding and π-π interactions [16-18].

Statistical analysis

The results are presented as mean ± SEM (n = 3) and were analyzed by Microsoft Excel 2010. The results for 50 % inhibitory concentration (IC_{50}) and minimum inhibitory concentration (MIC) were obtained at different dilutions (5 - 30 µg/well) and analyzed by EZ-Fit software (Perrella Scientific Inc., Amherst, USA).

RESULTS

1-(3,5-Dichloro-2-ethoxybenzenesulfonyl)-*N′*-(arylidene)piperidin-4-carbohydrazides, **8a-h**, were prepared according to the protocol

described in Scheme 1. The synthesized compounds exhibited substantial α-glucosidase inhibitory activity and antibacterial activity.

Ethyl 1-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]piperidin-4-carboxylate (3)

White powder; Yield: 87 %; IR (KBr): υ_{max}: 3247 (O-H), 3110 (Ar-H), 2860 (C-H), 1750 (>C=O), 1610 (Aromatic C=C), 1386 (-SO_2); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 8.92 (s, 1H, HO-2'), 7.77 (d, *J* = 2.8 Hz, 1H, H-6'), 7.55 (d, *J* = 2.4 Hz, 1H, H-4'), 4.12 (q, *J* = 6.8 Hz, 2H, H-7), 3.80-3.77 (m, 2H, H_e-2 & H_e-6), 3.34-3.04 (m, 1H, H-4), 2.88-2.83 (m, 2H, H_a-2 & H_a-6), 2.05-1.96 (m, 2H, H_e-3 & H_e-5), 1.86-1.77 (m, 2H, H_a-3 & H_a-5), 1.47 (t, *J* = 6.8 Hz, 3H, H-8); EIMS: *m/z* 381 $[M]^{\bullet+}$, 352 $[C_{12}H_{12}Cl_2NO_5S]^+$, 336 $[C_{12}H_{12}Cl_2NO_4S]^+$, 308 $[C_{11}H_{12}Cl_2NO_3S]^+$, 224 $[C_7H_6Cl_2O_3S]^{\bullet+}$, 156 $[C_8H_{14}NO_2]^{\bullet+}$, 144 $[C_6H_2Cl_2]^{\bullet+}$, 111 $[C_6H_9NO]^{\bullet+}$, 83 $[C_5H_9N]^+$.

Ethyl 1-[(3,5-dichloro-2-ethoxyphenyl)sulfonyl]piperidin-4-carboxylate (5)

White powder; Yield: 80 %; IR (KBr): υ_{max}: 3045 (Ar-H), 2975 (C-H), 1617 (Aromatic C=C), 1369 (-SO_2), 1170 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 7.79 (d, *J* = 2.8 Hz, 1H, H-6'), 7.53 (d, *J* = 2.4 Hz, 1H, H-4'), 4.22 (q, *J* = 6.8 Hz, 2H, H-7'), 3.88 (q, *J* = 6.4 Hz, 2H, H-7), 3.82-3.57 (m, 2H, H_e-2 & H_e-6), 2.32-2.04 (m, 1H, H-4), 2.77-2.73 (m, 2H, H_a-2 & H_a-6), 2.10-1.96 (m, 2H, H_e-3 & H_e-5), 1.80-1.72 (m, 2H, H_a-3 & H_a-5), 1.42 (t, *J* = 6.8 Hz, 3H, H-8'), 1.31 (t, *J* = 6.4 Hz, 3H, H-8); EIMS: *m/z* 409 $[M]^{\bullet+}$, 380 $[C_{14}H_{16}Cl_2NO_5S]^+$, 364 $[C_{14}H_{16}Cl_2NO_4S]^+$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 188 $[C_8H_7Cl_2O]^{\bullet+}$, 156 $[C_8H_{14}NO_2]^+$, 144 $[C_6H_2Cl_2]^{\bullet+}$, 111 $[C_6H_9NO]^{\bullet+}$, 83 $[C_5H_9N]^{\bullet+}$.

1-(3,5-Dichloro-2-ethoxyphenylsulfonyl) piperidin-4-carbohydrazide (6)

Grey powder; Yield: 79 %; IR (KBr): υ_{max}: 3332 (CON-H), 3029 (Ar-H), 1639 (>C=O), 1610 (Aromatic C=C), 1378 (-SO_2), 1159 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 8.37 (br s, CON-H), 7.77 (d, *J* = 2.8 Hz, 1H, H-6'), 7.56 (d, *J* = 2.4 Hz, 1H, H-4'), 3.88 (q, *J* = 6.4 Hz, 2H, H-7'), 3.81-3.77 (m, 2H, H_e-2 & H_e-6), 2.88-2.83 (m, 1H, H-4), 2.67-2.53 (m, 2H, H_a-2 & H_a-6), 2.05-1.96 (m, 2H, H_e-3 & H_e-5), 1.86-1.77 (m, 2H, H_a-3 & H_a-5), 1.47 (t, *J* = 7.2 Hz, 3H, H-8'); EIMS: *m/z* 395 $[M]^{\bullet+}$, 365 $[C_{14}H_{17}Cl_2NO_4S]^{\bullet+}$, 364 $[C_{14}H_{16}Cl_2NO_4S]^+$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 142 $[C_6H_{12}N_3O]^+$, 111 $[C_6H_9NO]^+$, 83 $[C_5H_9N]^+$.

1-(3,5-Dichloro-2-ethoxyphenylsulfonyl)-*N'*-(4-methoxybenzylidene)piperidin-4-carbohydrazide (8a)

White powder; Yield: 96 %; IR (KBr): υ_{max}: 3025 (Ar-H stretching), 2869 (C-H), 1678 (C=N), 1508 (Aromatic C=C), 1360 (-SO_2), 1162 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 8.47 (br s, 1H, CONH), 8.38 (s, 1H, H-7''), 7.80 (d, *J* = 2.7 Hz, 1H, H-6'), 7.58 (d, *J* = 2.5 Hz, 1H, H-4'), 7.71 (d, *J* = 8.2 Hz, 2H, H-2'' & H-6''), 7.21 (d, *J* = 8.4 Hz, 2H, H-3'' & H-5''), 4.23 (q, *J* = 6.4 Hz, H-7'), 3.81 (s, 3H, OCH_3-8''), 3.89-3.84 (m, 2H, H_e-2 & H_e-6), 3.36-3.20 (m, 1H, H-4), 2.86-2.80 (m, 2H, H_a-2 & H_a-6), 1.96-1.80 (m, 4H, H-3 & H-5), 1.43 (t, *J* = 6.4 Hz, 3H, H-8'); EIMS: *m/z* 513 $[M]^{\bullet+}$, 379 $[C_{14}H_{17}Cl_2N_2O_4S]^+$, 364 $[C_{14}H_{16}Cl_2NO_4S]^+$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 260 $[C_{14}H_{18}N_3O_2]^{\bullet+}$, 147 $[C_5H_9NO_2S]^+$, 134 $[C_8H_8NO_2]^{\bullet+}$, 107 $[C_7H_7O]^{\bullet+}$, 83 $[C_5H_9N]^{\bullet+}$.

1-(3,5-Dichloro-2-ethoxyphenylsulfonyl)-*N'*-(4-hydroxybenzylidene)piperidin-4-carbohydrazide (8b)

Off-white powder; Yield: 96 %; IR (KBr): υ_{max}: 3135 (Ar-H), 2892 (C-H), 1680 (C=N), 1588 (Aromatic C=C), 1395 (-SO_2), 1134 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ*9.95 (br s, 1H, CONH), 9.93 (s, 1H, OH-8''), 8.29 (s, 1H, H-7''), 7.90 (d, *J* = 2.8 Hz, 1H, H-6'), 7.83 (d, *J* = 2.4 Hz, 1H, H-4'), 7.51 (d, *J* = 8.8 Hz, 2H, H-2'' & H-6''), 6.80 (d, *J* = 8.4 Hz, 2H, H-3'' & H-5''), 4.23 (q, *J* = 6.5 Hz, 2H, H-7'), 3.68-3.56 (m, 2H, H_e-2 & H_e-6), 3.42-3.20 (m, 1H, H-4), 2.86-2.82 (dt, *J* = 11.2 Hz, 2H, H_a-2 & H_a-6), 1.95-1.84 (m, 4H, H_e-3 & H_e-5, H_a-3 & H_a-5), 1.43 (t, *J* = 3.2 Hz, 3H, H-8'); EIMS: *m/z* 499 $[M]^+$, 379 $[C_{14}H_{17}Cl_2N_2O_4S]^+$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 246 $[C_{13}H_{16}N_3O_2]^+$, 252 $[C_8H_7Cl_2O_3S]^{\bullet+}$, 163 $[C_8H_7N_2O_2]^{\bullet+}$, 120 $[C_7H_6NO]^+$, 118 $[C_8H_8N]^{\bullet+}$, 83 $[C_5H_9N]^+$.

1-(3,5-Dichloro-2-ethoxyphenylsulfonyl)-*N'*-(3-nitrobenzylidene)piperidin-4-carbohydrazide (8c)

White powder; Yield: 96 %; IR (KBr): υ_{max}: 3015 (Ar-H), 2910 (C-H), 1645 (C=N), 1598 (Aromatic C=C), 1406 (-SO_2), 1122 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 8.71 (s, 1H, H-2''), 8.32 (dd, *J* = 8.4, 1.6 Hz, 1H, H-4''), 8.30 (s, 1H, H-7''), 7.94 (d, *J* = 7.6 Hz, 1H, H-6''), 7.80 (d, *J* = 2.8 Hz, 1H, H-6'), 7.72 (t, *J* = 8.0 Hz, 1H, H-5''), 7.58 (d, *J* = 2.4Hz, 1H, H-4'), 4.24 (q, *J* = 6.4 Hz, 2H, H-7'), 3.69-3.56 (m, H_e-2 & H_e-6), 3.40-3.20 (m, 1H, H-4), 2.90-2.86 (m, 2H, H_a-2 & H_a-6), 1.96-1.88 (m, 4H, H-3 & H-5), 1.46 (t, *J* = 6.4 Hz, 3H, H-8'); EIMS: *m/z* 528 $[M]^{\bullet+}$, 379 $[C_{14}H_{17}Cl_2N_2O_4S]^+$, 364 $[C_{14}H_{17}Cl_2NO_4S]^{\bullet+}$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 275

$[C_{13}H_{15}N_4O_3]^{\bullet+}$, 149$[C_7H_5N_2O_2]^{\bullet+}$, 126$[C_6H_{10}N_2O]^+$, 83 $[C_5H_9N]^+$.

1-(3,5-Dichloro-2-ethoxyphenylsulfonyl)-*N′*-(2,4-dichlorobenzylidene)piperidin-4-carbohydrazide (8d)

White powder; Yield: 96 %; IR (KBr): υ_{max}: 3035 (Ar-H), 2967 (C-H), 1635 (C=N), 1595 (Aromatic C=C), 1425 (-SO_2), 1112 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 8.41 (s, 1H, H-7''), 7.80 (d, *J* = 2.8 Hz, 1H, H-6'), 7.58 (d, *J* = 8.0 Hz, 1H, H-6''), 7.57 (dd, *J* = 8.0, 2.0 Hz, 1H, H-5''), 7.55 (d, *J* = 2.4 Hz, 1H, H-4'), 7.32 (d, *J* = 2.0 Hz, 1H, H-3''), 4.23 (q, *J* = 6.4 Hz, 2H, H-7'), 3.69-3.56 (m, 2H, H_e-2 & H_e-6), 3.40-3.20 (m, 1H, H-4), 2.86-2.80 (m, 2H, H_a-2 & H_a-6), 1.96-1.84 (m, 4H, H-3 & H-5), 1.43 (t, *J* = 6.8 Hz, 3H, H-8'); EIMS: *m/z* 551 $[M]^{\bullet+}$, 379 $[C_{14}H_{17}Cl_2N_2O_4S]^{\bullet+}$, 364 $[C_{14}H_{17}Cl_2NO_4S]^{\bullet+}$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 298 $[C_{13}H_{14}Cl_2N_3O]^{\bullet+}$, 172 $[C_7H_4Cl_2O]^{\bullet+}$, 145 $[C_6H_3Cl_2]^{\bullet+}$, 126 $[C_6H_{10}N_2O]^+$, 83 $[C_5H_9N]^+$.

1-(3,5-Dichloro-2-ethoxyphenylsulfonyl)-*N′*-(2-hydroxybenzylidene)piperidin-4-carbohydrazide (8e)

Off white powder; Yield: 99 %; IR (KBr): υ_{max}: 3135 (Ar-H), 2892 (C-H), 1680 (C=N), 1588 (Aromatic C=C), 1395 (-SO_2), 1134 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 8.59 (s, 1H, OH-8''), 8.54 (s, 1H, H-7''), 7.90 (d, *J* = 2.8 Hz, 1H, H-6'), 7.81 (d, *J* = 7.6 Hz, 1H, H-6''), 7.56 (d, *J* = 2.4 Hz, 1H, H-4'), 7.53 (dd, *J* = 7.6, 1.6 Hz, 1H, H-3''), 7.23 (dt, *J* = 7.6, 2.0 Hz, 1H, H-4''), 6.90 (dt, *J* = 7.6, 2.0 Hz, 1H, H-5''), 4.22 (q, *J* = 6.8 Hz, 2H, H-7'), 3.69-3.56 (m, 2H, H_e-2 & H_e-6), 3.40-3.22 (m, 1H, H-4), 2.86-2.78 (m, 2H, H_a-2 & H_a-6), 1.96-1.74 (m, 4H, H-3 & H-5), 1.43 (t,*J* = 6.4 Hz, 3H, H-8'); EIMS: *m/z* 499 $[M]^{\bullet+}$, 379 $[C_{14}H_{17}Cl_2N_2O_4S]^{\bullet+}$, 364 $[C_{14}H_{17}Cl_2NO_4S]^{\bullet+}$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 246 $[C_{13}H_{16}N_3O_2]^+$, 120 $[C_7H_6NO]^+$, 83 $[C_5H_9N]^+$.

1-(3,5-Dichloro-2-ethoxyphenylsulfonyl)-*N′*-(4-dimethylamino)benzylidene) piperidin-4-carbohydrazide (8f)

Off white powder; Yield: 100 %; IR (KBr): υ_{max}: 3128 (Ar-H), 3000 (C-H), 1665 (C=N), 1597 (Aromatic C=C), 1405 (-SO_2), 1102 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 8.42 (s, 1H, H-7''), 7.93 (d, *J* = 2.8 Hz, 1H, H-6'), 7.89 (d, *J* = 2.4 Hz, 1H, H-4'), 7.35 (d, *J* = 8.4 Hz, 2H, H-2'' & H-6''), 6.66 (d, *J* = 8.8 Hz, 2H, H-3'' & H-5''), 4.25 (q, *J* = 6.8 Hz, 2H, H-7'), 3.02 (s, 6H, H-8'' & H-9''), 3.69-3.56 (m, 2H, H_e-2 & H_e-6), 3.40-3.20 (m, 1H, H-4), 2.86-2.80 (m, 2H, H_a-2 & H_a-6), 1.96-1.84 (m, 4H, H-3 & H-5), 1.43 (t, *J* = 6.4 Hz, 3H, H-8'); EI-MS: *m/z* 526 $[M]^{\bullet+}$, 379 $[C_{14}H_{17}Cl_2N_2O_4S]^{\bullet+}$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 273$[C_{15}H_{21}N_4O]^{\bullet+}$, 147 $[C_9H_{11}N_2]^+$, 83 $[C_5H_9N]^+$.

1-(3,5-Dichloro-2-ethoxyphenylsulfonyl)-*N′*-(3,4-dimethoxybenzylidene)piperidine-4-carbohydrazide (8g)

Light grey powder; Yield: 96 %; IR (KBr): υ_{max}: 3075 (Ar-H), 2881 (C-H), 1664 (C=N), 1588 (Aromatic C=C), 1395 (-SO_2), 1145 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 8.47 (br s, 1H, CONH), 8.41 (s, 1H, H-7''), 7.85 (d, *J* = 2.8 Hz, 1H, H-6'), 7.78 (d, *J* = 2.4 Hz, 1H, H-4'), 7.54 (d, *J* = 1.6 Hz, 1H, H-2''), 7.36 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6''), 7.03 (d, *J* = 8.4 Hz, 1H, H-5''), 4.23 (q, *J* = 7.2 Hz, 2H, H-7'), 3.89 (s, 3H, OCH_3-8''), 3.87 (s, 3H, OCH_3-9''), 3.79-3.56 (m, 2H, H_e-2 & H_e-6), 3.40-3.20 (m, 1H, H-4), 2.86-2.80 (m, 2H, H_a-2 & H_a-6), 1.96-1.74 (m, 4H, H-3 & H-5), 1.43 (t, *J* = 6.8 Hz, 3H, H-8'); EIMS: *m/z* 543 $[M]^{\bullet+}$, 379 $[C_{14}H_{17}Cl_2N_2O_4S]^+$, 364 $[C_{14}H_{17}Cl_2NO_4S]^+$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 290 $[C_{15}H_{20}N_3O_3]^+$, 126 $[C_9H_{10}N_2O]^{\bullet+}$, 164 $[C_9H_{10}NO_2]^{\bullet+}$, 147 $[C_5H_9NO_2S]^{\bullet+}$, 83 $[C_5H_9N]^+$.

1-(3,5-Dichloro-2-ethoxyphenylsulfonyl)-*N′*-(2-nitrobenzylidene)piperidine-4-carbohydrazide (8h)

Light green powder; Yield: 96 %; IR (KBr): υ_{max}: 3015 (Ar-H), 2910 (C-H), 1645 (C=N), 1598 (Aromatic C=C), 1406 (-SO_2), 1122 (C-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 8.47 (br s, 1H, CONH), 8.30 (s, 1H, H-7''), 8.03 (d, *J* = 8.0 Hz, 1H, H-6''), 7.94 (d, *J* = 7.8 Hz, 1H, H-3''), 7.80 (d, *J* = 2.8 Hz, 1H, H-6'), 7.58 (d, *J* = 2.4 Hz, 1H, H-4'), 7.67-7.61 (m, 2H, H-4'' & H-5''), 4.03 (q, *J* = 6.8 Hz, 2H, H-7'), 3.69-3.56 (m, H_e-2 & H_e-6), 3.40-3.20 (m, 1H, H-4), 2.86-2.80 (m, 2H, H_a-2 & H_a-6), 1.96-1.84 (m, 4H, H-3 & H-5), 1.43 (t, *J* = 6.4 Hz, 3H, H-8'); EI-MS: *m/z* 528 $[M]^{\bullet+}$, 379 $[C_{14}H_{17}Cl_2N_2O_4S]^+$, 364 $[C_{14}H_{17}Cl_2NO_4S]^{\bullet+}$, 336 $[C_{13}H_{16}Cl_2NO_3S]^+$, 275 $[C_{13}H_{15}N_4O_3]^{\bullet+}$, 149 $[C_7H_5N_2O_2]^{\bullet+}$, 126 $[C_6H_{10}N_2O]^+$, 83 $[C_5H_9N]^+$.

Biological studies

The results for *in vitro* antibacterial activity against *Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus* are presented in Table 1 and those for *in vitro* α-glucosidase inhibitory activity are presented in Table 2.

Molecular docking

With regard to hydroxyl-containing compounds (**8b** and **8e**), the docking conformation of **8e** (*ortho* analogue) showed good interaction network as well as good docking score compared

Table 1: Antibacterial activity of synthesized compounds

Compound	Inhibition (%)				
	S. typhi (-)	***E. coli (-)***	***P. aeruginosa (-)***	***B. subtilis (+)***	***S. aureus (+)***
Inhibition (%)					
3	86.56±0.78	78.35±0.65	56.20±1.00	70.75±1.15	77.30±0.50
5	81.33±0.67	75.95±0.75	33.00±0.20	70.55±0.25	71.65±0.55
6	78.33±0.11	83.85±0.55	58.45±0.96	73.95±0.85	68.35±0.25
8a	72.78±0.33	74.30±0.60	55.65±1.15	62.25±0.55	64.50±0.80
8b	51.72±1.39	73.10±0.47	40.60±0.43	65.05±0.75	48.20±1.00
8c	78.50±1.09	83.55±0.27	65.05±0.55	79.20±0.30	75.40±0.60
8d	71.33±0.22	46.25±0.89	56.50±0.11	59.70±0.70	64.10±0.50
8e	83.44±0.11	81.20±0.70	66.85±1.05	73.40±1.10	71.20±0.40
8f	69.72±0.61	78.75±0.25	51.75±0.35	73.10±0.75	59.35±0.95
8g	75.28±0.54	75.50±1.00	51.50±1.90	70.85±1.55	69.90±0.90
8h	67.50±0.83	79.10±1.00	31.05±0.45	73.00±0.90	35.65±0.25
Ciprofloxacin	92.87±0.91	92.27±0.64	92.34±0.35	91.63±0.05	90.57±0.35
Minimum inhibitory concentration (MIC, µg/mL)					
3	7.99±0.86	8.31±0.05	15.78±0.69	9.79±0.86	8.43±0.79
5	8.87±0.37	8.42±0.43	-	9.80±0.81	9.46±0.89
6	8.38±0.53	8.05±0.52	14.79±0.86	9.22±0.65	9.24±0.69
8a	9.32±1.09	9.11±0.77	15.84±0.58	14.68±0.57	10.75±1.00
8b	17.98±0.82	9.39±0.64	-	12.08±0.58	-
8c	8.96±0.68	8.79±0.37	10.68±0.76	8.56±0.63	8.86±0.29
8d	9.89±0.58	-	16.98±0.89	13.86±0.84	10.47±0.80
8e	8.00±0.54	8.64±0.47	10.12±0.58	9.48±0.89	9.54±0.89
8f	9.41±0.63	8.21±0.83	17.89±0.59	9.20±0.89	13.65±0.87
8g	9.01±0.90	8.43±0.65	17.98±0.78	9.75±0.98	9.90±0.87
8h	10.00±0.79	8.26±0.48	-	9.01±0.58	-
Ciprofloxacin	7.12±0.21	7.05±0.28	7.41±0.61	7.65±0.48	7.89±0.27

Note: *MIC (minimum inhibitory concentration) values of compounds were calculated using EZ–Fit Enzyme Kinetics software (Perella Scientific Inc. Amherst, USA). Results are expressed as mean ± SEM.*

Table 2: α-Glucosidase activity (% inhibition and IC_{50}) of synthesized compounds

Compound	Inhibition (%) at 0.5 mM	IC_{50} (µM)
3	94.81±0.28	187.57±0.12
5	98.47±0.19	97.86±0.07
6	85.73±0.23	312.82 ±0.15
8a	24.62±0.12	-
8b	83.76±0.11	90.81±0.02
8c	11.91±0.14	-
8d	97.71±0.12	48.64±0.08
8e	98.57±0.18	40.62±0.07
8f	95.41±0.29	94.32±0.03
8g	83.76±0.11	342.72±0.12
8h	7.45±0.16	-
Acarbose	**92.23±0.14**	**38.25±0.12**

IC_{50} (50% inhibitory concentration) values of compounds were calculated using EZ–Fit Enzyme Kinetics software (Perella Scientific Inc. Amherst, USA). Results are expressed as mean ± SEM

to **8b** (*para* analogue). The binding mode of **8e** with a docking score of **-10.4311** displayed two hydrogen bonds with Asn 241 and Asp 408, an arene-arene interaction with Phe 157 and arene-arene interaction with Arg 312 residue of the enzyme (Figure 2a). Compound **8b** with a docking score of **-7.2386** established a hydrogen bond with Asn 241 and an arene-arene interaction with Phe 157 residue of the enzyme (Figure 2b). In case of dimethoxy substituted compound **8g**, the docking conformation showed moderate interaction pattern.

The two methoxy groups present at the *meta* and *para* position at adjacent carbon atoms displayed poor interaction, which might have been due to higher steric strain. The docking conformation of compound **8g** showed two arene-arene interactions with Phe 157 and His 239 residues of the enzyme (Figure 2c).

DISCUSSION

The synthesis of compound **8a** yielded a white powder. Its molecular formula, $C_{22}H_{25}Cl_2N_3O_5S$, was determined by EI-MS with $[M]^+$ at *m/z* 513; along with two distinct peaks at *m/z* 336 for 1-[(3,5-dichloro-2-ethoxyphenyl)sulfonyl]piperidine-4-yl cation and *m/z* 260 for base ion peak *N′*-(4-methoxybenzylidene)piperidine-4-carbohydrazide-1-yl cation. The proposed fragmentation pattern is sketched in Figure - 1 for this compound. Characteristic bands appeared in the IR spectrum confirming the sulfonyl group (1360 cm^-

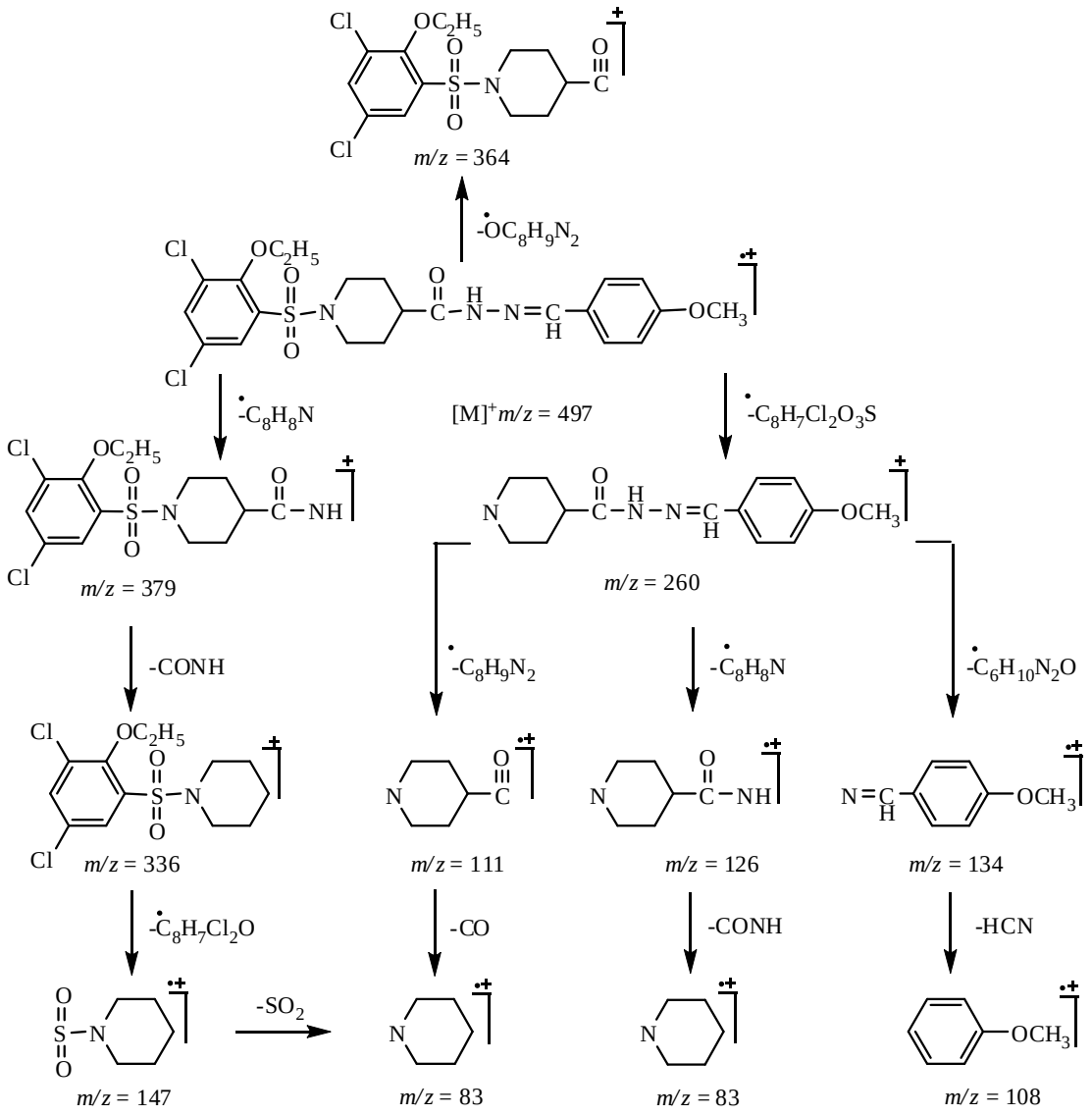


Figure 1: Mass fragmentation pattern of 1-[(3,5-dichloro-2-ethoxyphenyl)sulfonyl]-*N′*-(4-methoxybenzylidene)piperidin-4-carbohydrazide (**8a**)

^{1}) and –N=CH- (1678 cm^{-1}). In the aromatic region of the ^{1}H-NMR spectrum, two *m*-coupled signals of the phenylsulfonyl group with one proton integration, a *J*-value of 2.4 and 2.8 Hz, appeared at *δ* 7.58 (*para* proton to sulfonyl group) and *δ* 7.80 (*ortho* proton to sulfonyl group). The signals resonating at *δ* 7.71 (d, *J* = 8.2 Hz, 2H, H-2'' & H-6'') and 7.21 (d, *J* = 8.4 Hz, 2H, H-3'' & H-5'') were assigned to the *para*-substituted benzene ring due to the large coupling constant and symmetry of the molecule. In the aliphatic section, the multiplets appearing at *δ* 3.89-3.84 (m, 2H, H_e-2 & H_e-6), 3.36-3.20 (m, 1H, H-4), 2.86-2.80 (m, 2H, H_a-2 & H_a-6) and 1.96-1.80 (m, 4H, H-3 & H-5) for nine protons were assigned to the piperidine ring. The two quartet and triplet signals at *δ* 4.23 (q, *J* = 6.4 Hz, 2H, H-7') and *δ* 1.43 (t, *J* =6.4 Hz, 3H, H-8') were assigned to five protons of the ethoxy group, *ortho* to sulfonyl group. A chemical shift value at *δ* 3.81 was assigned to singlet of the methoxy group attached to one of the benzene rings. The singlet appearing at *δ* 8.38 (s, 1H, H-7''), due to a downfield shift, was attributed to a proton of the imine group. The structure of **8a** was substantiated and designated 1-[(3,5-dichloro-2-ethoxyphenyl)sulfonyl]-*N′*-(4-methoxybenzylidene)piperidin-4-carbohyrazide. The structure of the other compounds was likewise established.

The screening of all these synthesized compounds against Gram-positive and -negative bacterial strains showed potent antibacterial activity in all but a few. Compounds **8e** and **8c** exhibited good inhibition percentage and MIC values against all bacterial strains, possibly due to the presence of a 2-hydroxyphenyl and 3-nitrophenyl group, respectively, in comparison to ciprofloxacin, taken as reference standard.

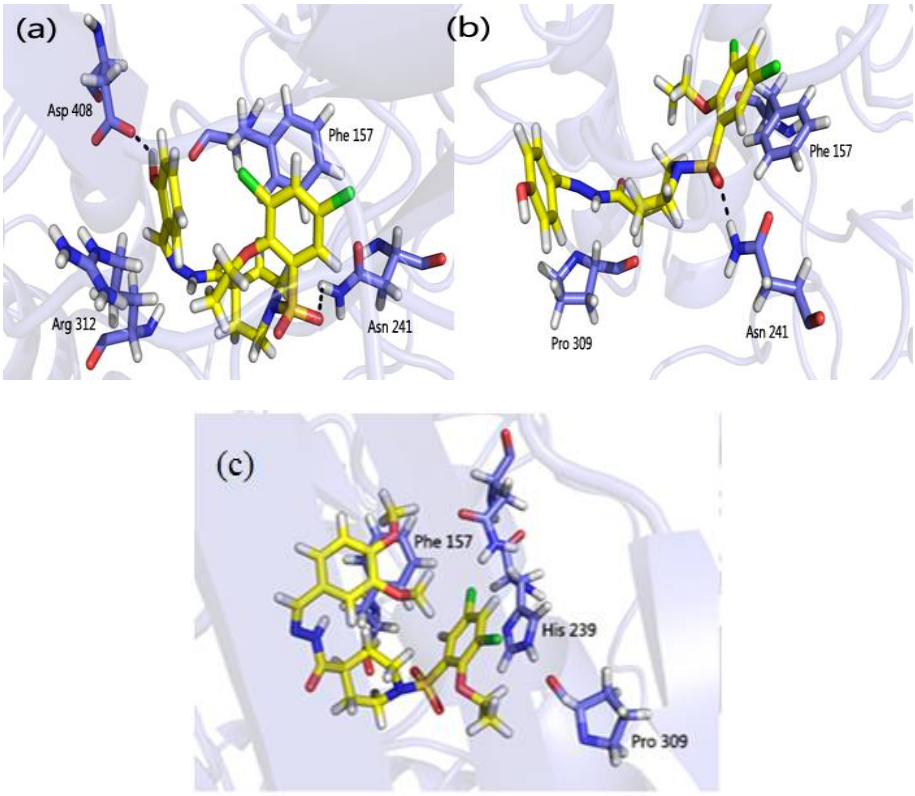


Figure 2: Binding models of compounds; **(a):** Compound **8e** nicely binds to α-glucosidase through Asn 241 and Asp and two π-π interactions with Phe 157 and Arg 312**. (b):** Compound **8b** binds well to *α*-glucosidase through Asn 241and one π-π interaction with Phe 157. **(c):** Compound **8g** binds to α-glucosidase and shows two π-π interactions with Phe 157 and His 239

Compounds **8a**, **8b**, **8d**, **8f**, **8g** and **8h** demonstrated good to moderate activity against both Gram-positive and -negative bacterial strains. *S. typhi* was best inhibited by molecule **3** (ethyl ester) with a MIC of 7.99 ± 0.86 µM and **8e** (bearing 2-hydroxyphenyl group) with a MIC of 8.00 ± 0.54 µM relative to 7.12 ± 0.21 µM for ciprofloxacin. Against *E. coli*, molecule **6** (carbohydrazide) with a MIC of 8.05 ± 0.52 µM and **8f** (bearing a 4-(dimethylamino)phenyl group) with a MIC of 8.21 ± 0.83 µM were the most effective in comparison to 7.05 ± 0.28 µM for the reference. The synthesized compounds showed relatively moderate activity against *B. subtilis* with the lowest MIC for **8c** (bearing a 3-nitrophenyl group) at 8.56 ± 0.63 µM as compared to 7.65 ± 0.48 µM for the standard. The moderate to excellent activity against *Staphylococcus aureus* rendered **3** (ethyl ester) and **8c** (bearing a 3-nitrophenyl group) the best ones with MIC values of 8.43 ± 0.79 and 8.86 ± 0.29 µM in comparison to 7.89 ± 0.27 µM.

Similarly, evaluation of α-glucosidase inhibitory activity of all synthesized compounds showed moderate activity except **8a, 8c** and **8h**, the inactive ones. Good activity was shown by **8e** and **8d** with respective IC_{50} values of 40.62 ± 0.07 and 48.64 ± 0.08 µM in comparison to 38.25 ± 0.12 µM for acarbose, the positive control. The activity of **8e** and **8d** was probably due to the presence of a hydroxyl and chloro group in these compounds. Compounds **8b, 5** and **8f** were less active, and **3**, **6** and **8g** had very low poorly active. However, **8a** and **8b** showed outstanding activity and could be further evaluated for the treatment of type-2 diabetes.

CONCLUSION

The biological activity data obtained demonstrate that the target compounds are significant inhibitors of bacterial growth and α-glucosidase activity. On the basis of the aforementioned results, these newly synthesized compounds may be further developed for the treatment of type-2 diabetes and bacterial infections.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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