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Synthesis, Analytical Characterization and Anti-Diabetic Activity of Some Heterocycles of Quinazolin-4(3H)one

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Authors' contributions

This work was carried out in collaboration among all authors. Author PGM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MHJ and LJP managed the analyses of the study. Author LJP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Novel quinazolin-4(3H)-one heterocycles were synthesized and assessed for their anti-diabetic activity. Non-enzymatic glycosylation of haemoglobin assay was carried out to identify their potential as anti-diabetic. The cyclization of quinazolinone-4(3H)-one heterocycles was achieved, whereas carbon-carbon cross coupling reactions were carried out using Sonogashira and Suzuki-Miyaura reaction conditions and characterized with analysis. This synthesis method afforded corresponding 2, 3 and 6 substituted quinazolin-4(3H)-ones **(3a to 3m)** with excellent yields.
Methods: 2-Amino-6-bromobenzoic acid was used as a substrate which was converted to corresponding benzamide derivatives (1a-1b) by reaction with benzylamine or cyclohexylamine using acid-amine reaction, followed by cyclization and oxidation using suitable aldehyde in DMSO under microwave condition to give bromo substituted quinazolin-4(3H)-ones (2a-2c), which were cross coupled to suitable terminal alkyne with palladium catalyst as well as copper co-catalyst using Sonogashira condition to obtain desired (3a-3h) and suitable boronic acid with palladium catalyst using Suzuki-Miyaura condition to obtain desired (3i-3m). All synthesized compounds were characterized by FTIR, proton NMR, LC-MS analysis and evaluated for *in vitro* anti-diabetic activity

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using non-enzymatic glycosylation of haemoglobin assay.
Results: Compounds **3m** showed good inhibition of glycosylation of haemoglobin which in turn suggest good anti-oxidant potential on metabolism of glucose and hence lower glucose concentration. It showed IC50 value of 35.91±0.82 µg/mL which was comparable to the standard alpha tocopherol (34.47±0.87µg/mL).
Conclusion: *In-vitro* non-enzymatic glycosylation of haemoglobin method is one of important assays to judge the control of diabetes. The haemoglobin present in RBCs has an affinity to bind with glucose. The greater the glucose level in blood, more amount of glucose-bound (called glycosylated) haemoglobin will be formed. Accordingly, presence of lower concentration of glycosylated haemoglobin is a sure guide to the lower concentration of glucose in the blood. Synthesized compounds (3a-3m) lower the blood glucose level and **3m** has highest potential among those which can be further developed as potent anti-diabetic.

Keywords: Quinazolin-4(3H)-one; heterocycles; Sonogashira reaction; cyclization; glycosylation; anti-diabetic.

ABBREVIATIONS

RBCs : Red Blood Cells
TLC : Thin Layer Chromatography.
FTIR : Fourier Transform Infrared.
NMR : Nuclear Magnetic Resonance.
LC-MS :Liquidchromatography–Mass Spectrometry
IC50 : The half maximal Inhibitory Concentration

1. INTRODUCTION

Currently, 366 million people worldwide affected diabetes mellitus, and this number is estimated to increase to 522 million by 2030 [1]. Diabetes mellitus is a chronic endocrine disorder affecting the metabolism of carbohydrates, proteins, fat, electrolytes and water. It includes a group of metabolic diseases which are characterized by hyperglycemia, where the blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells fail to respond to the produced insulin [2]. Therefore, a primary therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia [3]. Current antidiabetic drugs to treat this disease include metformin, insulin, thiazolinediones, alpha-glucosidase inhibitors, sulfonylureas and DPPIV inhibitors. Despite these multiple treatment options, it is difficult to effectively treat T2D by single treatment option in the long term [4-6]. Therefore, there is an unmet medical need for the development of new, safe and effective antidiabetic therapies with novel and multiple mode of action.

Quinazolin-4(3H)-ones exhibited a wide range of pharmacological properties such as antimicrobial [7,8], antibacterial [9-12], cytotoxicity [13], analgesic [14,15], antifungal [9,16,17], anti-inflammatory [18-20], poly(ADP-ribose)polymerase-1 inhibitors [21], anticonvulsant [22] and hypolipidemic agents [23]. A series of quinazolinone-1,2,3-triazole-acetamide hybrids was synthesized using by molecular hybridization of the potent α-glucosidase inhibitor pharmacophores and evaluated for their anti-diabetic activity [24]. The novel quinazoline derivatives containing acetic acid and 2-methyl propanoic acid as pharmacophore were synthesized for their hypoglycemic and hypolipidemic activity [25]. piperidine-substituted quinazolinone derivatives were identified as a new class of small molecule GHS-R1a antagonists. In vivo efficacy evaluation of selected compounds revealed suppression of food intake and body weight reduction as well as glucose-lowering effects mediated by glucose-dependent insulin secretion [26]. Balaglitazone, a quinazolinone- thiazolidinedione derivative with antihyperglycemic activity, exerts partial peroxisome proliferator-activated receptor (PPAR) gamma agonistic activity and appears to be associated with fewer side effects as compared to full PPAR gamma agonists. Balaglitazone has been used in trials studying the treatment of Type 2 Diabetes Mellitus [27]. Quinazolinone derivatives were synthesized for their inhibitory activities on α-glucosidase enzyme. Two compounds: 2-(4-chlorophenyl)-quinazolin-4(3*H*)-one (CQ) and 2-(4-bromophenyl)-quinazolin-4(3*H*)-one (BQ) were found to be potent inhibitors of α-glucosidase [28].

In the present work, we synthesized novel quinazolin-4(3H)-one heterocycles and evaluated for non-enzymatic glycosylation of haemoglobin assay for anti-diabetic activity.

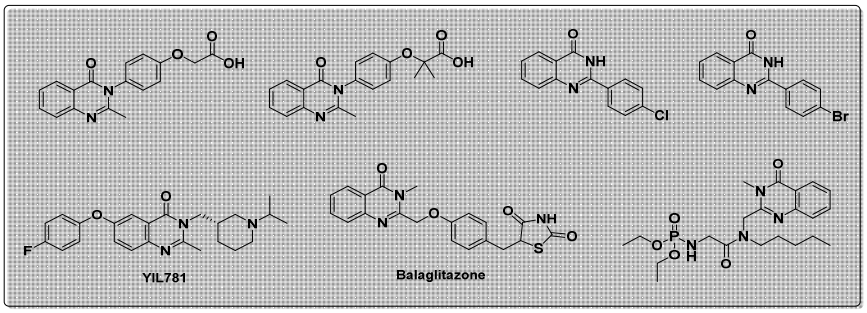

Fig. 1. Some quinazolinone drugs having anti-diabetic potential

2. MATERIALS AND METHODS

The synthesis scheme for the title compounds (3a-3m) is outlined in Fig. 2, 3 and 4. Melting points of all synthesized compounds were determined in open capillaries using Veego melting point apparatus, Model VMP-D (Veego Instrument Corporation) and were uncorrected. Infrared spectra were recorded on SHIMADZU-IR Affinity-1S Fourier Transform Infrared (FTIR) spectrophotometer using attenuated total reflection (ATR) technique. LC–MS analysis for all samples were carried out using WATERS ACQUITY UPLC H class spectrometer with PDA and SQ detector. Samples were prepared in 2mM ammonium acetate and injected into the BEH C18 (502.1 mm) 1.7µm column for detection using 0.1% formic acid in water: acetonitrile as mobile phase with. 1H-NMR spectra were recorded on Brucker 400 MHz Avance III HD instrument with 5mm PABBO BB/19F-1H/D Z-GRD Z108618 probe using DMSO D6 as a solvent and data were processed using Topspin 3.2 software. TLC was performed on precoated alumina silica gel 60 F254 (Merck) using different polarity ratios of ethylacetate: n-hexane as mobile phase and detection was done using UV light. The resulting compounds were purified by recrystallization using suitable solvent or by flash column chromatography.

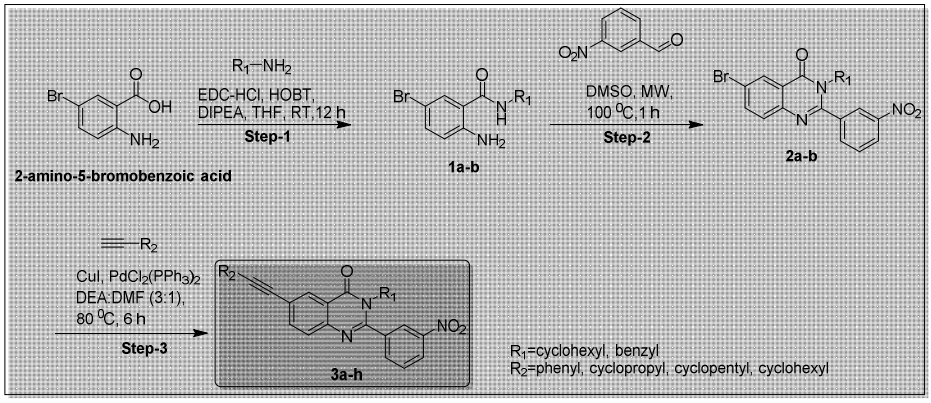

Fig. 2. Synthetic scheme of 2, 3 and 6-trisubstituted quinazolin-4(3*H*)-one (3a-3h)

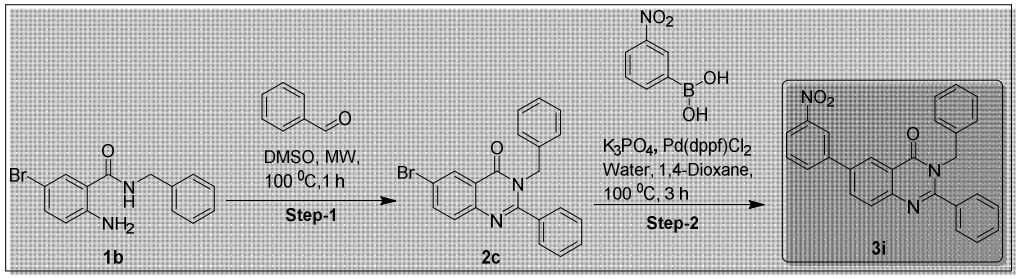

Fig. 3. Synthetic scheme of 3-benzyl-6-(3-nitrophenyl)-2-phenylquinazolin-4(3H)-one (3i)

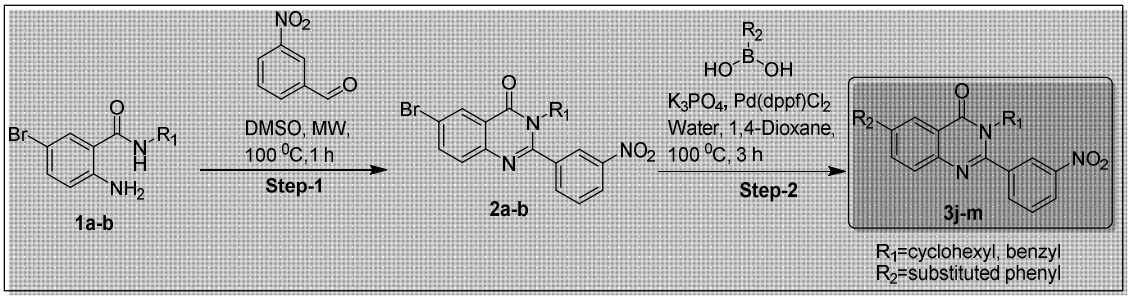

Fig. 4. Synthetic scheme of 2, 3 and 6-trisubstituted quinazolin-4(3*H*)-one (3j-3m)

General synthetic procedures used for the preparation of the target compounds are described below.

2.1 Synthesis of 2-Amino- N-Substituted-5-Bromobenzamide (1a-1b)

Synthesis of 1a and 1b was carried out using the earlier described method [29]. To the mixture of 2-amino-5-bromobenzoic acid (1.0 mmol), cyclohexyl amine or benzyl amine (1.0 mmol) in tetrahydrofuran (15 time), N, N-diisopropylethylamine (2.0 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.5 mmol) and 1-hydroxybenzotriazole (0.5 mmol) were added successively and stirred for 12 h at room temperature. The progression of the reaction was monitored with TLC using ethylacetate: n-hexane (4:1) as mobile phase. The reaction mixture was poured to water, extracted with ethylacetate, washed with brine, dried over sodium sulfate and concentrated under reduced pressure to get the crude product which was purified by trituration using diethyl ether and dried to obtain the desired product as 2-amino-5-bromo-N-cyclohexyl benzamide **(1a)** or 2-amino-N-benzyl-5-bromobenzamide **(1b)**.

2.2 Synthesis of 3-Substituted-6-Bromo-2-(3-Nitrophenyl)Quinazolin-4(3*h*)-One (2a-2b)

To a solution of 2-amino-5-bromo-N-cyclohexyl benzamide **(1a)** or 2-amino-N-benzyl-5-bromobenzamide **(1b)** (1.0 mmol) in dimethylsulphoxide (10 time) was added 3-nitrobenzaldehyde (1.5 mmol) and stirred for 1 h at 100 ^{0}C under microwave irradiation. Consumption of 1a or 1b was checked with TLC using ethylacetate: n-hexane (1:1) as mobile phase, the reaction mixture was poured to water, extracted with ethylacetate, washed with brine, dried over sodium sulfate and concentrated under reduced pressure to get the crude product which was purified by flash column chromatography using 20-30% ethylacetate in n-hexane as mobile phase, eluting the desired product as 6-bromo-3-cyclohexyl-2-(3-nitrophenyl)quinazolin-4(3H)-one **(2a)** or 3-benzyl-6-bromo-2-(3-nitrophenyl) quinazolin-4(3H)-one **(2b)**.

2.3 Synthesis of 3-Benzyl-6-Bromo-2-Phenylquinazolin-4(3h)-One (2c)

To a solution of 2-amino-N-benzyl-5-bromobenzamide **(1b)** (1.0 mmol) in dimethylsulphoxide (10 time) was added benzaldehyde (1.5 mmol) and stirred for 1 h at 100 ^{0}C under microwave irradiation. Consumption of 1b was checked with TLC using ethylacetate: n-hexane (1:1) as mobile phase, the reaction mixture was poured to water, extracted with ethylacetate, washed with brine, dried over sodium sulfate and concentrated under reduced pressure to get the crude product which was purified by flash column chromatography using 20-30 % ethylacetate in n-hexane as mobile phase, eluting the desired product as 3-benzyl-6-bromo-2-phenylquinazolin-4(3H)-one **(2c)**.

2.4 Synthesis Of 2, 3 And 6 Substituted Quinazolin-4(3*h*)-One (3a To 3h)

To the mixture of 2a or 2b (1.0 mmol) and ethynyl derivative (1.5 mmol) in diethylamine: N, N-dimethylformamide (3:1) (10 time) was added copper iodide (0.1 eq) in a glass sealed tube and the reaction mixture was degassed with nitrogen for 15 min, followed by the addition of dichlorobis(triphenylphosphine)palladium (II) ($PdCl_2(PPh_3)_2$) (0.05 eq) and heated to 80 ^{0}C for 6h. The reaction was monitored with TLC using ethylacetate: n-hexane as mobile phase. The

reaction mixture was poured to water, extracted with ethylacetate, washed with brine, dried over sodium sulfate, concentrated under reduced pressure to give the crude product which was purified by flash column chromatography using ethylacetate: n-hexane as mobile phase to give the desired product (3a-3h) with good yield.

2.5 Synthesis Of 3-Benzyl-6-(3-Nitrophenyl)-2-Phenylquinazolin-4(3h)-One (3i)

To the mixture of 2c (1.0 mmol) and 3-nitrophenyl boronic acid derivative (1.5 mmol) in water:1, 4-dioxane (1:3) (20 time) was added potassium phosphate (3.0 mmol) in a glass sealed tube and the reaction mixture was degassed with nitrogen for 15 min, followed by the addition of [1,1′-Bis(diphenylphosphino)ferrocene]dichloro palladium (II) ($Pd(dppf)Cl_2$) (0.05 mmol) and heated to 100 ^{0}C for 3 h. The reaction was monitored with TLC using ethylacetate: n-hexane as mobile phase. The reaction mixture was poured to water, extracted with ethylacetate, washed with brine, dried over sodium sulfate, concentrated under reduced pressure to give the crude product which was purified by flash column chromatography using ethylacetate: n-hexane as mobile phase to give the desired product 3-benzyl-6-(3-nitrophenyl)-2-phenylquinazolin-4(3H)-one (3i).

2.6 Synthesis Of 2, 3 And 6 Substituted Quinazolin-4(3*h*)-One (3j To 3m)

To the mixture of 2a or 2b (1.0 mmol) and 3-nitrophenyl boronic acid derivative (1.5 mmol) in water:1, 4-dioxane (1:3) (20 time) was added potassium phosphate (3.0 mmol) in a glass sealed tube and the reaction mixture was degassed with nitrogen for 15 min, followed by the addition of [1,1′-Bis(diphenylphosphino) ferrocene]dichloro palladium (II) ($Pd(dppf)Cl_2$) (0.05 mmol) and heated to 100 ^{0}C for 3 h. The reaction was monitored with TLC using ethylacetate: n-hexane as mobile phase. The reaction mixture was poured to water, extracted with ethylacetate, washed with brine, dried over sodium sulfate, concentrated under reduced pressure to give the crude product which was purified by flash column chromatography using ethylacetate: n-hexane as mobile phase to give the desired product (3j-3m) with good yield.

2.7 Non-Enzymatic Glycosylation Of Haemoglobin Assay (Anti-Diabetic Activity)

2.7.1 Chemicals & reagents

Solutions of glucose (2%), Haemoglobin (0.06%), Gentamicin (0.02%)
Phosphate Buffer (pH 7.4), Alpha-Tocopherol (Standard).

2.7.2 Instrument

Colorimeter

2.7.3 Experimental method

Solutions of glucose (2%), hemoglobin (0.06%) and Gentamicin (0.02%), were freshly prepared in phosphate buffer (0.01 M, pH 7.4). One mL of each of above-mentioned solution were mixed. One mL of each concentration of different synthesized Compound (PM1-PM30) (10-50 µg/mL) were added to the prepared mixture. Then, the test tubes containing reaction mixture were incubated in dark place at room temperature for three days. The degree of glycosylation of hemoglobin was observed colorimetrically at 520nm. The percentage of inhibition was calculated applying this formula:

$$\text{Inhibition activity (\%)} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

where; Abs (control) is the absorbance of the control reaction (containing all reagents except the test sample) and Abs (sample) is the absorbance of synthesized compounds (3a to 3m). Alpha-Tocopherol was used as a standard drug. Experiments were carried out in triplicates.

3. RESULTS AND DISCUSSION

In the first step, benzamide derivatives **(1a-1b)** were synthesized from anthranilic acid using acid-amine coupling condition. In the second step, substituted benzamide derivatives were cyclized using nitrobenzaldehyde or benzaldehyde in DMSO under microwave condition at 100 ^{0}C to give bromo substituted quinazolin-4(3H)-ones **(2a-2c)**, which were cross coupled to suitable ethynyl derivative under Sonogashira conditions and to suitable boronic acid using Suzuki-Miyaura condition in third step to obtain desired compounds **(3a-3m)**. All

synthesized compounds were characterized and confirmed with physical parameters like melting point, IR, LC-MS and ^{1}H-NMR spectroscopy.

3.1 Physical and Spectral Data of Synthesized Compounds

- **2-amino-5-bromo-N-cyclohexylbenzamide (1a)**

Off white solid product; MP: 195-199°C; Rf: 0.55 (TLC, Ethylacetate: n-hexane = 4: 1); Yield: 70%.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.12 (d, J=7.8 Hz, 1H), 7.63 (d, J=2.3 Hz, 1H), 7.25 (dd, J=8.7, 2.3 Hz, 1H), 6.65 (d, J=8.8 Hz, 1H), 6.50 (s, 2H), 3.70 (s, 1H), 1.76 (dd, 22.6, 7.7 Hz, 4H), 1.61 (d, J=12.8 Hz, 1H), 1.28 (t, J=9.6 Hz, 4H), 1.12 (d, J=11.7 Hz, 1H).

LC-MS m/z = 297/299 $[M]^+$.

- **2-amino-N-benzyl-5-bromobenzamide (1b)**

Off white solid product; MP: 204-208°C; Rf: 0.62 (TLC, Ethylacetate: n-hexane = 4: 1); Yield: 79%.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (t, J=6.0 Hz, 1H), 7.73 (d, J=2.4 Hz, 1H), 7.37-7.20 (m, 6H), 6.68 (d, J=8.7 Hz, 1H), 6.63 (s, 2H), 4.40 (d, J=5.9 Hz, 2H).

LC-MS m/z = 305/307 $[M]^+$.

- **6-bromo-3-cyclohexyl-2-(3-nitrophenyl) quinazolin-4(3*H*)-one (2a)**

Cream solid product; MP: 192-196°C; Rf: 0.44 (TLC, Ethylacetate: n-hexane = 1: 1); Yield: 68%.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 (s, 1H), 8.43 (d, J=8.4 Hz, 1H), 8.27 (d, J=2.4 Hz, 1H), 8.12 (d, J=7.7 Hz, 1H), 8.01 (dd, J=8.8, 2.5 Hz, 1H), 7.87 (t, J=8.0 Hz, 1H), 7.64 (dd, J=8.6 Hz, 2.3 Hz, 1H), 3.66 (m, 1H), 1.82 (d, J=12.4 Hz, 2H), 1.70 (d, J= 13.2 Hz, 2H), 1.49 (d, J=13.0 Hz, 1H), 1.24 (d, J=13.0 Hz, 1H), 1.09 (d, J=13.3 Hz, 1H), 0.93-0.86 (m, 3H).

LC-MS m/z = 428/430 $[M]^+$.

- **3-benzyl-6-bromo-2-(3-nitrophenyl) quinazolin-4(3*H*)-one (2b)**

Cream solid product; MP: 201-205°C; Rf: 0.48 (TLC, Ethylacetate: n-hexane = 1: 1); Yield: 80%.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.39-8.30 (m, 2H), 8.25 (d, J=8.3 Hz, 1H), 8.13-8.03 (m, 1H) 7.87 (t, J=7.8 Hz, 1H), 7.71 (q, J=7.9 Hz, 2H), 7.26-7.16 (m, 3H), 6.94 (dd, J=10.1, 5.0 Hz, 2H), 5.18 (s, 2H).

LC-MS m/z = 436/438 $[M]^+$.

- **3-benzyl-6-bromo-2-phenylquinazolin-4(3H)-one (2c)**

White solid product; MP: 180-184°C; Rf: 0.42 (TLC, Ethylacetate: n-hexane = 1: 1); Yield: 85%.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, J=2.4 Hz, 1H), 8.05 (dd, J=8.7, 2.4 Hz, 1H), 7.69 (d, J=8.7 Hz, 1H), 7.56-7.39 (m, 5H), 7.22 (d, J=6.4 Hz, 3H), 6.96=-6.89 (m, 2H), 5.18 (s, 2H).
LC-MS m/z = 391/393 $[M]^+$.

- **3-benzyl-2-(3-nitrophenyl)-6-(phenylethynyl)quinazolin-4(3H)-one (3a)**

White solid product; MP: 207-210°C; Rf: 0.42 (TLC, Ethylacetate: n-hexane = 1: 4); Yield: 48%.

IR (u_{max}, cm^{-1}): 2212 (C≡C), 1681 (C=O), 1616 (C=N), 1570 (Ar. C=C), 1529 (N-O asymmetrical), 1494, 1340 (N-O symmetrical).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40-8.31 (m, 2H), 8.26 (s, 1H), 8.04 (dd, J=8.4, 2.0 Hz, 1H), 7.88 (d, J=7.7 Hz, 1H), 7.79 (d, J=8.5 Hz, 1H), 7.77-7.62 (m, 3H), 7.51-7.44 (m, 3H), 7.22 (dd, J=7.9, 3.4 Hz, 3H), 6.98-6.91 (m, 2H), 5.19 (s, 2H).

LC-MS m/z = 458.68 $[M+H]^+$.

- **3-benzyl-6-(cyclopropylethynyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one (3b)**

Cream solid product; MP: 182-185°C; Rf: 0.46 (TLC, Ethylacetate: n-hexane = 1: 4); Yield: 39%.

IR (u_{max}, cm^{-1}): 2223 (C≡C), 1681 (C=O), 1616 (C=N), 1587 (Ar. C=C), 1521 (N-O asymmetrical), 1481, 1346 (N-O symmetrical).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (d, J=8.3 Hz, 1H), 8.23 (s, 1H), 8.15 (S, 1H), 7.84 (t, J=7.3 Hz, 2H), 7.70 (t, J=8.0 Hz, 2H), 7.20 (d, J=5.2 Hz, 3H), 6.95-6.88 (m, 2H), 5.16 (s, 2H), 1.62 (tt, J=8.3, 4.9 Hz, 1H), 0.94 (dq, J=6.6, 3.8 Hz, 2H), 0.82 (dq, J=7.1, 4.2 Hz, 2H).
LC-MS m/z = 422.28 $[M+H]^+$.

- **3-benzyl-6-(cyclopentylethynyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one (3c)**

Off white solid product; MP: 190-193°C; Rf: 0.42 (TLC, Ethylacetate: n-hexane = 1: 4); Yield: 43%.

IR (u_{max}, cm^{-1}): 2947, 2866 (Ar. C-H), 2225 (C≡C), 1691 (C=O), 1618 (C=N), 1577 (Ar. C=C), 1535 (N-O asymmetrical), 1483, 1342 (N-O symmetrical).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (dd, J=8.2, 2.3 Hz, 1H), 8.24 (t, J=2.0 Hz, 1H), 8.17 (d, J=2.0 Hz, 1H), 7.93-7.82 (m, 2H), 7.76-7.67 (m, 2H), 7.21(dd, J=5.1, 1.9 Hz, 3H), 6.93 (dd, J=6.7, 2.7 Hz, 2H), 5.18 (s, 2H), 2.95 (p, J=7.4 Hz, 1H), 2.10-1.97 (m, 2H), 1.81-1.73 (m, 2H), 1.74 (s, 1H), 1.69 (dtd, J=11.6, 6.4, 2.8 Hz, 1H), 1.66-1.57 (m, 2H).

LC-MS m/z = 450.51 $[M+H]^+$.

- **3-benzyl-6-(cyclohexylethynyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one (3d)**

Light brown solid product; MP: 203-207°C; Rf: 0.36 (TLC, Ethylacetate: n-hexane = 1: 4); Yield: 38%.

IR (u_{max}, cm^{-1}): 2929, 2852 (Ar. C-H), 2225 (C≡C), 1691 (C=O), 1577 (Ar. C=C), 1533 (N-O asymmetrical), 1481, 1344 (N-O symmetrical).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (dd, J=8.3, 2.3 Hz, 1H), 8.24 (s, 1H), 8.16 (d, J=2.0 Hz, 1H), 7.90-7.81 (m, 2H), 7.70 (dt, J=8.0, 3.8 Hz, 2H), 7.25-7.17 (m, 3H), 6.95 -6.88 (m, 2H), 5.17 (s, 2H), 2.72 (s, 1H), 1.87-1.85 (m, 2H), 1.72-1.70 (m, 2H), 1.54-1.52 (m, 3H), 1.38-1.36 (m, 3H).

LC-MS m/z = 464.68 $[M+H]^+$.

- **3-cyclohexyl-6-(cyclopentylethynyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one (3e)**

Off white solid product; MP: 187-191°C; Rf: 0.48 (TLC, Ethylacetate: n-hexane = 1: 4); Yield: 46%.

IR (u_{max}, cm^{-1}): 2924, 2864 (Ar. C-H), 2222 (C≡C), 1683 (C=O), 1587 (Ar. C=C), 1537 (N-O asymmetrical), 1490, 1344 (N-O symmetrical).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.44 (d, J=8.3 Hz, 1H), 8.16-8.06 (m, 2H), 7.92-7.75 (m, 2H), 7.63 (d, J=8.4, 1H), 3.66-3.63 (m, 1H), 2.92 (p, J=7.4 Hz, 1H), 2.38 (s, 2H), 2.03 (d, J=11.1 Hz, 2H), 1.83 (d, J=12.4 Hz, 2H), 1.76-1.57 (m, 8H), 1.50 (d, J=13.0 Hz, 1H), 1.10 (d, J=13.3 Hz,1H), 0.93-0.86 (m, 2H).

LC-MS m/z = 442.70 $[M+H]^+$.

- **3-cyclohexyl-6-(cyclohexylethynyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one (3f)**

Cream solid product; MP: 198-202°C; Rf: 0.42 (TLC, Ethylacetate: n-hexane = 1: 4); Yield: 35%.

IR (u_{max}, cm^{-1}): 2926, 2852 (Ar. C-H), 2360, 2341 (C≡C), 1674 (C=O), 1583 (Ar. C=C), 1529 (N-O asymmetrical), 1485, 1346 (N-O symmetrical).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.43 (dd, J=8.2, 2.2 Hz, 1H), 8.15-8.06 (m, 2H), 7.91-7.75 (m, 2H), 7.63 (d, J=8.4, 1H), 3.68-3.62 (m, 1H), 2.70 (s, 1H), 2.57 (s, 2H), 1.84-1.80 (m, 4H), 1.72-1.70 (m, 4H), 1.52-1.47 (m, 4H), 1.36 - 1.34(m, 3H), 1.13-0.98 (m, 1H), 0.91 (t, J=13.7 Hz, 2H).

LC-MS m/z = 456.51 $[M+H]^+$.

- **3-cyclohexyl-6-(cyclopropylethynyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one (3g)**

White solid product; MP: 190-194 ^{0}C; Rf: 0.45 (TLC, Ethylacetate: n-hexane = 1: 4); Yield: 49%.

IR (u_{max}, cm^{-1}): 2927, 2852 (Ar. C-H), 2360, 2220 (C≡C), 1674 (C=O), 1583 (Ar. C=C), 1531 (N-O asymmetrical), 1485, 1346 (N-O symmetrical).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 1H), 8.46-8.39 (m, 1H), 8.14-8.05 (m, 2H), 7.86 (t, J=8.0 Hz, 1H), 7.77 (dd, J=8.4, 2.0 Hz, 1H), 7.61 (d, J=8.3 Hz, 1H), 3.63 (d, J=12.0 Hz, 1H), 2.57 (s, 2H), 1.82 (d, J=13.2 Hz, 2H), 1.77-1.68 (m, 2H), 1.62-1.58 (m, 1H), 1.49 (d, J=12.9 Hz, 1H), 1.07 (t, J=13.2 Hz, 1H), 0.98-0.86 (m, 4H), 0.80 (dt, J=6.9, 3.4 Hz, 2H).

LC-MS m/z = 414.6 $[M+H]^+$

- **3-cyclohexyl-2-(3-nitrophenyl)-6-(phenylethynyl)quinazolin-4(3H)-one (3h)**

White solid product; MP: 196-200°C; Rf: 0.42 (TLC, Ethylacetate: n-hexane = 1: 4); Yield: 45%.

IR (u_{max}, cm^{-1}): 2927, 2852 (Ar. C-H), 2222 (C≡C), 1674 (C=O), 1583 (Ar. C=C), 1529 (N-O asymmetrical), 1485, 1346 (N-O symmetrical).
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.44 (d, J=8.5 Hz, 1H), 8.29 (d, J=2.0 Hz, 1H),

8.13 (d, J=7.6 Hz, 1H), 7.98 (d, J=8.5 Hz, 1H), 7.88 (t, J=8.0 Hz, 1H), 7.72 (d, J=8.4 Hz, 1H), 7.64 (dd, J=6.6 Hz, 3.0 Hz, 2H), 7.51-7.44 (m, 3H), 3.68-3.66 (m, 1H), 2.55 (s, 2H), 1.84 (d, J=12.3 Hz, 2H), 1.71 (d, J=13.0 Hz, 2H), 1.50 (d, J=12.9, 1H), 1.11 (d, J=12.6 Hz, 1H), 0.93 (t, J=13.0 Hz, 2H).

LC-MS m/z = 450.62 $[M+H]^+$.

- **3-benzyl-6-(3-nitrophenyl)-2-phenylquinazolin-4(3H)-one (3i)**

Light yellow solid product; MP: 180-184°C; Rf: 0.26 (TLC, Ethylacetate: n-hexane = 1: 4); Yield: 49%.

IR (u_{max}, cm^{-1}): 3062, 2968 (Ar. C-H), 1678 (C=O), 1581 (Ar. C=C), 1533 (N-O asymmetrical), 1471, 1346 (N-O symmetrical).

^1H NMR (400 MHz, DMSO-d_6) δ 8.59-8.55 (m, 2H), 8.35-8.29 (m, 3H), 7.88-7.81 (m, 2H), 7.53-7.44 (m, 5H), 7.24 (q, J=6.8 Hz, 3H), 6.97 (d, J=6.8 Hz, 2H), 5.24 (s, 2H).

LC-MS m/z = 434.61 $[M+H]^+$.

- **3-benzyl-6-(4-hydroxyphenyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one (3j)**

Off white solid product; MP: 203-207°C; Rf: 0.22 (TLC, Ethylacetate: n-hexane = 3: 2); Yield: 46%.

IR (u_{max}, cm^{-1}): 3385 (O-H), 3076 (Ar. C-H), 1654 (C=O), 1604 (Ar. C=C), 1535 (N-O asymmetrical), 1485, 1340 (N-O symmetrical).

^1H NMR (400 MHz, DMSO-d_6) δ 9.74 (s, 1H, OH), 8.38 (s, 1H), 8.34 (d, J=6.8 Hz, 1H), 8.24 (s, 1H), 8.16 (d, J=8.4 Hz, 1H), 7.88 (d, J=7.6 Hz, 1H), 7.78 (d, J=8.5 Hz, 1H), 7.70 (dd, J=19.6, 8.0 Hz, 3H), 7.21 (d, J=6.2 Hz, 3H), 6.93 (dd, J=8.3, 4.4 Hz, 4H), 5.20 (s, 2H).

LC-MS m/z = 450.65 $[M+H]^+$.

- **3-(3-benzyl-2-(3-nitrophenyl)-4-oxo-3,4-dihydroquinazolin-6-yl)benzamide (3k)**

Cream solid product; MP: 212-216°C; Rf: 0.12 (TLC, Ethylacetate: n-hexane = 4: 1); Yield: 37%.

IR (u_{max}, cm^{-1}): 2360, 1670 (C=O), 1589 (Ar. C=C), 1529 (N-O asymmetrical), 1479, 1344 (N-O symmetrical).

^1H NMR (400 MHz, DMSO-d6) δ 8.59 (s, 1H), 8.34 (d, J=10.3 Hz, 3H), 8.27 (t, J=12.1 Hz, 2H), 8.04-7.84 (m, 4H), 7.72 (t, J=7.8 Hz, 1H), 7.63 (t, J=7.6 Hz, 1H), 7.51 (s, 1H), 7.22 (d, J= 6.2 Hz, 3H), 6.95 (d, J=6.9 Hz, 2H), 5.22 (s, 2H).

LC-MS m/z = 477.67 $[M+H]^+$.

- **3-benzyl-2-(3-nitrophenyl)-6-(4-(trifluoromethoxy)phenyl)quinazolin-4(3H)-one (3l)**

Off white solid product; MP: 184-188°C; Rf: 0.42 (TLC, Ethylacetate: n-hexane = 3: 2); Yield: 43%.

IR (u_{max}, cm^{-1}): 1676 (C=O), 1585 (Ar. C=C), 1531 (N-O asymmetrical), 1481, 1348 (N-O symmetrical), 1255 (C-F), 1182 (C-F), 1151 (C-F).

^1H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, J=2.3 Hz, 1H), 8.39-8.31 (m, 1H), 8.27 (d, J=6.8 Hz, 2H), 7.99 (d, J=8.4 Hz, 2H), 7.88 (t, J=9.4 Hz, 2H), 7.72 (t, J=7.9 Hz, 1H), 7.54 (d, J=8.2 Hz, 2H), 7.22 (d, J= 6.1 Hz, 3H), 6.98-6.91 (m, 2H), 5.21 (s, 2H)

LC-MS m/z = 518.36 $[M+H]^+$.

- **3-(3-cyclohexyl-2-(3-nitrophenyl)-4-oxo-3,4-dihydroquinazolin-6-yl)benzaldehyde (3m)**

White solid product; MP: 192-196°C; Rf =0.3 (TLC, Ethylacetate: n-hexane = 3: 2); Yield: 45%.

IR (u_{max}, cm^{-1}): 2927, 2856 (Ar. C-H), 1697 (C=O), 1674 (C=O), 1585 (Ar. C=C), 1533 (N-O asymmetrical), 1473, 1340 (N-O symmetrical).
^1H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H, CHO), 8.57 (s, 1H), 8.51 (s, 1H), 8.50-8.43 (m, 1H), 8.37 (d, J=1.8 Hz, 1H), 8.27 (dd, J= 8.5, 2.3 Hz, 1H), 8.19-8.13 (m, 2H), 7.97 (d, J=7.6 Hz, 1H), 7.90-7.86 (m, 1H), 7.82-7.75 (m, 2H), 3.72-3.67 (m, 1H), 2.62-2.54 (m, 2H), 1.86 (d, J=12.3 Hz, 2H), 1.73 (d, J=13.2 Hz, 2H), 1.51 (d, J=13.0 Hz, 1H), 1.20-1.04 (m, 1H), 0.96 (d, J=13.2 Hz, 2H).

LC-MS m/z = 454.31 $[M+H]^+$.

3.2 Non-enzymatic Glycosylation Of Haemoglobin Assay (Anti-Diabetic Activity)

Compounds (3a to 3m) were tested for non-enzymatic glycosylation of haemoglobin assay and results were compared with Alpha-Tocopherol (Positive control). The inhibitory effects of the test compounds on are indicated by IC50 value in Table 1.

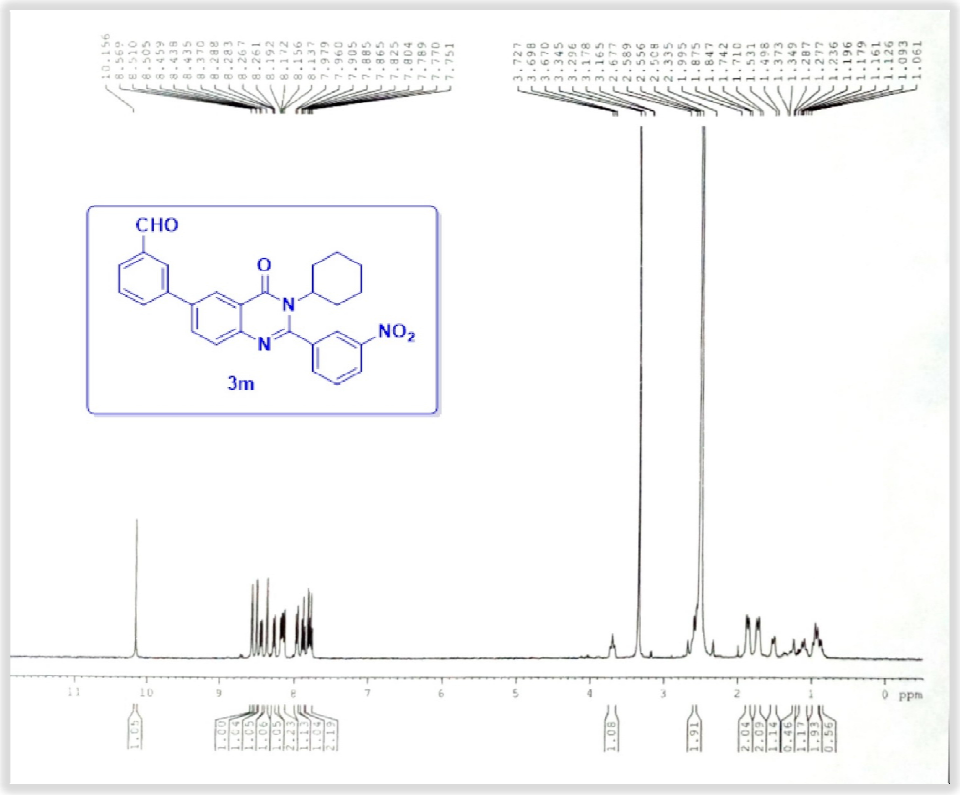

Fig. 5. ^{1}H-NMR Spectrum of 3-(3-cyclohexyl-2-(3-nitrophenyl)-4-oxo-3,4-dihydroquinazolin-6-yl) benzaldehyde (3m)

Table 1. Inhibition of glycosylation (IC50 values)

Compounds	IC50 (µg/mL) Mean ± SEM	Compounds	IC50 (µg/mL) Mean ± SEM
3a	56.91±2.45	3h	43.74±1.78
3b	41.96±1.62	3i	90.43±2.97
3c	47.58±0.96	3j	73.86±1.25
3d	50.82±2.76	3k	46.53±1.67
3e	55.75±2.33	3l	53.65±1.22
3f	53.68±1.45	3m	35.91±0.82
3g	49.04±2.64	Standard	34.47±0.87

Mean ± S.E.M = Mean values ± Standard error of means, standard=Alpha-Tocopherol

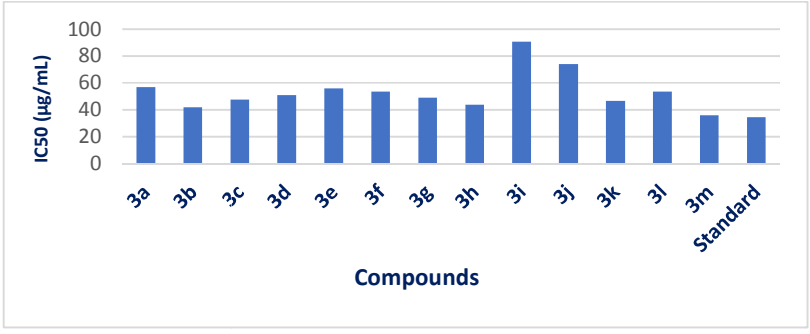

Fig. 6. Inhibition of glycosylation

Alpha-tocopherol is an essential vitamin-micronutrient with antioxidant potential that provide a complementary treatment for patients with chronic diseases. Lowering glycated hemoglobin (HbA1c) to below 7% has been shown to be one of the primary endpoints in reducing microvascular complications of diabetes mellitus and possibly macrovascular disease [30]. Many evidences demonstrates that oxidative stress plays an important role in the pathogenesis of chronic diseases such as DM [31,32] and may diminish the antioxidative defense system of the body, increasing the oxidative load [33]. Some studies have shown that individuals with low concentrations of antioxidants are at increased risk of diabetes complications [32,34,35] and that T2DM is associated with endothelial dysfunction [36]. These conditions may develop into macro and microvascular diseases such as retinopathy, nephropathy, lower extremity amputations, coronary artery and cardiovascular diseases [37-39], which are the main causes of morbidity and mortality worldwide [40].

Our aim was to assess the efficiency of newly synthesized compounds for anti-oxidant property and possible glycemic control in type 2 diabetes mellitus. Evaluating from graph of percentage inhibition as a function of synthesized compounds **(3a to 3m)** at different concentration (20 to100 µg/mL). From percentage inhibition IC50 is calculated. IC50 Value was found to be **35.90±1.12** for **3m** which was close to the standard drug alpha-Tocopherol which has showed an IC50 value of **34.47±0.87 µg/mL.**

4. CONCLUSION

Newly synthesized compound **3m** can promote health benefits since it has antioxidant property and as discussed earlier diabetic patients have a high risk of experiencing micro and macrovascular complications, it provides an alternative stratagem for metabolic control, in addition to the combination of diet and exercise. Compound **3m** may represent a step forward in disease management. Further studies should be conducted to strengthen the results of this study and **3m** can be further explored as anti-diabetic drug.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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