RESEARCH ARTICLE

Synthesis, Characterization, and Antidiabetic Evaluation of N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl) carbamothioyl) Benzamide Derivatives

Hadeel Ahmed kenoosh[1*], Anto Emmanuel D P[2], Ramesh S Bhat[3], Janardhana Nayak[3], Alphonsus D'souza[2]. Arun Kumar R[4]

[1]*Al-Maarif University College -Anbar-Iraq.*
[2]*Department of Studies in Chemistry, St. Philomena College (Autonomous) Mysuru-570015, Karnataka, India.*
[3]*Department of Chemistry, NMAM Institute of Technology (NMAMIT), NITTE (Deemed to be University), Karnataka, India.*
[4]*Holy Grace Academy of Pharmacy, Mala. Thrissur, India.*

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ABSTRACT

A series of novel N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl) carbamothioyl) benzamide derivatives (2a-2h) were synthesized and characterized for their potential antidiabetic properties. The synthesis involved multi-step reactions starting from 3-chloro-4-nitrobenzoic acid, and the final compounds were purified by recrystallization. The structures were confirmed using melting points, TLC, FTIR, ^{1}H-NMR, ^{13}C-NMR, and LC-MS. *In-silico* pharmacokinetic analysis using SwissADME indicated favorable drug-likeness properties for the synthesized compounds, particularly those with electron-withdrawing substituents. *In-vitro* assays demonstrated significant α-amylase and α-glucosidase inhibitory activities, with compounds 2a, 2g, and 2h showing the highest potency (IC_{50} values: 23.19-28.61 µM for α-amylase and 23.25-28.25 µM for α-glucosidase), comparable to the standard drug acarbose. The presence of electron-withdrawing groups enhanced the inhibitory effects, while electron-donating groups reduced efficacy. These findings suggest that the synthesized compounds, particularly 2a, 2g, and 2h, hold promise as effective antidiabetic agents.

Keywords: Diabetes, oxadiazoles, acarbose and swiss ADME.

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INTRODUCTION

Diabetes mellitus is a complex, progressive metabolic disorder characterized by chronic hyperglycemia resulting from impaired processing of fats, proteins, and carbohydrates. This dysregulation leads to consistently elevated blood sugar levels, which can cause a range of complications if left unmanaged.[1-2] This sustained hyperglycemia poses grave risks to various bodily systems, including the heart, kidneys, eyes, nerves, and blood vessels, potentially culminating in dysfunction and enduring harm.[3] Its prevalence is swiftly escalating worldwide, with projections suggesting it will afflict approximately 693 million adults by 2045.[4,5] The complications of diabetes, encompassing both macrovascular (such as cardiovascular diseases) and microvascular (like diabetic kidney diseases, retinopathy, and neuropathy), significantly contribute to heightened mortality rates, vision impairment, and renal failure. Diagnosis primarily relies on detecting elevated blood glucose levels, indicative of the body's failure to produce or utilize insulin effectively, often attributed to beta-cell dysfunction or insulin resistance.[6,7] Insulin resistance impedes glucose transport into cells, perpetuating hyperglycemia and adversely affects pancreatic beta-cell function, thereby diminishing insulin, secretion and overall metabolic efficiency.[8,9] The islets of Langerhans within the pancreas house beta-cells responsible for insulin production in response to glucose stimulation.[10] Type 2 diabetes, commonly discussed as (NIDDM) non-insulin-dependent diabetes, manifests as a prevalent subtype characterized by concurrent insulin resistance and inadequate insulin secretion as pivotal contributors to its onset.[11] Research tracking high-risk cohorts and individuals with impaired glucose tolerance underscores the early emergence of insulin resistance in the disease progression.[12]

**Author for Correspondence: hadeel.kenoush@uoa.edu.iq*

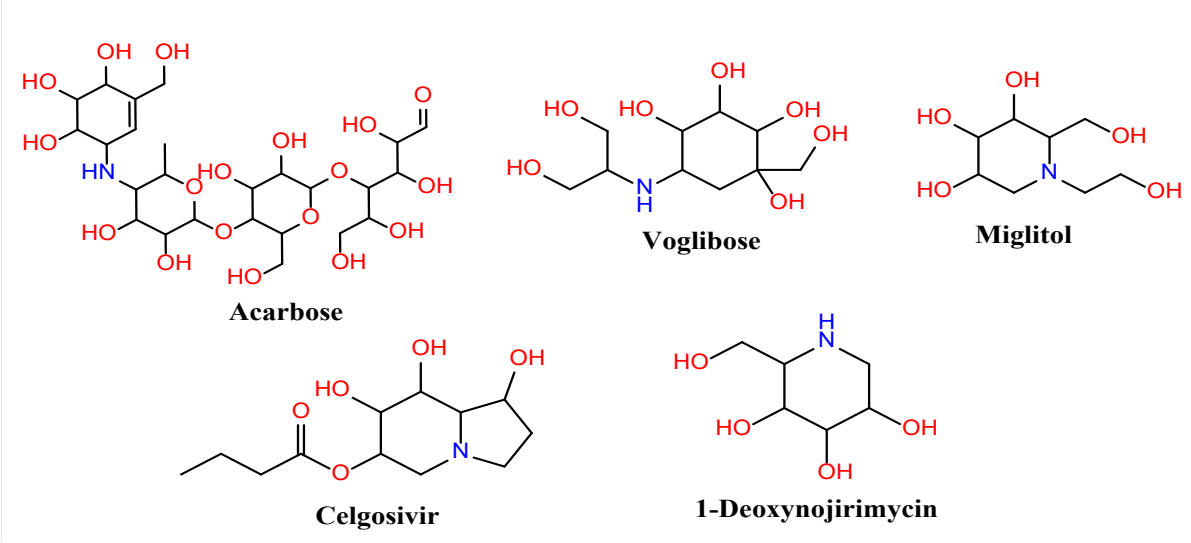


Figure 1: Important clinically used α-amylase and α-glucosidase inhibitors[20]

α-Amylase is a pivotal enzyme responsible for the hydrolysis of starch, a complex carbohydrate, into simpler sugars like maltose and glucose. It plays an essential role in various biological processes, particularly in digestion in humans and other animals. Additionally, α-amylase is widely utilized in industrial applications, including food processing, where it is used to break down starches during the production of products such as syrups, bread, and alcoholic beverages.[13,14]

In this context, α-glucosidase plays a critical role in carbohydrate digestion by breaking down oligosaccharides into monosaccharides, which contributes to elevated blood glucose levels and hyperglycemia. This enzyme is also involved in the synthesis of glycoproteins, which are important targets in the diagnosis and treatment of type 2 diabetes mellitus (T2DM). Unlike β-glucosidase, which hydrolyzes 1,4-β bonds, α-glucosidase specifically targets the 1,4-α bonds in oligosaccharides and is primarily located in the epithelial cells of the small intestine, making it a key factor in postprandial hyperglycemia.[15-17]

As a result, inhibiting α-glucosidase has become a potent strategy in T2DM management. Various chemotherapeutic agents, such as α-glucosidase inhibitors (AGIs), reduce the enzyme's activity. However, the hypoglycemic compounds remain often linked with gastrointestinal side effects, affecting more than 50% of patients. The fermentation of unabsorbed carbohydrates produces gas, resulting bloating in abdomen, increases flatulence, diarrhea, and cramping. As a result, individuals with acute or chronic gastrointestinal conditions are generally not prescribed AGIs. Existing drugs such as acarbose and miglitol, which are shown in Figure 1, are known to have contraindications, particularly in patients with renal impairment and hepatic dysfunction.[18,19]

In addition to being a crucial focus in drug discovery, 1,3,4-oxadiazoles have demonstrated significant therapeutic potential across various areas, including weight loss management, diabetic treatment and cancer therapy. Given the importance of 1,3,4-oxadiazole moieties in these therapeutic fields, this study introduces the preparation of heterocyclic compounds and explores their potential bioactive properties. The design process employs an innovative approach, and the activities of these new drugs are assessed through in vitro studies to evaluate their inhibitory effects on α-amylase and α-glucosidase.[21-24]

MATERIAL AND METHODS

Chemistry

Reagents and chemicals were sourced from Sigma Aldrich, HI media, and Fine Chemicals, Mumbai, India. The homogeneity of composites was assessed using TLC(thin-layer chromatography) with a solvent system of ethyl acetate and n-hexane in a 3:2 ratio. The spots were visualized on a pre-coated Silica GF254 plate under UV light. Melting points were measured using an Equiptronics digital melting point apparatus, with values reported as uncorrected. FTIR spectra (cm^{-1}) were documented on an IR spectrometer, Alpha Bruker. Both ^{1}H-NMR and ^{13}C-NMR spectra are obtained using a Jeol ECZ 400 FT-NMR spectrometer at 100 MHz, with trimethylsilyl (TMS) as the internal standard (δ ppm). Mass spectra were recorded on a Shimadzu LC-MS-8030 series.

General Procedure for the synthesis of 5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-amine:

Methyl 3-chloro-4-nitrobenzoate was synthesized by reacting 0.01 mole of 3-chloro-4-nitrobenzoic acid in 20 ml of methanol. The reaction mixture was microwaved for 10 to 15 minutes at 340 watts while under reflux, and drops of H2SO4 was added as an activator. The resulting compounds were separated and utilized to make 3-chloro-4-nitrobenzohydrazide in the following stage. The separated solid (0.01 mole) was subsequently subjected to a microwave reflux reaction for 5 minutes at 340 watts with 2 mL of 99% hydrazine hydrate. TLC was used to track the course of the reaction, and after it

was finished, the compounds were dried and with methanol recrystallized. Lastly, 15 milliliters of methanol were used to dissolve 0.01 mole of 3-chloro-4-nitrobenzohydrazide, which then interacted with 0.01 mole of cyanogen bromide (Figure 2). For 5 to 15 minutes, the reaction mixture was refluxed at 340 watts while being stirred. After TLC verified that the process was finished, the mixture was dried and nullified using Sodium bicarbonate. After that, the compound was cleaned, dried, and recrystallized.[25]

The general protocol for the preparation of isothiocyanates replaced with benzoyl

A solution of ammonium thiocyanate (5 mmol) in 15 mL of acetone was dropwise mixed with a solution of substituted benzoyl chloride (5 mmol) in 25 mL of acetone (Figure 2). Next, the reaction mixture was heated for five to ten minutes at 340 watts while under reflux. Using TLC, the reaction's development was tracked. Upon completion, the mixture was allowed to cool to room temperature, and the resulting precipitate (NH_4Cl) was filtered off. The filtrate contained the desired aroyl isothiocyanate derivative.[26]

General Procedure of Synthesis of N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl)carbamothioyl)benzamide Derivatives (2a-2h):

A solution of 5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-amine (5 mmol) in 5 mL of acetone was added to the aroyl isothiocyanate derivative, and the resulting mixture was stirred under reflux at 340 watts for 10-15 minutes. The reaction's completion was confirmed using TLC. After that, the product was cleaned with H2O and then again with CH3OH to refine it further.

N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl) carbamothioyl)-4-methoxybenzamide(2a):

Molecular formula: $C_{17}H_{12}ClN_5O_5S$, FTIR (cm^{-1}): 3453(-NH), 3001 (Ar, CH), 2967 (CH_2), 1737 (C=O), 1584 (C=N), 1516 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 3.34(s, CH3, 3H), 6.91-8.12 (m, Ar-H, 7H), 11.26 and 12.12 (s, NH, 2H): ^{13}CNMR (400MHz, DMSOd_6, δ, ppm): 54.32, 121.63, 124.38, 128.65, 130.67, 134.65, 152.33, 157.76, 158.85, 161.65, 162.96, 163.14, 164.37, 166.18, 166.76, 167.56 and 172.85: LC-MS (m/z): calculated for $C_{17}H_{12}ClN_5O_5S$ is 433.825; found: 433.7682 (M+).

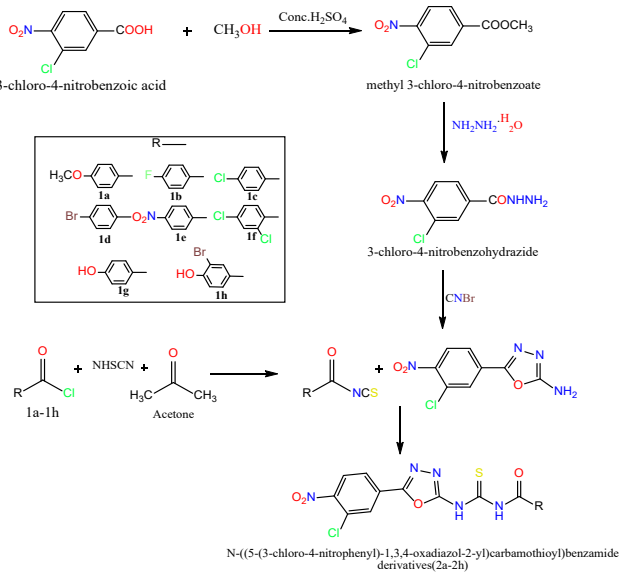


Figure 2: Synthetic scheme

N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl) carbamothioyl)-4-fluorobenzamide (2b)

Molecular formula: $C_{16}H_9ClFN_5O_4S$, FTIR (cm^{-1}): 3453(-NH), 3001 (Ar, CH), 2967 (CH_2), 1737 (C=O), 1584 (C=N), 1516 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 6.91-8.12 (m, Ar-H, 7H), 11.26 and 12.12 (s, NH, 2H): ^{13}CNMR (400MHz, DMSOd_6, δ, ppm):

120.43, 123.32, 127.65, 131.67, 134.15, 152.87, 157.23, 158.45, 161.15, 162.56, 164.14, 165.30, 166.06, 166.86, 167.44 and 172.65: LC-MS (m/z): calculated for $C_{16}H_9ClFN_5O_4S$ is 421.79; found: 421.4528 (M+).

4-chloro-N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl)carbamothioyl)benzamide (2c)

Molecular formula: $C_{16}H_9Cl_2N_5O_4S$, FTIR (cm^{-1}): 3482(-NH), 3011 (Ar, CH), 2976 (CH_2), 1734 (C=O), 1581 (C=N), 1514 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 6.82-8.22 (m, Ar-H, 7H), 11.14 and 12.14 (s, NH, 2H): ^{13}CNMR (400MHz, DMSOd_6, δ, ppm): 121.43, 124.32, 128.75, 132.33, 133.10, 152.45, 156.23, 157.46, 160.15, 162.12, 162.14, 163.30, 165.06, 165.93, 167.23 and 173.26: LC-MS (m/z): calculated for $C_{16}H_9Cl_2N_5O_4S$ is 438.244; found: 438.32 (M+).

4-bromo-N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl)carbamothioyl)benzamide (2d)

Molecular formula: $C_{16}H_9BrClN_5O_4S$, FTIR (cm^{-1}): 3482(-NH), 3012 (Ar, CH), 2980 (CH_2), 1730 (C=O), 1584 (C=N), 1516 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.58-8.14 (m, Ar-H, 7H), 11.38 and 12.85 (s, NH, 2H): ^{13}CNMR (400MHz, DMSOd_6, δ, ppm): 121.47, 122.35, 126.54, 130.31, 132.55, 151.32, 155.23, 157.46, 160.01, 163.06, 162.15, 163.34, 165.01, 166.78, 167.25 and 172.22: LC-MS (m/z): calculated for $C_{16}H_9BrClN_5O_4S$ is 482.695; found: 482.690 (M+).

N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl) carbamothioyl)-4-nitrobenzamide (2e)

Molecular formula: $C_{16}H_9ClN_6O_6S$, FTIR (cm^{-1}): 3480(-NH), 3010 (Ar, CH), 2975 (CH_2), 1738 (C=O), 1582 (C=N), 1517 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.67-8.04 (m, Ar-H, 7H), 11.30 and 12.15 (s, NH, 2H): ^{13}CNMR (400MHz, DMSOd_6, δ, ppm): 122.47, 123.39, 127.84, 131.32, 132.10, 151.22, 156.23, 158.46, 160.01, 162.06, 162.15, 163.34, 165.01, 165.99, 167.21 and 173.22: LC-MS (m/z): calculated for $C_{16}H_9ClN_6O_6S$ is 448.797; found: 448.653 (M+).

2,4-dichloro-N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl) carbamothioyl) benzamide (2f)

Molecular formula: $C_{16}H_{10}ClN_5O_5S$, FTIR (cm^{-1}): 3480(-NH), 3010 (Ar, CH), 2975 (CH_2), 1738 (C=O), 1582 (C=N), 1517 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 6.82-8.22 (m, Ar-H, 6H), 11.34 and 12.11 (s, NH, 2H): ^{13}CNMR

(400MHz, DMSOd_6, δ, ppm): 120.43, 123.32, 127.65, 131.33, 132.10, 150.22, 156.23, 158.46, 160.01, 162.06, 162.15, 163.34, 165.01, 165.99, 167.21 and 173.21: LC-MS (m/z): calculated for $C_{16}H_{10}ClN_5O_5S$ is 472.689; found: 472.0567 (M+).

N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl) carbamothioyl)-4-hydroxybenzamide(2g)

Molecular formula: $C_{16}H_8Cl_3N_5O_4S$, FTIR (cm-^{1}): 3495(-NH), 3332(-OH), 3007 (Ar, CH), 2965 (CH_2), 1730 (C=O), 1585 (C=N), 1517 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 6.67-8.3 (m, Ar-H, 7H), 9.32 (s, OH, 1H), 11.34 and 12.11 (s, NH, 2H): ^{13}CNMR (400MHz, DMSOd_6, δ, ppm): 120.43, 123.32, 127.65, 130.33, 132.10, 150.22, 156.23, 158.46, 160.01, 162.06, 162.15, 163.34, 164.01, 165.99, 167.21 and 172.22: LC-MS (m/z): calculated for $C_{16}H_8Cl_3N_5O_4S$ is 419.798; found: 419.674 (M+).

3-bromo-N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl)carbamothioyl)-4-hydroxybenzamide(2h)

Molecular formula: $C_{12}H_8N_4O_5S$, FTIR (cm-^{1}): 3432(-NH), 3345(-OH), 3005 (Ar, CH), 2955 (CH_2), 1734 (C=O), 1581 (C=N), 1512 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.67-8.19 (m, Ar-H, 6H), 9.41 (s, OH, 1H), 11.32 and 12.03 (s, NH, 2H): ^{13}CNMR (400MHz, DMSOd_6, δ, ppm): 119.76, 123.42, 128.65, 129.33, 135.10, 149.22, 155.23, 159.46, 160.93, 161.01, 162.15, 163.29, 164.0, 164.99, 166.23 and 171.23: LC-MS (m/z): calculated for $C_{12}H_8N_4O_5S$ is 433.825; found: 433.8012 (M+).

Swiss ADME's Pharmacokinetics and Drug-likeness Prediction

The Swiss ADME predictor, a free web tool for evaluating small compounds' pharmacokinetics, drug-likeness, and medicinal chemistry friendliness, was used to conduct the ADME investigation. The project aimed to create molecules that meet predetermined standards for drug-likeness, such as having a molecular weight of less than 500 g/mol, fewer than five donors and acceptors of hydrogen bonds, and fewer than ten rotatable bonds. The Swiss ADME tool provided detailed results on key parameters such as solubility, gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, and bioavailability of the synthesized molecules.[27,28]

Additionally, the study considered Log P, a measure of a molecule's lipophilicity, defined as the logarithm of the concentration ratio of a drug between two solvents in its unionized form. According to Lipinski's rule of five, a Log P value below 5 is recommended for drug-like compounds, with lower Log P values indicating higher lipophilicity. The aqueous solubility of a compound is also critical, as it significantly influences its absorption and distribution characteristics.[29,30]

Biological Activity

Assay for α-Amylase Inhibitory: 20 µL of α-amylase solution (0.5 mg/mL) and 200 µL of sodium phosphate buffer (0.02 M, pH 6.9) made up the reaction mixture. Following the addition of 250 µL of test samples (10–50 µg/mL), the mixture was allowed to sit at room temperature for ten minutes. Next, 200 µL of a 1% starch solution was added, and the mixture was left to incubate at 25°C for an extra 10 minutes. 400 µL of dinitrosalicylic acid (DNS) reagent was added to stop the reaction, and the mixture was then incubated for five minutes at 70°C in a water bath. At 540 nm, absorbance was determined using an ELISA microplate reader.

The standard drug was acarbose, and each experiment was run in triplicate. The reaction mixture sans the test sample made up the control.

The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = [(C - S)/C] \times 100 \quad \text{(equation 1)}$$

where C is the absorbance of the control and
S is the absorbance of the sample.

***In-Vitro* α-Glucosidase Inhibitory Assay**

A 96-well plate was used to generate the reaction mixture, which included 20 µL of test samples (concentration range: 10-50 µg/mL), 10 µL of α-glucosidase enzyme, and 50 µL of 0.1 M phosphate buffer (pH 6.8). For 15 minutes, the mixture was incubated at 37°C. Next, 20 µL of p-nitrophenyl D-glucopyranoside solution was added as the substrate, and it was incubated at 37°C for an additional 20 minutes. About 50 µL of 0.1 M NaCO3 was added to halt the reaction. An ELISA microplate reader was then used to measure the amount of freed p-nitrophenol at 405 nm. The standard was acarbose, and concurrent control trials were carried out without the test drug. Every experiment was carried out three times.

The percentage inhibition was calculated using the formula: as shown in Equation 1

RESULT AND DISCUSSION

Scheme 1 shows how the named compounds (2a-2h) are synthesized. The purification process involved sequentially recrystallizing all newly produced chemicals and intermediates from ethanol. The compounds' purity was confirmed by measuring their melting points and by employing thin-layer chromatography (TLC) with n-hexane and ethyl acetate (2:3) as the solvent system and silica gel G plates as the stationary phase. The resulting synthesized compounds' physicochemical characteristics are shown in Table 1. FTIR, ^{1}H NMR, ^{13}C NMR, and LC-MS spectrum data were utilized to corroborate the structures of every molecule.

SwissADME's pharmacokinetics and drug-likeness prediction

The outcomes shows that the compounds coded as 2a through 2f, each featuring different substituent groups, exhibit the most promising drug-like properties. These compounds demonstrate favorable absorption, distribution, metabolism, and excretion (ADME) characteristics, suggesting their high potential for drug development. Detailed evaluation using the SwissADME search engine further supports these findings, with the specific ADME parameters for each compound summarized comprehensively (Table 2).

Table 1: Compounds (5a-5j) Physicochemical Properties

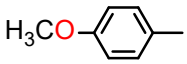
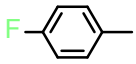
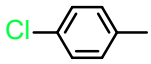
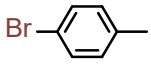
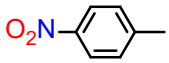
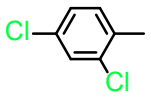
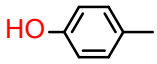
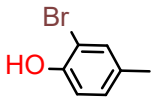
Compound	*R*	*Molecular formula*	*Molecular weight*	*MP(°C)*	*Yield (%)*
2a		$C_{17}H_{12}ClN_5O_5S$	433.825	204–206	88
2b		$C_{16}H_9ClFN_5O_4S$	421.79	241–243	76
2c		$C_{16}H_9Cl_2N_5O_4S$	438.244	255–257	74
2d		$C_{16}H_9BrClN_5O_4S$	482.695	234–236	69
2e		$C_{16}H_9ClN_6O_6S$	448.797	251–253	89
2f		$C_{16}H_8Cl_3N_5O_4S$	472.689	293–295	74
2g		$C_{16}H_{10}ClN_5O_5S$	419.798	234–236	83
2h		$C_{16}H_9BrClN_5O_5S$	498.695	288–290	86

Table 2: Swiss ADME's predictions on the pharmacokinetics and drug-likeness of the produced compounds (2a–2h)

Code	*Mol.Wt*	*Solubility*	*GI absorption*	*Log Kp (cm/sec)*	*BBB permeability*	*Bioavailability*
2a	433.825	Moderately soluble	High	-4.149	No	0.13
2b	421.79	Moderately soluble	High	-4.119	No	0.13
2c	438.244	Moderately soluble	High	-4.153	No	0.13
2d	482.695	soluble	High	-4.134	No	0.13
2e	448.797	Moderately soluble	High	-5.883	No	0.13
2f	472.689	soluble	High	-4.223	No	0.13
2g	419.798	soluble	High	-5.03	No	0.13
2h	498.695	Moderately soluble	High	-5.009	No	0.13

In-vitro Antidiabetic Activity

The antidiabetic potential of all newly synthesized compounds (2a-2h) was evaluated using in vitro tests that measured the inhibition of α-amylase and α-glucosidase. Acarbamazeone served as the usual reference medication for the tests, which examined the substances at 10 and 50 µg/mL concentrations. Table 2 provides a summary of the findings. Compounds 2a, 2g, and 2h demonstrated significant inhibitory action in the α-amylase inhibition experiment, with IC50 values of 28.62 ± 0.31 µM, 24.84 ± 0.15 µM, and 23.11 ± 0.27 µM, in that order. These values are comparable to acarbose, which has an IC50 of 24.41 ± 1.43 µM. The high potency of these compounds indicates their potential as effective α-amylase inhibitors. Compounds containing electron-withdrawing groups generally showed the greatest inhibitory activity. Conversely, compounds 2b (IC50 55.24 ± 0.22 µM), 2c (IC50 34.05 ± 0.39 µM),

Table 3: Compounds (2a-2h) In-vitro antidiabetic activity

Compounds	*IC_{50} Values*	
	α-Amylase (µM/mL)	*α-Glucosidase (µM/mL)*
2a	28.62 ± 0.31	27.52 ± 0.19
2b	52.63 ± 0.34	55.24 ± 0.22
2c	34.05 ± 0.39	37.26 ± 0.23
2d	55.41 ± 0.55	45.24 ± 0.79
2e	38.44 ± 0.46	33.53 ± 0.45
2f	39.25 ± 0.41	51.27 ± 0.83
2g	24.84 ± 0.15	23.27 ± 0.24
2h	23.11 ± 0.27	28.21 ± 0.31
Acarbose	24.41 ± 1.43	23.43 ± 1.21

All results done in triplicate (n = 3, ± SD)

2d (IC_{50} 55.41 ± 0.55 µM), and 2f (IC_{50} 39.25 ± 0.41µM) demonstrated moderate to weak inhibition. The presence of electron-donating groups on the aromatic ring is probably the cause of this decreased efficacy, which may impair the compounds' interaction with the enzyme's active site. Overall, the structural variations among the synthesized compounds significantly affect their α-amylase inhibitory activity, with electron-withdrawing groups enhancing and electron-donating groups diminishing their antidiabetic potential.

In the α-glucosidase inhibition assay, compounds 2a, 2g, and 2h exhibited exceptionally strong activity, with inhibitory concentration 50 of 27.52 ± 0.19 µM, 23.27 ± 0.24 µM, and 28.21 ± 0.31 µM, correspondingly. These values reflect high potency, particularly in comparison to the standard inhibitor, acarbose, which has an IC50 value of 23.43 ± 1.21 µM. These compounds' increased inhibitory activity is thought to be caused by the presence of electron-withdrawing groups on the aromatic ring, such as chloro (Cl) and bromo (Br).

Conversely, compounds 2b (IC50 55.24 ± 0.22 µM), 2c (IC50 37.26 ± 0.23 µM), 2d (IC50 45.24 ± 0.79 µM), and 2f (IC50 51.27 ± 0.83 µM) showed moderate to weak inhibition. The presence of electron-donating groups on the aromatic ring, which seem to lessen the compounds' inhibitory ability, is most likely the cause of this decreased efficiency. The findings suggest that the inhibitory activity against α-glucosidase is significantly influenced by the chemical makeup of the substituents on the aromatic ring, with electron-donating and electron-withdrawing groups increasing and decreasing the inhibitory effects, respectively.

CONCLUSION

In this study, we synthesized a series of novel N-((5-(3-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl)carbamothioyl) benzamide derivatives (2a-2h) and assessed their potential as antidiabetic agents. Efficient synthetic routes were employed, utilizing microwave-assisted reactions and conventional purification methods. The structures of the synthesized products were confirmed through comprehensive spectroscopic analysis, including FTIR, ^{1}H-NMR, ^{13}C-NMR, and LC-MS. Pharmacokinetic profiles, predicted using the SwissADME tool, indicated favorable drug-likeness properties for most compounds, aligning with Lipinski's rule of five. These in silico results were corroborated by promising ADME characteristics, suggesting potential suitability for further drug development. In vitro antidiabetic assays demonstrated significant inhibitory activity of the compounds against α-glucosidase enzymes and α-amylase. Compounds 2a, 2g, and 2h showed potent inhibition, with IC50 values comparable to the standard drug acarbose, indicating strong potential as antidiabetic agents. On the aromatic rings, the presence of electron-withdrawing groups was linked to increased inhibitory activity and the presence of electron-donating groups decreased efficacy.

AUTHOR CONTRIBUTIONS STATEMENT

H. A. K oversaw the project design, while A.E.D.P. authored the methodology. Formal analysis was carried out by R.S.B, J.N, A.K.R and A.D with validation of results conducted by both. All authors. performed software analysis. All authors contributed to the original draft preparation, final review, and editing, and approved the submitted version.

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