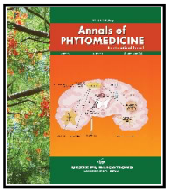
Annals of Phytomedicine 12(1): 632-637, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.1.109

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Synthesis and biological evaluation of some substituted pyrazolopyrimidines derivatives as potential α-glucosidase inhibitors

Mukesh Chandra Sharma◆
School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshila Campus, Indore-452001, Madhya Pradesh, India.

Article Info

Article history
Received 26 May 2023
Revised 22 June 2023
Accepted 23 June 2023
Published Online 30 June-2023

Keywords
Pyrazolo [1,5-α] pyrimidine
α-glucosidase activity
Type 2 diabetes mellitus

Abstract

Present communication deals with the synthesis of a novel series of substituted ethyl 6-substituted (2-phenyldiazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-a]pyrimidine-5-carboxylate compounds were synthesized and evaluated for invitro a-glucosidase inhibitory activity embracing pyrazolopyrimidines nucleus in their moiety. The compounds were synthesized in excellent yields and their structures were collaborated on the basis of FT-IR, 1H NMR, and mass analyses data. Their purity has been ascertained by thin layer chromatographic method. The maximum alpha-glucosidase inhibitory activity was reported with 3c, 3d, 3g, 3i and 3k, with inhibition 82.96%, 88.37%, 85.79%, 89.43% and 94.61%, respectively.

1. Introduction

Diabetes is a severe public health issue that has reached pandemic proportions across the world. Almost 80% of all adult diabetics are in developing countries. In terms of the number of people with diabetes, India is in the top 10 in the world, measured at 40.9 million, followed by China at 39.8 million. Behind them come USA; Russia; Germany; Japan; Pakistan; Brazil; Mexico and Egypt (Tabish, 2007). At least 462 million individuals worldwide have type 2 diabetes; with that number of people suffering from diabetes is expected to reach 693 million by 2045 globally (Allahabi and Singh, 2022).

Diabetes mellitus is developed due to long term existence of hyperglycaemia in the body. Increased blood glucose levels above the standard range lead to hyperglycaemia and this situation may arise due to defects in insulin secretion, action, or both (Singh and Singh, 2021; WHO, 2002). Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia leading to abnormalities in insulin secretion, insulin action, or both. Type 2 diabetes mellitus (T2DM) is a complex and chronic metabolic disease due to insulin resistance in body tissue (Matthews *et al.,* 1985). Type 2 diabetes accounts for 90% of the total diabetes cases and the overall rise in prevalence over the years (Saeedi *et al*., 2019). It is characterized by insulin resistance and beta-cells dysfunction leading to reduced glucose uptake and postprandial hyperglycaemia (Kahn, 2003). The insulin resistance leads to hyperglycaemia, which can damage many of organs (Kawahito *et al.,* 2009). α-glucosidase, found in the brush-border surface membrane of intestinal cells, plays catalysing role in the carbohydrate digestion process by which the postprandial blood glucose levels increases. Preventing the glucose release in the bloodstream, the α-glucosidase inhibitors control T2DM (Poovitha and Parani, 2016). The effects of diabetes mellitus include long-term damage, dysfunction, and failure of various organs including the kidney, nerves, heart, and gastrointestinal tract, retina, foot ulcer bacteria (Kaur and Valecha, 2014; Shirumalla *et al*., 2021; Gupta and Kori, 2022; Singh *et al*., 2022; Arora *et al*., 2022). Pyrazolo [1,5-α] pyrimidine moiety was fused, rigid, and planar N heterocyclic system that contains both pyrazole and pyrimidine rings (Castillo and Portilla, 2018). Pyrazolo[1,5-α] pyrimidine pharmacophores, privileged scaffolds, and an outstanding heterocyclic compound with wide ranges of pharmacological activities due to different mechanisms of action. It has several potent biological implementations as antischistosomal, antimetabolites in purine biochemical interactions, sedative and antitrypanosomal (Novinson *et al.,* 1976). The pyrazolopyrimidine pyrimidine ring is a versatile scaffold. A class of isomeric heterocyclic chemical compounds known as pyrazolopyrimidines (pyrazolo [1,5-α] pyrimidine) with the molecular formula $C_6H_5N_3$.Pyrazolo[1,5- α] pyrimidines have a wide range of biological and pharmaceutical activities, which have been patented for herbicide, insecticide, and sterilization uses (Feurer *et al.,* 2004). Pyrazolo[1,5-α] pyrimidines, in particular, have exhibited valuable pharmaceutical applications including various kinase inhibitors (Ren *et al.,* 2012), COX-2 inhibitors (Almansa *et al.,* 2001), antiviral activity (Hwang *et al.,* 2012; Sun *et al.,* 2016), antimicrobial activity (Hassan *et al.,* 2017), anxiolytic activity (Childress *et al.,* 2019), and positron emission tomography tumour imaging agents (Xu *et al.,* 2011). Among various approaches, alpha glucosidase was chosen as the target because of the less postprandial hyperglycaemic and ultimately less insulin secretion.

A series of biologically active ethyl 6-substituted (2-phenyldiazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α] pyrimidine-5-carboxylate derivatives (3a-l) were synthesized by initial diazotisation of various substituted anilines (1a-l) under temperature-controlled conditions (0-5°C). Furthermore, the structures of synthesized compounds were confirmed based on IR, ^{1}H NMR, and mass spectroscopy.

Corresponding author: Dr. Mukesh Chandra Sharma
Assistant Professor, School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshila Campus, Indore-452001, Madhya Pradesh, India.
E-mail: drmukeshcsharma@gmail.com
Tel.: +91-9836372944; 9713513574

2. Materials and Methods

2.1 Chemistry

All the chemicals and reagents used in the synthesis and biological activity determination were purchased from commercial sources and used without further purification. The solvents for chemical reaction, crystallization and chromatography are used as procured. All the synthesized compounds were found in good agreement with the elemental analysis. The structural assignments are also in accordance with FT-IR and ^{1}H NMR spectral data. The sample was analyzed and spectral data was collected from instrumentation facility, School of Chemical Sciences, Devi Ahilya Vishwavidyalaya Indore. The ^{1}H NMR spectra and mass spectra of the synthesized compound were processed on LC-MS as single quadrupole spectrometer from Agilent and the data were collected from Central instrumentation facility IISER, Bhopal. The Veego corporation (Model: VMP-D) melting point apparatus was used to determine the melting point of synthesized compounds. The melting points reported here are uncorrected. The synthesis of substituted ethyl 6-(2-phenyl diazenyl) -4,5-dihydro-7-methylpyrazolo[1,5-α] pyrimidine-5-carboxylate derivatives (3a-l) was accomplished by step procedure.

2.2 General procedure for ethyl 6-(2-phenyldiazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α]pyrimidine-5-carboxylate derivatives

Pertinent aniline (1a-l) derivatives (0.01M) were dissolved in a mixture of 5.0 ml of concentrated HCl and 4.0 ml distilled water, now the contents were kept at freezing temperature. To this, an aqueous solution of sodium nitrite (0.01M) in 5 ml of distilled water was added drop wise with continuous stirring and keeping the temperature of the reaction vessel in the vicinity of 0-5^{o}C. Meanwhile, in another beaker 7 g (0.01 M) of sodium acetate, ethyl 4-phenyl-2,4-dioxobutanoate (0.01 M), (Fadnavis and Radhika, 2004) in 25 ml of ethyl alcohol was taken and cooled in a ice bath. To this, now the diazotized solution was added to this solution by drop wise under through stirring. The reaction mixture was left for overnight period, filtered through suction, washed with plenty of water and dried to get product (2a-l).

The intermediate formed in above step was taken as starting material (2a-l) for synthesis of these derivatives. A mixture of compound 2a-l (0.01mol) and amino pyrazoles (0.01 mol) in solvent in suitable round flask was heated for hrs with constant stirring. Further, the reaction was monitored by using thin layer chromatography technique, the reaction mixture was poured into ice cold water, and the white precipitate was filtered off, dried, and crystallized from ethanol to give the derivative yield (95%) as shown below in Figure 1. Structures of all the synthesized compounds were established based on spectral assignments. The spectroscopic data for the synthesized pyrazolo [1, 5-α] pyrimidine derivatives (3a-l) are given below:

2.2.1 Ethyl 6-(2-phenyldiazenyl)-4,5-dihydro-7-methylpyrazolo [1,5-α]pyrimidine-5-carboxylate (3a)

IR (–, cm^{-1}); 3356 (N-H stretching), 3071 (C-H, sp^2), 2821 (C-H, sp^3), 1603 (C=C;C=N), 1522 (N=N),1628,1543,1396 (C–C, ring str.), 732 (CH_2),713 (sub. phenyl). ^{1}HNMR (δ ppm); 1.86 (s, 3H, CH_3), 2.30 (s,3H, CH_3), 4.16 (s,1H, CH), 4.98 (s, 2H, CH_2), 5.26 (bs, NH), 6.3-7.6 (m, 7H, Ar-H), mp (ºC) 101-102; yield (%) 72; molecular formula- $C_{16}H_{17}N_5O_2$, mass [m/z] =311.07

2.2.2 Ethyl6-(2-(3-chlorophenyl)diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α]pyrimidine-5-carboxylate (3b)

IR (–, cm^{-1}); 3348 (N-H stretching), 3065 (C-H, sp^2), 2895 (C-H, sp^3), 1611 (C=C;C=N), 1526 (N=N),1620,1555,1391 (C–C, ring str.), 755 (CH_2), 751 (sub. phenyl), 596 (C-Cl). ^{1}HNMR (δ ppm); 1.74 (s, 3H, CH_3), 2.09 (s,3H, CH_3), 4.11 (s,1H, CH), 4.88 (s, 2H, CH_2), 5.20 (bs, NH), 6.4-7.6 (m, 6H, Ar-H), mp (ºC) 111-112; Yield (%) 65; molecular formula- $C_{16}H_{17}ClN_5O_2$, mass [m/z] = 346.11

2.2.3 Ethyl6-(2-(4-chlorophenyl)diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α]pyrimidine-5-carboxylate(3c)

IR (–, cm^{-1}); 3329 (N-H stretching), 3081 (C-H, sp^2), 2877 (C-H, sp^3), 1618 (C=C;C=N), 1520 (N=N),1636,1533,1382 (C–C, ring str.), 766 (CH_2), 742 (sub. phenyl), 611 (C-Cl). ^{1}HNMR (δ ppm); 1.74 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 4.11 (s, 1H, CH), 4.88 (s, 2H, CH_2), 5.20 (bs, NH), 6.4-7.6 (m, 6H, Ar-H), mp (ºC) 128-129; yield (%) 72; molecular formula- $C_{16}H_{17}ClN_5O_2$, mass [m/z] = 345.88

2.2.4 Ethyl 6-(2-(3-hydroxyphenyl)diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α] pyrimidine-5-carboxylate(3d)

IR (–, cm^{-1}); 3521(OH), 3298 (N-H stretching), 3043 (C-H, sp^2), 2858 (C-H, sp^3),1626 (C=C; C=N), 1517 (N=N), 1613, 1568, 1382 (C–C, ring str.), 747(CH_2), 759 (sub. phenyl). ^{1}HNMR (δ ppm); 1.79 (s, 3H, CH_3), 2.15 (s,3H, CH_3), 4.17 (s,1H, CH), 4.91 (s, 1H, OH), 4.69 (s, 2H, CH_2), 5.20 (bs, NH), 6.4-7.6 (m, 6H, Ar-H), mp (ºC) 145-146; yield (%) 70; molecular formula- $C_{16}H_{17}N_5O_4$, mass [m/z] = 327.35

2.2.5 Ethyl 6-(2-(4-hydroxyphenyl)diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α] pyrimidine-5-carboxylate(3e)

IR (–, cm^{-1}); 3498 (OH), 3253 (N-H stretching), 3055 (C-H, sp^2), 2849 (C-H, sp^3), 1640 (C=C; C=N), 1519 (N=N), 1611, 1564, 1352 (C–C, ring str.), 739(CH_2), 754 (sub. phenyl). ^{1}HNMR (δ ppm); 1.73 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 4.07 (s, 1H, CH), 4.96 (s, 1H, OH), 4.60 (s, 2H, CH_2), 5.24 (bs, NH), 6.3-7.6 (m, 6H, Ar-H), mp (ºC) 105-106; Yield (%) 68; molecular formula- $C_{16}H_{17}N_5O_4$, mass [m/z] = 327.35

2.2.6 Ethyl 6-(2-(4-bromophenyl)diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α]pyrimidine-5-carboxylate(3f)

IR (–, cm^{-1}); 3323 (N-H stretching), 3066 (C-H, sp^2), 2872 (C-H, sp^3), 1627 (C=C;C=N), 1520 (N=N),1629,1531,1356 (C–C, ring str.),750 (CH_2), 742 (sub. phenyl), 601 (C-Br). ^{1}HNMR (δ ppm); 1.74 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 4.11 (s, 1H, CH), 4.88 (s, 2H, CH_2), 5.20 (bs, NH), 6.4-7.6 (m, 6H, Ar-H), mp (ºC) 138-139; yield (%) 75; molecular formula- $C_{16}H_{16}BrN_5O_2$, mass [m/z] = 389.18

2.2.7 Ethyl 6-(2-m-tolyldiazenyl)-4,5-dihydro-7-methylpyrazolo [1,5-α]pyrimidine-5-carboxylate (3g)

IR (–, cm^{-1}); 3311,(N-H stretching), 3055 (C-H, sp^2), 2849 (C-H, sp^3), 1640(C=C; C=N), 1511(N=N), 1611, 1564, 1352 (C–C, ring str.),739(CH_2), 754 (sub. phenyl). ^{1}HNMR (δ ppm); 1.89 (s,6H, CH_3 x 2), 2.42 (s,3H, CH_3), 4.15 (s,1H, CH), 4.56 (s, 2H, CH_2), 5.14 (bs, NH), 6.3-7.6 (m, 6H, Ar-H), mp (ºC) 131-132; Yield (%) 71; molecular formula- $C_{17}H_{19}N_5O_2$, mass [m/z] = 325.05

2.2.8 Ethyl 6-(2-p-tolyldiazenyl)-4,5-dihydro-7-methylpyrazolo [1,5-α]pyrimidine-5-carboxylate (3h)

IR (–, cm^{-1}); 3315,(N-H stretching), 3072 (C-H, sp^2), 2863 (C-H, sp^3), 1657(C=C; C=N), 1518 (N=N),1619, 1571, 1359 (C═C, ring str.),748 (CH_2), 751(sub. phenyl). ^{1}HNMR (δ ppm); 1.77 (s,6H, CH_3 x 2), 2.37 (s,3H, CH_3), 4.12 (s,1H, CH), 4.41 (s, 2H, CH_2), 5.08 (bs, NH), 6.3-7.6 (m, 6H, Ar-H), mp (ºC) 125-126; Yield (%) 60; molecular formula- $C_{17}H_{19}N_5O_2$, mass [m/z] = 325.05

2.2.9 Ethyl6-(2-(3-nitrophenyl)diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α]pyrimidine-5-carboxylate(3i)

IR (–, cm^{-1}); 3324 (N-H stretching), 3058 (C-H, sp^2), 2874 (C-H, sp^3), 1652 (C=C; C=N), 1524 (N=N), 1589(-NO_2), 1621, 1555, 1367 (C═C, ring str.),751 (CH_2), 781 (sub. phenyl). ^{1}HNMR (δ ppm); 1.77 (s,3H, CH_3), 2.31 (s,3H, CH_3), 4.17 (s,1H, CH), 4.50 (s, 2H, CH_2), 5.11 (bs, NH), 7.2-8.4 (m, 6H, Ar-H), mp (ºC) 162-163; Yield (%) 77; molecular formula- $C_{16}H_{16}N_6O4$, mass [m/z] = 356.25

2.2.10 Ethyl6-(2-(4-nitrophenyl)diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α]pyrimidine-5-carboxylate(3j)

IR (–, cm^{-1}); 3312 (N-H stretching), 3070 (C-H, sp^2), 2851 (C-H, sp^3),1662 (C=C; C=N), 1523 (N=N), 1591(-NO_2), 1630, 1545, 1360 (C═C, ring str.),748(CH_2), 761 (sub. phenyl). ^{1}HNMR (δ ppm); 1.72 (s,3H, CH_3), 2.41 (s,3H, CH_3), 4.11 (s,1H, CH), 4.58 (s, 2H, CH_2), 5.14 (bs, NH), 7.2-8.4 (m, 6H, Ar-H), mp (ºC) 156-157; Yield (%) 70; molecular formula- $C_{16}H_{16}N_6O4$, mass [m/z] = 356.18

2.2.11 Ethyl 6-(2-(3-methoxyphenyl)diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α]pyrimidine-5-carboxylate(3k)

IR (–, cm^{-1}); 3325 (N-H stretching), 3064(C-H, sp^2), 2861 (C-H, sp^3), 1535 (N=N), 1658 (C=C; C=N), 1635, 1536, 1367 (C═C, ring str.), 758 (CH_2), 755 (sub. phenyl). ^{1}HNMR (δ ppm); 1.77 (s,3H, CH_3), 2.44 (s,3H, CH_3), 3.26 (s, 3H, OCH_3), 4.16 (s,1H, CH), 4.41 (s, 2H, CH_2), 5.01 (bs, NH), 6.2-7.3 (m, 6H, Ar-H), mp (ºC) 150-151; Yield (%) 62; molecular formula- $C_{17}H_{19}N_5O3$, mass [m/z] = 341.08

2.2.12 Ethyl6-(2-(4-methoxyphenyl)diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α]pyrimidine-5-carboxylate (3l)

IR (–, cm^{-1}); 3316 (N-H stretching), 3049(C-H, sp^2), 2851 (C-H, sp^3), 1656 (C=C; C=N), 1526 (N=N), 1631, 1516, 1357 (C═C, ring str.), 748 (CH_2), 754 (sub. phenyl). ^{1}HNMR (δ ppm); 1.74 (s,3H, CH_3), 2.40 (s,3H, CH_3), 3.21 (s, 3H, OCH_3), 4.18 (s,1H, CH), 4.48 (s, 2H, CH_2), 5.08 (bs, NH), 6.2-7.3 (m, 6H, Ar-H), melting point (ºC) 148-149; yield (%) 65; molecular formula- $C_{17}H_{19}N_5O3$, mass [m/z] = 341.35

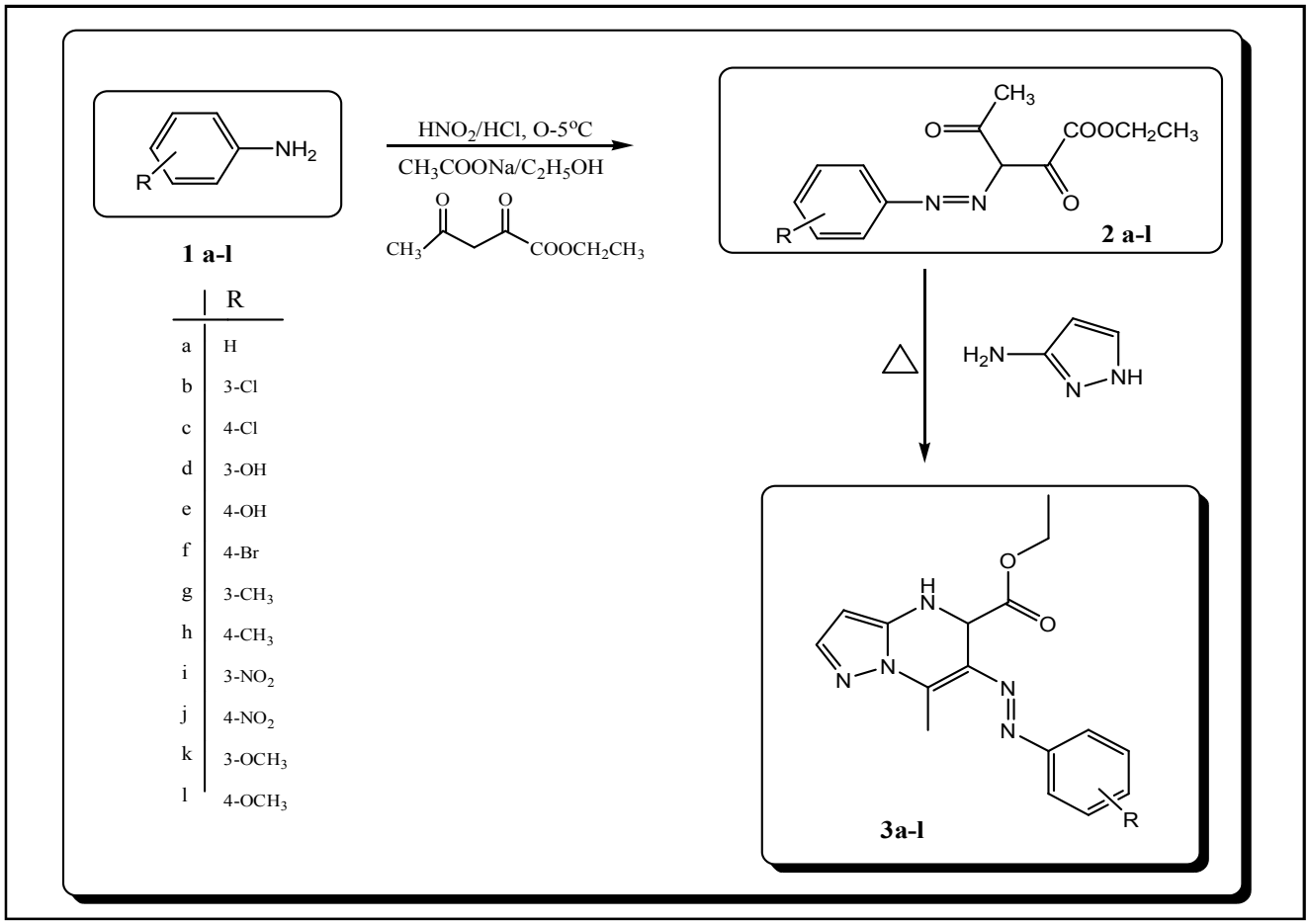


Figure 1: Synthetic pathway of ethyl 6-substituted (2-phenyldiazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α] pyrimidine-5-carboxylate derivatives.

2.3 Antidiabetic activity used *in vitro* α-glucosidase inhibitory activity

The *in vitro* α-glucosidase (*Saccharomyces cerevisiae*) inhibitory activity of the synthesized compounds was performed. The determination of maximum wavelength, incubation time and also substrate optimization was performed in enzyme solutions 2U/ml. The α-glucosidase enzyme (2U/ml) was premixed with 20µl of plant aqueous extract at a concentration of 1000 µg/ml and incubated for 5 min at 37°C (Zawawi *et al.,* 2016). Then, 1 mM p-nitrophenyl gluco-pyranoside (pNPG) (20 µl) in 50 mM of phosphate buffer (pH 6.8) was added to initiate the reaction. The mixture was incubated at 37 °C for 20 min. The reaction was terminated by the addition of 50 µl of 1 mM sodium carbonate. The enzyme inhibitory rates of samples were calculated as follows.

Percentage inhibition = [(absorbance of control – absorbance of the test sample)/absorbance of control] × 100

The α-glucosidase activity was determined at 405 nm. (Table 1 and Figure 2).

Table 1: Percentage alpha-glucosidase inhibition

S. No.	Compound	% Alpha-glucosidase inhibition
1	3a	60.72
2	3b	71.34
3	3c	82.96
4	3d	88.37
5	3e	65.51
6	3f	62.84
7	3g	85.79
8	3h	72.06
9	3i	83.88
10	3j	70.46
11	3k	94.61
12	3l	69.47

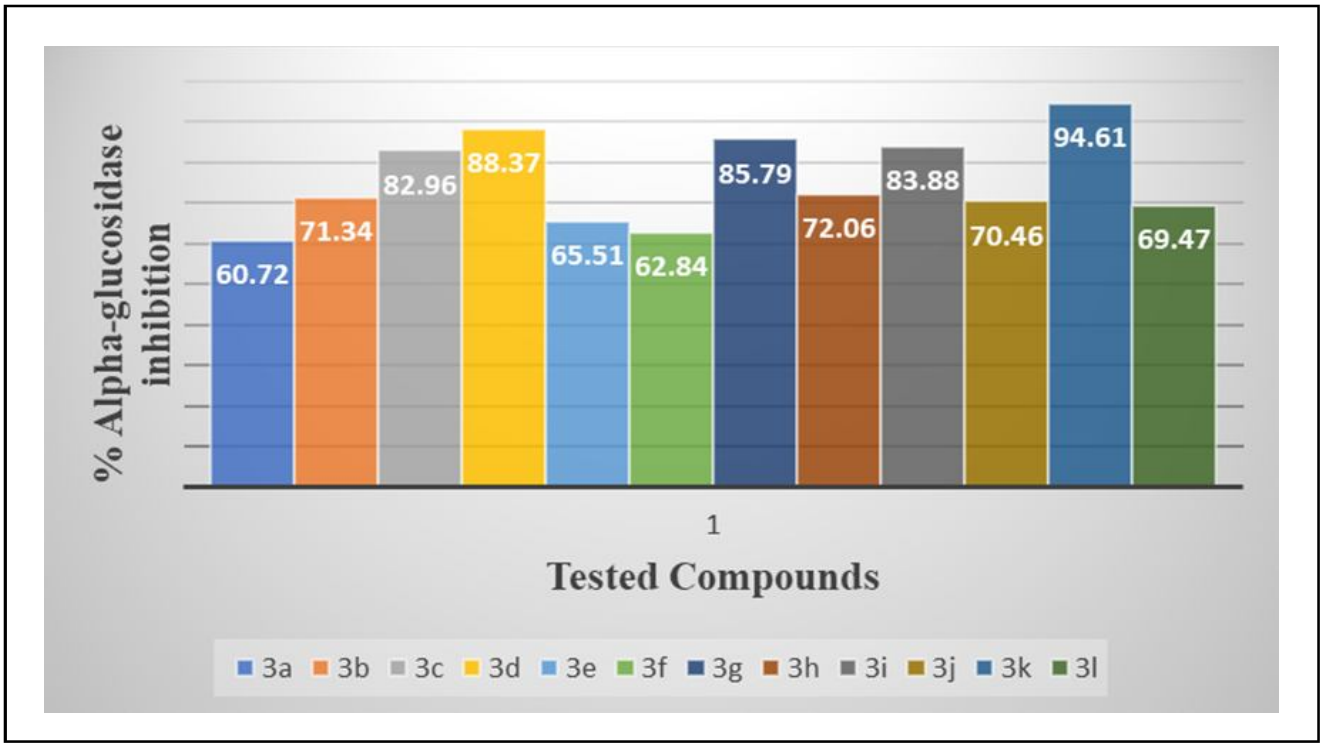


Figure 2: Alpha-glucosidase inhibition activity of synthesized compounds.

3. Results

The synthesis of substituted ethyl 6-(2-phenyldiazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α] pyrimidine-5-carboxylate derivatives (3a-l) was accomplished by step procedure. The synthesized compounds were considered by analyzing them physically and achieved their melting point, Rf value, FT-IR spectrum, MASS and ^{1}H NMR spectra were explained to set the characterized of synthesizes compounds. Infrared spectrum of a representative member belonging to compound, *viz.*, ethyl 6-(2-(4-hydroxyphenyl) diazenyl)-4,5-dihydro-7-methylpyrazolo[1,5-α] pyrimidine-5-carboxylate is occurrence of well-defined following infrared peaks 3498 cm^{-1}, 3253 cm^{-1}, 3055 cm^{-1}, 2849 cm^{-1} , 1519 cm^{-1} , 1611 cm^{-1}, 1352 cm^{-1}, 1640 cm^{-1} 1611 cm^{-1}, 1564 cm^{-1} 1352 cm^{-1} and 754 cm^{-1} . ^{1}H NMR spectrum of ethyl 6-(2-(4-hydroxyphenyl) diazenyl)-4,5-dihydro-7-methyl pyrazolo[1,5-α] pyrimidine-5-carboxylate is well defined singlet at δ 1.73, δ 2.10, δ 2.13, δ 4.07 and 5.24, δ 7.15 and 7.65 corresponding to six protons can be accounted to the aromatic protons present in the benzene ring.

The maximum alpha–glucosidase inhibitory activity was reported with 3c, 3d, 3g, 3i and 3k, with inhibition 82.96%, 88.37%, 85.79%,

89.43% and 94.61%, respectively. Whereas, the lowest alpha-glucosidase inhibitory activity was observed 3a, 3e and 3f as shown in Figure 2 and Table 1.

4. Discussion

Structural characterization of synthesized compounds confirmed the formation of substituted pyrazolo[1,5-α] pyrimidine derivatives. Biological evaluation of synthesized compounds have been carried out α-glucosidase inhibition activity. The pyrazolo [1,5-α] pyrimidine scaffold leads the activity of the synthesized and evaluated compounds against α-glucosidase inhibitory activity. Infrared spectrum of a representative member belonging to compound, *viz*., ethyl 6-(2-(4-hydroxyphenyl) diazenyl)-4,5-dihydro-7-methyl pyrazolo[1,5-α] pyrimidine-5-carboxylate is occurrence of well-defined peaks around 3498 and 3253 cm^{-1} can be assigned to the OH and NH stretching vibrations, respectively. Existence of a peak at 3055 cm^{-1} is attributed to the C-H stretching of sp^2 carbon atom. Appearance of peaks at 2849 cm^{-1} can be assigned to the asymmetric and symmetric vibrations of sp^3 carbons. A significant peak in the range of 1519 cm^{-1} is assignable to the vibrations of N=N moiety. Typical peaks at 1611 and 1352 cm^{-1} can be assigned for carbon-carbon aromatic ring stretching modes. Moreover, a number of peaks in the fingerprint region accounts for the substitution pattern in the phenyl ring. Furthermore, a peak at 1640 cm^{-1} is assignable to C=C or C=N entities, whereas aromatic ring stretching vibrations are noticeable at 1611, 1564 and 1352 cm^{-1}. Other bending vibrational modes at 754 cm^{-1} are characteristic of substitution pattern in the phenyl ring. ^{1}H NMR spectrum of ethyl 6-(2-(4-hydroxyphenyl) diazenyl)-4,5-dihydro-7-methy lpyrazolo[1,5-α] pyrimidine-5-carboxylate is well define singlet at δ 1.73 corresponding to three equivalent protons is assignable to the methyl protons. Existence of a singlet at δ 2.10 is attributable to proton connected to pyridine ring. One another singlet at δ 2.13 can be assigned to the methyl protons. Further, two singlets at δ 4.07 and 5.24 can be attributed to the CH and NH protons, respectively. Appearance of complex multiplate at δ 7.15 - 7.65 corresponding to six protons can be accounted to the aromatic protons present in the benzene ring. The α-glucosidase inhibition activity was carried out at School of Pharmacy, Devi Ahilya Vishwavidyalaya Indore. The *in vitro* α-glucosidase inhibitory activity of the synthesized compounds was performed. The maximum α-glucosidase inhibitory activity was reported with 3c, 3d, 3g, 3i and 3k, with inhibition 82.96%, 88.37%, 85.79%, 89.43% and 94.61%, respectively. Whereas, the lowest α-glucosidase inhibitory activity was observed 3a, 3e and 3f. The maximum according to the tested pyrazolo[1,5-α] pyrimidine derivatives, most of the compounds showed significant inhibition. Compound 3k is a highly active molecule with methoxy substitution.

5. Conclusion

All twelve compounds were evaluated using alpha-glucosidase inhibition activity. Overall these compounds were so to moderate to good inhibition against yeast alpha-glucosidase enzyme. Electron donating and electron withdrawing groups such as methyl, methoxy, nitro and chloro have been used in the synthesis of the target compounds. All the compounds introduction of an electron withdrawing group chlorine, bromine to the moiety.

Acknowledgements

The author thanks Devi Ahilya Vishwavidyalaya (File No. Dev/ Seedmoney2.0/2020-21, Dated 20/01/22 for providing the financial support.

Conflicts of interest

The author declares no conflicts of interest relevant to this article.

References

Allahabi, Z. and Singh S. (2022). *In silico* approach for discovery of drug against the peroxisome proliferator-activated receptor gamma for diabetes treatment. Ann. Phytomed., **11**(1):299-310.

Almansa, C.; De arriba, A.F.; Cavalcanti, F.L.; Gomez, L.A.; Miralles, A.; Merlos, M.; García, R. J. and Forn, J. (2001). Synthesis and SAR of a new series of COX-2-selective inhibitors: pyrazolo[1,5-a] pyrimidines. J. Med. Chem., **44**(3):350-61.

Arora, A.; Chhajed S. and Jain P. (2022). Characterization of phyto-synthesized silver nanoparticles using of nigella sativa L. seed extract and evaluate antimicrobial efficacy against diabetic foot ulcer bacterial isolates. Ann. Phytomed., **11**(11):751-758.

Castillo, J.C. and Portilla, J. (2018). Recent advances in the synthesis of new pyrazole derivatives. Targets Heterocycl. Syst., **2**:194-223.

Childress, E.S.; Wieting, J.M.; Felts, A.S.; Breiner, M.M.; Long, M.F.; Luscombe, V.B.; Rodriguez, A.L.; Cho, H.P.; Blobaum, A.L.; Niswender, C.M.; Emmitte, K.A.; Conn, P.J. and Lindsley, C.W. (2019). Discovery of novel central nervous system penetrant metabotropic glutamate receptor subtype 2 ($mGlu_2$) negative allosteric modulators (NAMs) based on functionalized pyrazolo[1,5-a]pyrimidine-5-carboxamide and thieno[3,2- b]pyridine-5-carboxamide Cores. J. Med. Chem., **62**(1):378-384.

Fadnavis, N.W. and Radhika, K.R. (2004). Enantio- and regiospecific reduction of ethyl 4-phenyl-2,4-dioxobutyrate with baker's yeast: Preparation of (R)-HPB ester, Tetrahed. Asym., **15**(21) 3443-3447.

Feurer, A.; Luithle, J. and Wirtz, S. (2004). Novel 2,5-disubstituted pyrimidine derivatives. PCT inter. Appli. WO patent 2,004,009,589.

Gupta, V. and Kori, M. L. (2022). Assessment of hepato and nephroprotective potential of polyherbal combinations against STZ-induced diabetic liver and kidney complications in wistar rats. Ann. Phytomed., 11(1):311-319.

Hassan, A.S.; Masoud, D.M.; Sroor, F.M. and Ahmed A.A. (2017). Synthesis and biological evaluation of pyrazolo[1,5-*a*]pyrimidine-3-carboxamide as antimicrobial agents. Med. Chem. Res., **26**:2909-2919.

Hwang, J.Y.; Windisch, M.P.; Jo, S.; Kim, K.; Kong, S.; Kim, H.C.; Kim, S.; Kim, H.; Lee, M.E.; Kim, Y.; Choi, J.; Park, D.S.; Park, E.; Kwon, J.; Nam, J.; Ahn, S.; Cechetto, J.; Kim, J.; Liuzzi, M.; No, Z. and Lee, J. (2001). Discovery and characterization of a novel 7-aminopyrazolo[1,5-a]pyrimidine analog as a potent hepatitis C virus inhibitor. Bioorg. Med. Chem. Lett. **22**(24):7297-301.

Kahn, S.E. (2003). The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia, **46**(1):3-19.

Kaur, M. and Valecha, V. (2014). Diabetes and antidiabetic herbal formulations: An alternative to allopathy. Eur. J. Med., **6**:226-240.

Kawahito, S.; Kitahata, H. and Oshita, S. (2009). Problems associated with glucose toxicity: Role of hyperglycemia-induced oxidative stress. World J. Gastroenterol., **15**(33):4137-42.

Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F. and Turner, R.C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, **28**(7):412-9.

Novinson, T.; Bhooshan, B.; Okabe, T.; Revankar, G.R.; Robins, R.K.; Senga, K. and Wilson, H.R. (1976). Novel heterocyclic nitrofurfural hydrazones in vivo antitrypanosomal activity. J. Med. Chem., **19**:512-516.

Poovitha, S. and Parani, M. (2016). *Invitro* and in vivo α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC Complement. Altern. Med., **16**:1-8.

Ren, L.; Laird, E.R.; Buckmelter, A.J.; Dinkel, V.; Gloor, S. L.; Grina, J.; Newhouse, B.; Rasor, K.; Hastings, G.; Gradl, S.N. and Rudolph, J. (2012). Potent and selective pyrazolo[1,5-a]pyrimidine based inhibitors of B-Raf(V600E) kinase with favourable physicochemical and pharmacokinetic properties. Bioorg. Med. Chem. Lett., **22**(2):1165-8.

Saeedi, P.; Petersohn, I.; Salpea, P.; Malanda, B.; Karuranga, S.; Unwin, N.; Colagiuri, S.; Guariguata, L.; Motala, A.A.; Ogurtsova, K.; Shaw, J.E.; Bright, D. and Williams, R. (2019). IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, Diabetes Res Clin Pract., **157**(9):107843.

Shirumalla, A.; Perelli, L. P. and Vanapatla S. R. (2021). *Invitro* α-amylase, α-glucosidase and α-galactosidase inhibitory activity of echinochloa colona (L.) Link. Ann. Phytomed., **10**(2):195-199.

Singh, S. and Singh, D. (2021). Phytomedicine: Alternative safe vehicles on the pathway of Diabetes mllitus. Ann. Phytomed., **10**(1):114-122.

Singh, S.; Kushwaha, P. and Gupta, S. (2022). Development and evaluation of thermos-responsive insitu nanoemulgel of myricetin for diabetic retinopathy. Ann. Phytomed., **11**(1):320-326.

Sun, L.; Gao, P.; Zhan, P. and Liu, X. (2016). Pyrazolo[1,5-a]pyrimidine-based macrocycles as novel HIV-1 inhibitors: A patent evaluation of WO2015123182. Expert. Opin. Ther. Pat., **26**(9):979-86.

Tabish, S.A. (2007). Is Diabetes Becoming the Biggest Epidemic of the Twenty-first Century?. Int. J. Health. Sci., **1**(2):V-VIII.

WHO Expert Consultation, (2002). Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care, **25**:5-20.

Xu, J.; Liu, H.; Li, G; He, Y.; Ding, R.; Wang, X.; Feng, M.; Zhang, S.; Chen, Y.; Li, S.; Zhao, M.; Qi, C. and Dang, Y. (2011). Synthesis and biological evaluation of novel F-18 labeled pyrazolo[1,5-a] pyrimidine derivatives: potential PET imaging agents for tumor detection. Bioorg. Med. Chem. Lett., **21**(16):4736-41.

Zawawi, N.K.; Taha, M.; Ahmat, N.; Wadood, A.; Ismail, N.H.; Rahim, F.; Azam, S.S. and Abdullah, N. (2016). Benzimidazole derivatives as new α-glucosidase inhibitors and *in silico* studies. Bioorg. Chem., **64**:29-36.

Citation **Mukesh Chandra Sharma (2023). Synthesis and biological evaluation of pyrazolopyrimidines as potential a-glucosidase inhibitors. Ann. Phytomed., 12(1):632-637. http://dx.doi.org/10.54085/ap.2023.12.1.109.**