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REGULAR ARTICLE

Synthesis, biological evaluation and molecular docking of 3-substituted quinazoline-2,4(1*H*, 3*H*)-diones

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Abstract. The quinazoline-2,4-diones scaffold is found in bioactive compounds, commercial drugs and exhibit important biological activities. However, their antidiabetic activity is rarely explored. For this purpose, an easy one-pot three-components and straightforward synthesis of 3-substituted quinazoline-2,4-diones was designed, in both, the catalyst- and solvent-free conditions under microwave irradiation. Additionally, the synthesized compounds were screened for *in vitro* α-amylase and α-glucosidase inhibitory activity, as well as antioxidants and cytotoxicity. The quinazoline-2,4-diones were isolated, with yields in the range of 30-65%. The compounds **3d**, **3e**, **3g** and **3h** displayed moderate activity against α-amylase and/or α-glucosidase enzymes compared with the acarbose drug. The molecular *docking* study revealed that all active compounds displayed a different type of intermolecular interaction in the pocked site of these enzymes. Interestingly, in the *Artemia salina* assay, the compound **3d** exhibited a higher cytotoxic effect than 5-fluorouracil. All these results support the pharmacological potential of quinazoline-2,4-diones since all evaluated compounds behave as moderate inhibitors of the enzymes α-amylase and/or α-glucosidase.

Keywords. quinazoline-2; 4-dione; α-amylase; α-glucosidase; Antioxidant; docking.

1. Introduction

The quinazolinediones are found in a large number of bioactive molecules including serotonergic, dopaminergic and adrenergic ligands, as well as in aldose reductase, lipoxygenase, cyclooxygenase, collagenase, and carbonic anhydrase inhibitors.[1] In view of the biological significance of the quinazoline-2,4-diones and their derivatives, many methods of synthesis for these compounds have been reported, e.g., from tetrachlorophthalimide,[2,3] anthranilic acid,[4] anthranilates with isocyanate,[5] or aminocrotonamide,[6] 2-aminobenzonitrile,[7] 2-bromobenzoates[8], trifluorobenzoic acid[9] and oxadiazolones rearrangement.[10] However, some of

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these methods are associated with drawbacks such as multi-step reactions, expensive reagents, difficult reaction conditions, and difficulty in purification techniques, highly toxic reagents, and low yields. Even with these problems, the synthesis of quinazoline-2,4-diones is still attractive to improve their potential therapeutic applications, such as vasorelaxation,[11] antihypertensive,[12] anticonvulsant,[13] antidepressant,[14] sedative and hypotensive,[15] antibacterial,[16] anti-*Leishmania mexicana,*[17] antifungal,[18] phosphodiesterase 4 inhibitors,[19] phospholipase A2α inhibitors,[20] HIV inhibitors,[21] *Mycobacterium smegmatis,*[22] aminopeptidase inhibitors[23] and cytotoxic activities.[24] At present, some papers report the application of quinazoline-2,4-diones in type 2 diabetes treatment, since they act as inhibitors of glycogen phosphorylase[25] or phosphodiesterase 7.[26]

On the other hand, α-glucosidase and α-amylase are enzymes that belong to the glucoside hydrolase family, which are widely distributed in nature. In humans, these protein complexes perform their action in the intestinal enzyme package that is responsible for the hydrolysis of the α 1,4-glucosidic bonds of the dietary carbohydrates. This action generates glucose molecules that will be absorbed and distributed by the bloodstream to tissues where energy is needed.[27,28] Due to these action mechanisms, α-glucosidase and α-amylase are closely associated with some metabolic diseases like diabetes mellitus type 2; this relation occurs by the stimulation of the absorption of glucose to the bloodstream in patients with a high blood level of this molecule, promoting hyperglycemic state, which is one of the main risk factors for this type of pathology. Different studies show that the inhibition of these enzymes can prevent this risk factor by decreasing the postprandial rise in blood glucose; therefore, the use of α-glucosidase and α-amylase inhibitors is considered necessary in managing non-insulin-dependent diabetes.

Currently, our research is focused on the development of an accessible synthesis of small heterocyclic compounds with potential biological activities. Recently, we have reported the synthesis of heterocyclic compounds and their biological evaluations.[29–32] Herein, an easy one-step solvent- and catalyst-free synthesis of 3-substituted quinazoline-2,4-diones under microwave irradiation, biological and molecular docking studies are reported.

2. Experimental

2.1 *Materials and methods*

All reagents were purchased in the highest quality available and were used without further purification. Nuclear Magnetic Resonance of ^{1}H (400 MHz) and ^{13}C (100 MHz) spectra were recorded on a Bruker 400 MHz Spectrometer in DMSO-d_6 with TMS as an internal standard. Chemical Ionization Mass spectra were obtained at a GC-MS (Agilent 7890B) with an ion trap. The intensities were reported as a relative percentage to the base peak after the corresponding *m/z* value. Melting points were obtained on a Stuart apparatus model SMP30 (calibrated before use with phenolphthalein) and were uncorrected. Three individual experiments were averaged and reported. All describe reactions herein were conducted in Pyrex tubes sealed with a silicone septum in a single-mode microwave reactor (Discover-SP model 909150) equipped with an Explorer 12 hybrid model 909505 operated to 725 W of maximum power and 100 W of initial power.

2.2 *General procedure for the 3-substituted quinazolin2,4-diones synthesis*

To a microwave reactor vessel (10 mL) were added an isatoic anhydrides (1.0 mmol), a primary amine (1.2 mmol), ethyl chloroformate (1.2 mmol) and *N,N*-diisopropylethyl amine (DIPEA, 1.2 mmol). The mixture was heated at 220 °C for 20 min and then were cooled to room temperature. At resulting mixture, ^{i}PrOH (2 mL) was added and shaken until dissolved. Cool water (1 or 2 mL) was added and the resulting mixture was allowed to stand still until a fine solid was formed. The solid was filtered, washed (^{i}PrOH-H_2O 1:1 v/v) and allowed to dry at room temperature to obtain the quinazoline-2,4-diones in high purity. Characterization data are similar to those reported in the literature.

3-phenylquinazoline-2,4(1*H*, 3*H*)-dione (**3a**) Lit.,[33] yield 51%, white solid, M.p. 278-279 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H, NH), 7.95 (dd, J = 7.83, 1.22 Hz, 1H, ArH), 7.69 (ddd, J = 8.38, 7.03, 1.47 Hz, 1H, ArH), 7.46 (m, 3H, ArH), 7.33 (m, 2H, ArH), 7.24 (dd, J = 8.07, 7.34 Hz, 2H, ArH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 162.2 (CO, amide), 150.2 (CO, urea), 139.8, 135.7, 135.1, 129.1, 128.8, 128.1, 127.5, 122.5, 115.2, 114.3; GC-MS (CI^+) *m/z*: 239 $[M+H]^+$.

3-benzylquinazoline-2,4(1*H*, 3*H*)-dione (**3b**) Lit.,[34] yield 50%, white solid, M.p. 228.3-228.8 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.50 (s, 1H, NH), 7.94 (dd, J = 8.31, 1.47 Hz, 1H, ArH), 7.65 (td, J = 7.76, 1.59 Hz, 1H, ArH), 7.30 (m, 4H, ArH), 7.21 (m, 3H, ArH), 5.09 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 161.9 (CO, amide), 150.1 (CO, urea), 139.4, 137.3, 135.0, 128.2, 127.4, 127.4, 127.0, 122.5, 115.1, 113.6, 43.1. GC-MS (CI^+) *m/z*: 253 $[M+H]^+$.

3-(pyridin-2-yl)quinazoline-2,4(1*H*, 3*H*)-dione (**3c**) Lit.,[35] **y**ield 35%, pale yellow solid, M.p. 318-319 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.76 (s, 1H, NH), 8.63 (dt, J = 4.83 Hz, 1H, ArH), 8.05 (td, J = 7.76, 1.83 Hz, 1H, ArH), 7.93 (dd, J = 7.95 Hz, 1H, ArH), 7.71 (dd, J = 15.41, 1.47 Hz, 1H, ArH), 7.55 (m, 2H, ArH), 7.33 (d, J = 8.0 Hz, 1H, ArH), 7.23 (dd, J = 15.16 Hz, 1H, ArH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 162.1 (CO, amide), 149.8 (CO, urea), 148.9, 148.8, 140.0, 139.4, 135.6, 127.5, 124.8, 124.4, 122.8, 115.6, 114.1. GC-MS (CI^+) *m/z*: 240 $[M+H]^+$.

3-(pyridin-3-yl)quinazoline-2,4(1*H*, 3*H*)-dione (**3d**) Lit.,[17] yield 60%, white solid, M.p. 318-319 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.76 (s, 1H, NH), 8.63 (dt, J = 4.83 Hz, 1H, ArH), 8.05 (td, J = 7.76, 1.83 Hz, 1H, ArH), 7.93 (dd, J = 7.95 Hz, 1H, ArH), 7.71 (dd, J = 15.41, 1.47 Hz, 1H, ArH), 7.55 (m, 2H, ArH), 7.33 (d, J = 8.0 Hz, 1H, ArH), 7.23 (dd, J = 15.16 Hz, 1H, ArH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 162.1 (CO), 149.8 (CO, urea), 148.9, 148.8, 140.0, 139.4, 135.6, 127.5, 124.8, 124.4, 122.8, 115.6, 114.1. GC-MS (CI^+) *m/z*: 240 $[M+H]^+$.

3-propylquinazoline-2,4(1*H*, 3*H*)-dione (**3e**) Lit.,[36] **y**ield 46%, pale green solid, M.p. 176.2-177.1 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.37 (s, 1H, NH), 7.92 (dd, J = 7.7, 1.1 Hz, 1H, ArH), 7.63 (td, J = 7.76, 1.59 Hz, 1H, ArH), 7.18 (t, J = 7.58 Hz, 2H, ArH), 3.84 (dd, J = 8.1, 6.6 Hz, 2H, N-CH_2), 1.59 (sxt, J = 7.43 Hz, 2H, -CH_2-), 0.87 (t, J = 7.46 Hz, 3H, -CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 161.8 (CO, amide), 150.1 (CO, urea), 139.9, 134.7, 127.3, 122.3, 114.9, 113.7, 41.4, 20.6, 11.1. GC-MS (CI^+) *m/z*: 205 $[M+H]^+$.

3-cyclopentylquinazoline-2,4(1*H*, 3*H*)-dione (**3f**) Lit.,[34] yield 45%, white solid, M.p. 235.7-236.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (br. s., 1H, NH), 7.90 (br. s., 1H, ArH), 7.60 (br. s., 1H, ArH), 7.15 (br. s., 1H, ArH), 5.28 (br. s., 1H), 1.71 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1 (CO, amide), 150.0 (CO, urea), 139.2, 134.6, 127.3, 122.2, 114.7, 114.0, 52.0, 28.0, 25.3. GC-MS (CI^+) *m/z*: 231 $[M+H]^+$.

3-cyclohexylquinazoline-2,4(1*H*, 3*H*)-dione (**3g**) Lit.,[33] yield 65%; white solid, M.p. 272.7-273.8 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.28 (br. s, 1H, NH), 7.91 (dd, J = 7.95, 1.10 Hz, 1H, ArH), 7.62 (ddd, J = 8.13, 7.27, 1.47 Hz, 1H, ArH), 7.17 (dt, J = 8.07, 7.21 Hz, 2H, ArH), 4.74 (m, 1H, CH), 2.39 (qd, J = 12.47, 3.42 Hz, 2H, CH_2), 1.80 (d, J = 12.96 Hz, 2H, CH_2), 1.59 (dd, J = 12.72, 1.96 Hz, 2H, CH_2), 1.24 (m, 4H, CH_2); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 162.1 (CO, amide), 150.2 (CO, urea), 139.3, 134.7, 127.4, 122.2, 114.7, 114.1, 52.7, 28.3, 25.9, 25.0. GC-MS (CI^+) *m/z*: 245 $[M+H]^+$.

3-(*o*-tolyl)quinazoline-2,4(1*H*, 3*H*)-dione (**3h**) Lit.,[37] yield 41%, white solid, M.p. 245.8-247.1 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.58 (br. s, 1H, NH), 7.96 (dd, J = 8.07, 1H, ArH), 7.96 (ddd, J = 8.38, 7.15, 1.59 Hz, 1H, ArH), 7.29 (m, 6H, ArH) 2.05 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 161.8 (CO, amide), 149.7 (CO, urea), 139.9, 135.7, 135.3, 134.8, 130.3, 129.1, 128.4, 127.6, 126.6, 122.6, 115.3, 114.0, 16.9. GC-MS (CI^+) *m/z*: 253 $[M+H]^+$.

3-(*m*-tolyl)quinazoline-2,4(1*H*, 3*H*)-dione (**3i**) Lit.,[37] **y**ield 64%, white solid, M.p. 254.4-256.4 °C.. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H, NH), 7.94 (dd, J = 7.95, 1.10 Hz, 1H, ArH), 7.69 (ddd, J = 8.38, 7.15, 1.59 Hz, 1H, ArH), 7.36 (t, J = 7.70 Hz, 1H, ArH), 7.22 (m, 3H, ArH), 7.11 (m, 2H, ArH), 2.35 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 162.1 (CO, amide), 150.1 (CO, urea), 139.7, 138.1, 135.5, 135.0, 129.3, 128.6, 128.4, 127.4, 125.9, 122.4, 115.1, 114.2, 20.7. GC-MS (CI^+) *m/z*: 253 $[M+H]^+$.

3-(*p*-tolyl)quinazoline-2,4(1*H*, 3*H*)-dione (**3j**) Lit.,[37] **y**ield 53%, white solid, M.p. 241.8-242.9 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.48 (br. s, 1H, NH), 7.93 (dd, J = 8.07, 1.22 Hz, 1H, ArH), 7.69 (ddd, J = 8.38, 7.15, 1.59 Hz, 1H, ArH), 7.25 (m, 6H, ArH) 2.37 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 162.1 (CO, amide), 150.1 (CO, urea), 137.3, 137.1, 135.0, 132.9, 130.4, 129.1, 128.6, 122.4, 115.1, 114.2, 20.6. GC-MS (CI^+) *m/z*: 253 $[M+H]^+$.

6-methyl-3-phenylquinazoline-2,4(1*H*, 3*H*)-dione and 7-methyl-3-phenylquinazoline-2,4(1*H*, 3*H*)-dione (**3k**) Lit.,[17] yield 30%, white solid, M.p. 293-294 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.46 (d, J = 9.29 Hz, 1H, NH), 7.74 (s, 1H, ArH), 7.48 (m, 2H, ArH), 7.30 (d, J = 7.10 Hz, 4H, ArH), 7.05 (d, J = 8.07 Hz, 1H, ArH), 2.35 (s, 3H, -CH_3) for 6-methyl-3-phenylquinazoline-2,4(1*H*,3*H*)-dione; δ 11.46 (d, J = 9.29 Hz, 1H, NH), 7.82 (d, J = 8.07 Hz, 1H, ArH), 7.48 (m, 7H, ArH), 7.30 (d, J = 7.10 Hz, 4H, ArH), 7.14 (d, J = 8.31 Hz, 1H, ArH), 7.02 (s, 1H, ArH), 2.40 (s, 3H, -CH_3) for 7-methyl-3-phenylquinazoline-2,4(1*H*,3*H*)-dione. GC-MS (CI^+) *m/z*: 253 $[M+H]^+$.

6-fluoro-3-phenylquinazoline-2,4(1*H*,3*H*)-dione and 7-fluoro-3-phenylquinazoline-2,4(1*H*,3*H*)-dione (**3l**) Lit.,[17] yield 32%, white solid, M.p. 312-313 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.63 (br. s., 2H, 2xNH) 8.00 (dd, J = 8.8, 6.4 Hz, 1H) 7.62 (m, 1H) 7.46 (m, 7H) 7.29 (m, 5H) 7.07 (td, J = 8.7, 2.32 Hz, 1H) 6.96 (m, 1H). ^{13}C-NMR (100 MHz, DMSO-d6): for 6-fluoro-3-phenylquinazoline-2,4(1*H*,3*H*)-dione: δ 161.3 (CO-N), 157.3 (d, J = 239.9 Hz, C-F), 149.8 (N-CO-N), 136.5 (C-N), 135.5 (C_{ipso}), 128.9 (2xCH), 128.7 (CH), 128.1 (2xCH), 121.7 (CH), 118.1 (CH), 117.5 (C), 115.3 (CH); for 7-fluoro-3-phenylquinazoline-2,4(1*H*,3*H*)-dione: δ 165.8 (d, J = 250.8 Hz, C-F), 161.4 (CO-N), 150.1 (N-CO-N), 139.7 (C-N), 135.4 (Cipso), 130.7 (CH), 128.9 (2xCH), 128.6 (CH), 128.1 (2xCH), 110.5 (C), 110.3 (CH), 101.2 (CH); GC-MS m/z: 257 $[M+H]^+$.

2.3 *α-Amylase inhibitory activity*

The α-amylase activity of the synthetic quinazoline-2,4(1*H*,3*H*)-diones was assessed as described by

Adisakwattana *et al.* with slight modifications.[38] Porcine pancreatic α-amylase (EC 3.2.1.1) was dissolved in 0.1 M phosphate buffer at 6.9 pH (concentration of 0.5 mg mL^{-1}). In a 96-well flat-bottom plate, 75 µL of the enzyme solution and 75 µL of quinazoline-2,4(1*H*,3*H*)-diones solution (concentrations of 1, 10, 100, 500, 1000 µg mL^{-1} in methanol) were added. After 10 min of incubation at 25 °C, 75 µL of the starch solution (0.1% p/v in 0.1 M phosphate buffer at pH 6.9) was added to start the reaction and the mixture were incubated for 10 minutes at 25 °C. The reaction was stopped by adding 62.5 µL dinitrosalicylic (DNS) reagent (1% 3,5-dinitrosalicylic acid, 0.2% phenol, 0.05% Na_2SO_3 and 1% NaOH in aqueous solution) to the reaction mixture. The mixtures were heated at 100 °C for 5 min in order to stop the reaction. After cooling to room temperature, the absorbance was recorded at 580 nm using a microplate reader. The α-amylase inhibitory activity was expressed as percentage inhibition and was calculated as follows:

$$\%\,Inhibition = \frac{A_0 - A_1}{A_0} x100$$

Where: A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the quinazoline-2,4(1*H*,3*H*)-diones.

2.4 *α-Glucosidase inhibitory activity*

The α-glucosidase (EC 3.2.1.20) activity of the synthetic quinazoline-2,4(1*H*,3*H*)-diones was assessed as described by Kwon *et al.,* with slight modification.[39] In a 96-well flat-bottom plate, 100 µL of the enzyme solution (1 U mL^{-1} in 0.1 M of phosphate buffer at pH 7.0) and 50 µL of quinazoline-2,4(1*H*,3*H*)-diones solution (concentrations of 1, 10, 100, 500, 1000 µg mL^{-1} in methanol) were added. After 10 min of incubation at 25 °C, 50 µL of 5 mM *p*-nitro-phenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 7.0) was added, and the mixture was incubated at 25 °C for 5 min. After, the absorbance was recorded at 405 nm using a microplate reader. The α-glucosidase inhibitory activity was expressed as percentage inhibition and was calculated as follows:

$$\%\,Inhibition = \frac{A_0 - A_1}{A_0} x100$$

Where: A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the quinazoline-2,4(1*H*,3*H*)-diones.

2.5 *DPPH-scavenging activity*

The $DPPH^{\bullet}$ scavenging activities of the imines were assessed as described by Clarke *et al.,*[40] with slight modifications. This method is based on the reduction of DPPH in the presence of antioxidants; the antioxidant activity is detected as a change from purple to yellow color in the solution. In a 96-well flat-bottom plate, 20 µL of the imines (0.1 to 100 µg mL^{-1} in methanol) and 180 µL of DPPH solution (150 µM in methanol) were added. The mixture was shaken, incubated for 20 min at room temperature in darkness, and the absorbance was measured at 532 nm. The $DPPH^{\bullet}$-scavenging activity of the quinazoline-2,4-diones was calculated as follows:

$$DPPH - scavenging\,effect(\%) = \frac{A_0 - A_1}{A_0} x100$$

Where: A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the quinazoline-2,4(1*H*,3*H*)-diones. Vitamin C and gallic acid were used as standard (0.1 to 10 µg mL^{-1}). Calculated values corresponded to the mean ± standard deviation of two experiments by triplicate and were determined by Prism software V6 (GraphPad).

2.6 *Brine shrimp lethality bioassay*

The toxicities of the quinazoline-2,4-diones were evaluated by the brine shrimp larvae assay.[41] Briefly, dried brine shrimp eggs were incubated in a saline medium under light for 48 h. One-day-old larvae (10-12 per vial in 100 µL of saline solution) were transferred into 96-well plates and exposed to 100 µL of the quinazoline-2,4-diones at 100, 300, 500, 700 and 1000 µg mL^{-1}. Four replicates of each concentration were done. The death larvae were counted after 24 h of incubation, and the Median Lethal Concentration (LC_{50}) and 95% confidence intervals were determined by the probit analysis with the SPSS Statistics software v19 (IBM company). Evaluated compounds were classified by the LC_{50} values as follows: $LC_{50} \geq$ 1000 µg mL^{-1}, non-toxic; $100 < LC_{50} < 1000$, moderate toxic; and $10 < LC_{50} < 100$, very toxic.

2.7 *Molecular docking*[42-44]

The molecular docking studies were carried out using AutoDock Tool v1.5.6 and were guided to the active site of the enzyme.[45] First, the quinazoline-2,4-diones (ligands) were designed using ChemDraw V16 and

Spartan V14 was used for 3D optimization using MMFF94's for the optimization to lowest energy geometry including water phase for all ligand. Later, the structures were saved as SYBYL mol2 file format and Gasteiger charges were assigned to the ligands. Second, the crystal structure for human pancreatic α-amylase (pdb code: 5E0F) and human lysosomal acid-α-glucosidase (pdb code: 5NN8) was downloaded from the RCSB protein data bank. Before docking calculations, all water molecules, ligands and cations were removed from protein. Later, polar hydrogens and the *Kollman* charges were assigned for the protein. Then, the grid box site was set for human pancreatic α-amylase in -7.946, 10.438 and -21.863 Å (x, y and z) with a grid of 80, 72 and 66 points (x, y and z). For human lysosomal acid-α-glucosidase the grid box site was set at -12.175, -35.415 and 88.753 Å (x, y and z) with a grid of 74, 70 and 90 points (x, y and z) with 0.375 Å spacing. Docking calculations were performed using the Lamarckian genetic algorithm for ligands conformational searching. The docking for the ligands consisted of a total of 200 runs that were carried out with a population size of 150 individuals, a maximum of 25 million energies evaluations, a maximum of 270,000 generations, a gene mutation rate of 0.02 and a crossover rate of 0.8. Cluster analysis was performed on the docked results using a root mean square (RMS) tolerance of 0.5 Å.

The conformation with the lowest binding energy was examined by Accelrys, Discovery Studio Visualizer v17.2.0.16349 [Accelrys Inc., San Diego, CA (2007)] to determine their binding orientations, molecular modeling, evaluation of the hydrogen bonds and other interactions.

3. Results and Discussion

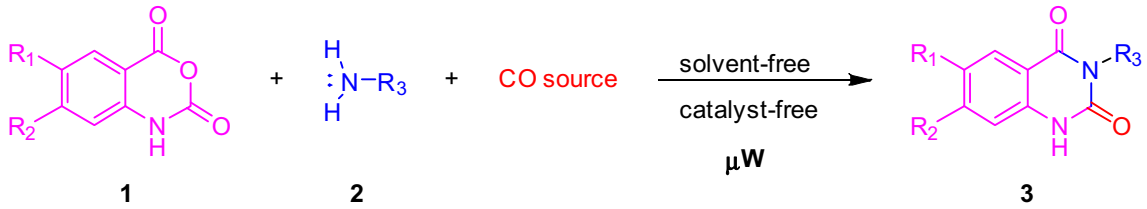
3.1 *Chemistry*

In continuation of our research interest, we report the synthesis of 3-substituted quinazolin-2,4(1*H*, 3*H*)-diones **3** by a multi-component solvent- and the catalyst-free reaction of the isatoic anhydrides **1**, primary amines **2** and a carbonyl source under microwave irradiation (Scheme 1).

This protocol is a tandem reaction that involves C–O/C–N bond cleavage, C–N bond formation, and a cyclizative carbonylation. Therefore, the initial efforts were devoted to the optimization of reaction conditions. For this purpose, compound **3a** was studied as a reaction model. The first studies were carried out setting the reaction conditions in 120 °C and 30 min with 1,1'-carbonyldiimidazole (CDI) as carbonyl source using straight conditions and some solvents (THF, DMF, DMSO, alcohols, acetonitrile, benzene, toluene, 1,4-dioxane and water). To our delight, the desired compound **3a** was isolated with higher yield under solvent-free conditions (11%). Later, an increase in the temperature (120 at 200 °C) showed an increase in yield up to 21%. The change of the carbonyl source by ethyl chloroformate (ECF) provided compound **3a** with 37% yield. Hence, some experiments where temperature (120 to 240 °C), time (5 to 30 min), carbonyl sources (ECF, CDI, di-*tert*butyldicarbonate and BTC) and bases (DIPEA, TEA and pyridine) were tested to establish the optimal conditions under solvent-free condition. From these experiments, the optimal conditions were set at 220 °C and 20 min. During the efficiency screening based on the isolated yield, it was found that triphosgene (BTC) provided compound **3a** with a likewise yield than ECF. Due to the problematic handling of BTC, it was decided to use ECF. It was observed that the absence of a base in the reaction decreases the yield up to 7%. The use of ECF as carbonyl source and DIPEA as a base were found to be the most efficient conditions for the synthesis of compound **3a** with 51% yield. Using this approach, the generality and feasibility were investigated by this method. The quinazolin-2,4-diones **3a-m** (Table 1) were prepared in 30-65% yields (Table 1), and their identities were confirmed by mass spectrometry and nuclear magnetic resonance analyses. Another advantage of this synthesis method resided in isolation by precipitation of all products, avoiding the need to purify them via column chromatography. A structure-yield analysis shows that the yield decreases when R^1 and/or R^2-positions are substituted (**3k-m**) compared with no substituted compound (**3a**). When R^3 is tolyl (**3h-j**), the yield follows the reactivity *meta* > *para* > *ortho*; the same behavior was observed when R^3 is pyridine (**3c-d**).

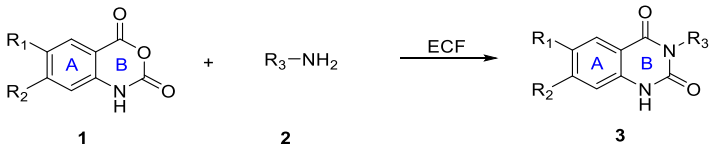
3.2 *Biological activities*

The enzymatic inhibition against α-glucosidase and α-amylase of the synthesized quinazoline-2,4(*1H*,*3H*)-diones **3a-m** is shown in Table 2 and were evaluated using acarbose as a reference standard. Among the analogs made, seven quinazoline-2,4(*1H*,*3H*)-diones displayed inhibitory activity against α-glucosidase, being the compound **3m** the most potent with 0.52- and 0.51-fold activity over **3d** and **3e**, respectively. Besides, it was 0.48-fold less active that acarbose at the concentration of 100 μg mL^{-1} and was evaluated



Scheme 1. The multicomponent reaction for the synthesis of 3-substituted quinazolin-2,4(1*H*, 3*H*)-diones under microwave irradiations.

Table 1. Yield of quinazoline-2,4-diones synthesized.



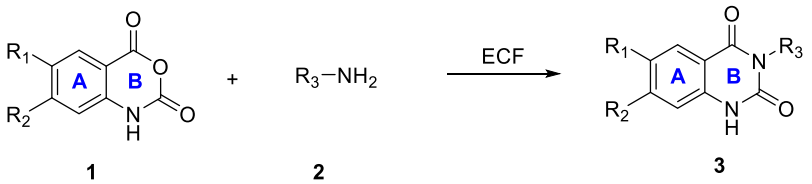
	R_1	R_2	R_3	yield (%)
3a	H	H	C_6H_5	51
3b	H	H	$C_6H_5CH_2$	50
3c	H	H	2-C_5H_3N	35
3d	H	H	3- C_5H_3N	60
3e	H	H	$CH_3CH_2CH_2$	46
3f	H	H	C_5H_9	45
3g	H	H	C_6H_{11}	65
3h	H	H	2- $CH_3C_6H_5$	41
3i	H	H	3- $CH_3C_6H_5$	64
3j	H	H	4- $CH_3C_6H_5$	53
3k	Me	H	C_6H_5	30
3l	F	H	C_6H_5	32

as a regioisomeric mixture (6- and 7-substitution). The enhanced activity was observed when R_3 substituent was *c*-hexyl, propyl or pyridyl group, in comparison with the phenyl group. When R_3 is a tolyl substituent, the *para*-methyl position was active while the *ortho*- and *meta*- positions were inactive (**3h-j**). Nonetheless, the substitution in the R_1 and/or R_2 positions by halogens (**3m**) displayed the highest enzymatic inhibition. These results suggest that the changes in R_1 and/or R_2 substituent on ring A of the quinazoline-2,4(*1H*,*3H*)-diones lead to new inhibitors compounds against α-glucosidase.

Conversely, compound **3g** displayed the highest enzymatic inhibition against α-amylase being 0.16-, 0.77-, 2.60- and 3.59-fold active over **3h**, **3e**, **3a** and **3i**, respectively. Also, it was 1.83-fold less active than acarbose at the concentration of 100 μg mL^{-1}. An increase in enzymatic inhibitory activity was observed when saturated substituents as *c*-hexyl (2.60-fold) or propyl (1.04-fold) are in R_3, compared with the phenyl group. If R_3 substituent is a tolyl group, the enzymatic inhibition followed the reactivity *ortho* > *meta* > *para*. However, the substitution of quinazoline-2,4(*1H*,*3H*)-diones in R_1 and/or R_2 positions of the ring A resulted in being inactive against α-amylase inhibition. These suggest that saturated substituents in R_3 of the quinazoline-2,4(*1H*,*3H*)-diones could lead to obtain inhibitors against α-amylase.

In the antioxidant activity (AA), all the synthetic quinazoline-2,4-diones behave as reducing agents over the $DPPH^{\bullet}$ radical, with **3h** as the best antioxidant (Table 2). Based on the structure-antioxidant activity relationship, the antioxidant activity can be related to substitution on ring B; e.g., if R^3 is a tolyl (**3h-j**), the $DPPH^{\bullet}$ radical scavenging follows the series *orto* > *meta* > *para* and was 1.21- and 6.49-fold more active, respectively. Compound **3h** displayed the best DPPH activity (10.045 ± 0.920 %) at 10 μg mL^{-1} being 1.00- and 1.21-fold better than **3f** and **3i**, respectively. However, all quinazoline-2,4-diones synthesized are inactive (IC_{50} >1000 μg mL^{-1}) compared with vitamin C (IC_{50}= 8.79 μg mL^{-1}) or gallic acid (IC_{50}= 4.08 μg mL^{-1}) by DPPH assay.

Table 2. Biological activities of quinazoline-2,4-diones synthesized.



	% AA (10 μg mL^{-1})	% αA[a]	% αG[a]	LC_{50}[b]
3a	2.412 ± 1.121	5.71 ± 4.83 *	7.77 ± 5.42 *	62.00
3b	2.333 ± 0.494	-[c]	-[c]	29,384
3d	2.703 ± 0.946	-[c]	21.72 ± 3.44 *	0.017
3e	1.898 ± 2.516	11.62 ± 5.34 *	21.90 ± 6.14 *	5.00
3f	5.022 ± 0.137	-[c]	-[c]	637
3g	1.712 ± 1.050	20.55 ± 0.34 *	18.17 ± 13.53 *	11,524
3h	10.045 ± 0.920	17.65 ± 0.37 *	-[c]	12.00
3i	4.547 ± 0.274	4.48 ± 0.32 *	-[c]	42,626
3j	1.341 ± 0.000	-[c]	13.23 ± 0.71 *	805
3l	1.13 ± 0.281	-[c]	33.03 ± 10.40 *	-
Gallic acid	89.24 ± 13.780			
Vitamin C	56.84 ± 3.211			
Acarbose		58.16 ± 0.95	49.01 ± 0.45	
5-fluorouracil				285

[a] % of activity obtained at 100 μg mL^{-1}; [b] lethal concentrations media (ng mL^{-1}) obtained in *Artemia salina L.*; [c] Not inhibition; * $P<0.05$ compared with acarbose group; αA: α-amylase; αG: α-glucosidase

In contrast, Rajabi *et al.,* reported that it is possible to evaluated cytotoxicity using *Artemia salina* L. as a primary routine assay before evaluation on cancer lines as a screening method for bioactive compounds.[46] Besides, our group previously reported the cytotoxicity of the compounds **3a** and **3d** in Raw 264.7 macrophages with CC_{50} between 97.71-106.1 μM.[17] In this sense, we evaluated the cytotoxicity of the synthesized quinazoline-2,4-diones using *Artemia salina* and observed that substitution in the ring A induces a decrease in the cytotoxicity compared to unsubstituted compound (**3a**). Similar behavior is shown with non-aromatic rings such as *c*-pentyl or *c*-hexyl in the R_3 position. When R_3 is a tolyl, the *ortho* position was the most active and was 22.8-fold more active than fluorouracil. Nevertheless, the highest cytotoxicity was observed with 3-pyridyl (**3d**) as a substituent at the R_3 position and it was 3646-, 293-, 705- and 16764-fold more cytotoxic that **3a**, **3e**, **3h** and fluorouracil, respectively.

3.3 *Molecular docking*

The quinazoline-2,4-diones with activity against α-glucosidase (αG) and α-amylase (αA) were analyzed by molecular docking studies in order to find the binding interactions with the active pocket size of the enzyme. For this purpose, X-ray structures of human pancreatic α-amylase (pdb code: 5E0F) and human lysosomal acid-α-glucosidase (pdb code: 5NN8) was selected as a template.

Since compound **3m** was obtained as a regioisomeric mixture, the docking study in the αG enzyme was discarded. Therefore, the compound **3d** is bound into receptor by polar H-bond between O···H-N of Arg600 (1.62 Å, 133.7°), N-H···O of Asp518 (2.00 Å, 120.5°) and a hydrophobic bond with Phe649 (4.84 Å). Compound **3e** is bound into the binding site by H-bond between O···H-N of Arg600 (1.92 Å, 141.3°) and N-H···O of Asp616 (1.90 Å, 110.0°). In addition, it is linked with Asp518 (3.91 Å) and Asp616 (3.18 Å) by an electrostatic interaction *via* ring B. The docking studies exhibited that compounds **3d** and **3e** mainly displayed polar H-bond on oxygen and/or nitrogen of the ring B, while the ring A or alkane chain exhibited hydrophobic interaction (π-π T-shaped or π-alkyl), Figure 1. However, the activity of the compounds **3d** and **3e** against αG is probably due to the resonance effect between the oxygen and nitrogen atoms of ring A, this produces a more electronegative oxygen atom than the oxygen atom of the glycosidic bond of the

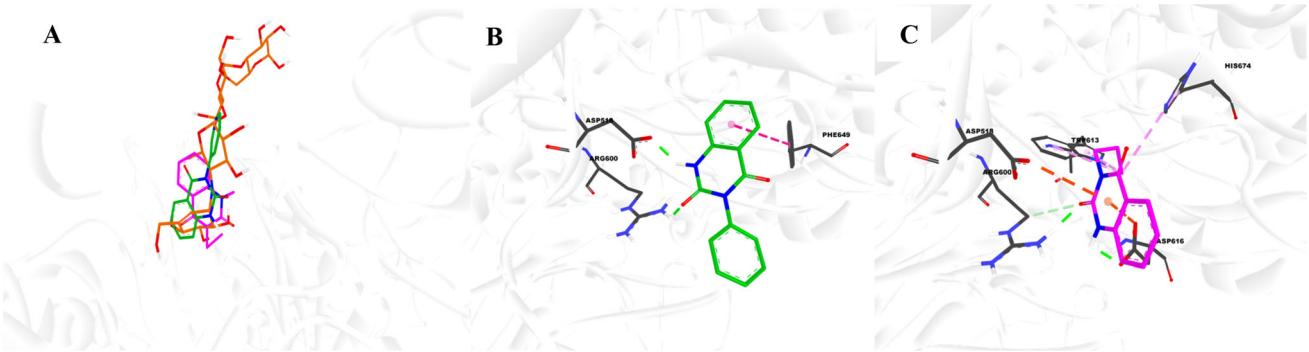


Figure 1. Molecular docking of acarbose drug (orange color), compounds **3d** (green color), and **3e** (pink color) in the active site of α-glucosidase. Putative binding interactions of the compounds **3d** (Figure B) and **3e** (Figure C). Hydrogen, electrostatic and hydrophobic bonds are shown as green, orange and pink dashed line, respectively.

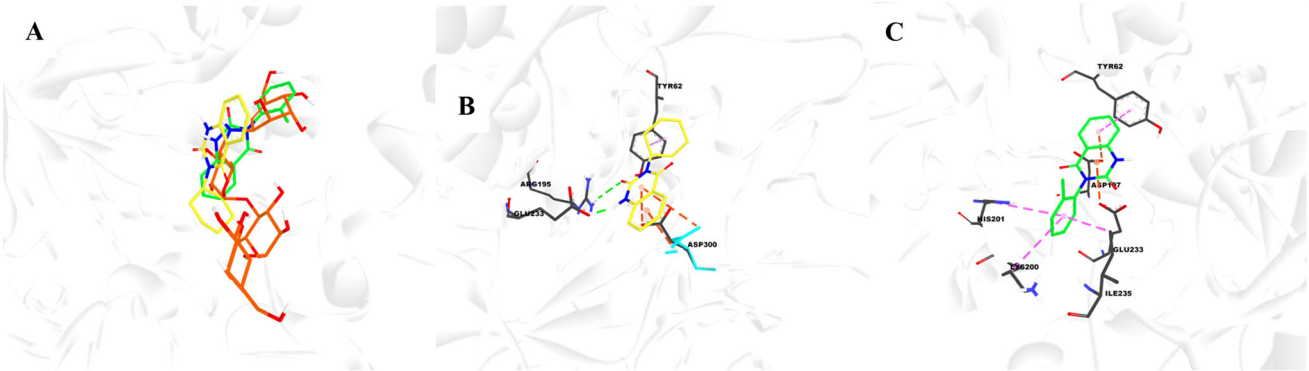


Figure 2. Molecular docking of acarbose drug (orange color), compounds **3g** (yellow color) and **3h** (green color) in the active site of α-amylase. Putative binding interactions of the compounds **3g** (Figure B) and **3h** (Figure C). Hydrogen, electrostatic and hydrophobic bonds are shown as green, orange and pink dashed line, respectively.

acorbose, causing synthetic compounds to be bound to H-N of the Arg600 better than the acarbose.

Molecular docking analysis exhibited several interactions between the inhibitors and the αA upon binding in the active site residues. In this sense, the compound **3g** that exhibited the highest *in vitro* activity against αA is bound into receptor site *via* hydrophilic binding by H-bond between O···H-N of Arg195 (2.48 Å, 160.2°) and N-H···O of Glu233 (1.69 Å, 152.6°). Other interactions result from other groups of the molecule as electrostatic interaction with Asp300, Figure 2. The second most active compound **3h** is bound into receptor site by electrostatic interaction *via* ring A with Asp197 (3.58 Å) and ring B with Asp197 and Glu233 (4.28 and 3.60 Å, respectively); however, it has no polar H-bonds. Similarly, the enzyme-ligand (E-L) complex for the active compounds **3a**, **3e** and **3i** are stabilized by H-bonds with the heteroatoms of ring B. Besides, they show other interactions that stabilize the E-L complex as electrostatic interaction with the catalytic amino acids Glu233 and Asp300. The docking studies display an *orto* > *meta* > *para* order in the interaction strength of the tolyl substituent compounds (**3h-j**), which corresponds to their *in vitro* activity, suggesting a good approximation between the docking studies and the experimental results. The molecular docking study revealed that all active compounds docked into the αA mainly displayed H-bond on oxygen and/or nitrogen of the ring B, while that rings A and R_3 mainly exhibited hydrophobic interaction (π-π T-shaped, π-alkyl, π-sigma, alkyl and π-π stacked).

4. Conclusions

We have developed a convenient one-pot microwave-assisted synthesis of quinazoline-2,4-diones from isatoic anhydrides under catalyst- and solvent-free conditions. The method offers an easy experimental work up. In contrast, all quinazolin-2,4-diones evaluated behave as moderate reducing agents over the $DPPH^{\bullet}$ radical and as moderated inhibitors of the enzymes α-amylase and/or α-glucosidase. The compound 3-(pyridin-3-yl)quinazoline-2,4(1*H*, 3*H*)-dione (**3d**) was found to be most active against α-glucosidase and in

the cytotoxic *Artemia salina* bioassay, while the compound 3-propylquinazoline-2,4(1*H*, 3*H*)-dione (**3e**) was most active against α-amylase. Compounds **3a**, **3d**, **3e** and **3h** are good candidates for cytotoxicity assays because they were most active than 5-fluorouracil against *Artemia salina* bioassay.

Supplementary Information (SI)

Supplementary information related to this article is available at www.ias.ac.in/chemsci.

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