




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

LUMADHAR SANTOS-BALLARDO^a, FERNANDO GARCÍA-PÁEZ^b, LORENZO A PICOS-CORRALES^a, ADRIÁN OCHOA-TERÁN^c, PEDRO BASTIDAS^d, LORANDA CALDERÓN-ZAMORA^e, GUADALUPE RENDÓN-MALDONADO^a, ULISES OSUNA-MARTÍNEZ^{a,*} and JUAN I SARMIENTO-SÁNCHEZ^{b,*} 

^aFacultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Blvd. de las Américas S/N, Campus Culiacán, 80040 Culiacán, Sinaloa, México

^bFacultad de Ingeniería Culiacán, Universidad Autónoma de Sinaloa, Blvd. de las Américas S/N, 80040 Culiacán, Sinaloa, México

^cTecnológico Nacional de México / IT de Tijuana, Blvd. Alberto Limón Padilla S/N, Mesa de Otay, 22500 Tijuana, BC, México

^dLaboratorio de Análisis de Residuos de Plaguicidas, Centro de Investigación en Alimentación y Desarrollo A.C., Carretera a El Dorado Km 5.5, Campo El Diez, 80129 Culiacán, Sinaloa, México

^eFacultad de Biología, Universidad Autónoma de Sinaloa, Blvd. de las Américas S/N, Campus Culiacán, 80040 Culiacán, Sinaloa, México

E-mail: ulises.osuna@uas.edu.mx; jsarmiento@uas.edu.mx

Ulises Osuna-Martínez and Juan I Sarmiento-Sánchez have contributed equally to this work.

MS received 11 September 2019; revised 17 February 2020; accepted 2 March 2020

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 pocket 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 quinazolinodiones 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-bromobenzoates⁸, trifluorobenzoic acid⁹ and oxadiazolones rearrangement.¹⁰ However, some of

*For correspondence

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 A2 α inhibitors,²⁰ HIV inhibitors,²¹ *Mycobacterium smegmatis*,²² aminopeptidase inhibitors²³ 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 phosphorylase²⁵ 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 ^1H (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 quinazolin-2,4-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. $^1\text{H-NMR}$ (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); $^{13}\text{C-NMR}$ (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 (Cl^+) m/z : 239 $[\text{M}+\text{H}]^+$.

3-benzylquinazoline-2,4(1*H*, 3*H*)-dione (**3b**) Lit.,³⁴ yield 50%, white solid, M.p. 228.3–228.8 °C. $^1\text{H-NMR}$ (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); $^{13}\text{C-NMR}$ (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 (Cl^+) m/z : 253 $[\text{M}+\text{H}]^+$.

3-(pyridin-2-yl)quinazoline-2,4(1*H*, 3*H*)-dione (**3c**) Lit.,³⁵ yield 35%, pale yellow solid, M.p. 318-319 °C. ¹H-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); ¹³C-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. ¹H-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); ¹³C-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 yield 46%, pale green solid, M.p. 176.2-177.1 °C. ¹H-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-CH₂), 1.59 (sxt, *J* = 7.43 Hz, 2H, -CH₂-), 0.87 (t, *J* = 7.46 Hz, 3H, -CH₃); ¹³C-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. ¹H 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); ¹³C 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. ¹H-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, CH₂), 1.80 (d, *J* = 12.96 Hz, 2H, CH₂), 1.59 (dd, *J* = 12.72, 1.96 Hz, 2H, CH₂), 1.24 (m, 4H, CH₂); ¹³C-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. ¹H-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); ¹³C-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 yield 64%, white solid, M.p. 254.4-256.4 °C. ¹H-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); ¹³C-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 yield 53%, white solid, M.p. 241.8-242.9 °C. ¹H-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); ¹³C-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. ¹H-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, -CH₃) 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, -CH₃) 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. ¹H-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). ¹³C-NMR (100 MHz, DMSO-*d*₆): 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 (C_{ipso}), 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 μ L of the enzyme solution and 75 μ L of quinazoline-2,4(1*H*,3*H*)-diones solution (concentrations of 1, 10, 100, 500, 1000 μ g mL⁻¹ in methanol) were added. After 10 min of incubation at 25 °C, 75 μ L 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 μ L dinitrosalicylic (DNS) reagent (1% 3,5-dinitrosalicylic acid, 0.2% phenol, 0.05% Na₂SO₃ 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:

$$\% \text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where: A₀ was the absorbance of the control and A₁ 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 μ L of the enzyme solution (1 U mL⁻¹ in 0.1 M of phosphate buffer at pH 7.0) and 50 μ L of quinazoline-2,4(1*H*,3*H*)-diones solution (concentrations of 1, 10, 100, 500, 1000 μ g mL⁻¹ in methanol) were added. After 10 min of incubation at 25 °C, 50 μ L 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:

$$\% \text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where: A₀ was the absorbance of the control, and A₁ was the absorbance in the presence of the quinazoline-2,4(1*H*,3*H*)-diones.

2.5 DPPH-scavenging activity

The DPPH[•] 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 μ L of the imines (0.1 to 100 μ g mL⁻¹ in methanol) and 180 μ L of DPPH solution (150 μ M 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[•]-scavenging activity of the quinazoline-2,4-diones was calculated as follows:

$$\text{DPPH} - \text{scavenging effect}(\%) = \frac{A_0 - A_1}{A_0} \times 100$$

Where: A₀ was the absorbance of the control, and A₁ 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 μ g mL⁻¹). Calculated values corresponded to the mean \pm 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 μ L of saline solution) were transferred into 96-well plates and exposed to 100 μ L of the quinazoline-2,4-diones at 100, 300, 500, 700 and 1000 μ g mL⁻¹. Four replicates of each concentration were done. The death larvae were counted after 24 h of incubation, and the Median Lethal Concentration (LC₅₀) and 95% confidence intervals were determined by the probit analysis with the SPSS Statistics software v19 (IBM company). Evaluated compounds were classified by the LC₅₀ values as follows: LC₅₀ \geq 1000 μ g mL⁻¹, non-toxic; 100 < LC₅₀ < 1000, moderate toxic; and 10 < LC₅₀ < 100, very toxic.

2.7 Molecular docking⁴²⁻⁴⁴

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

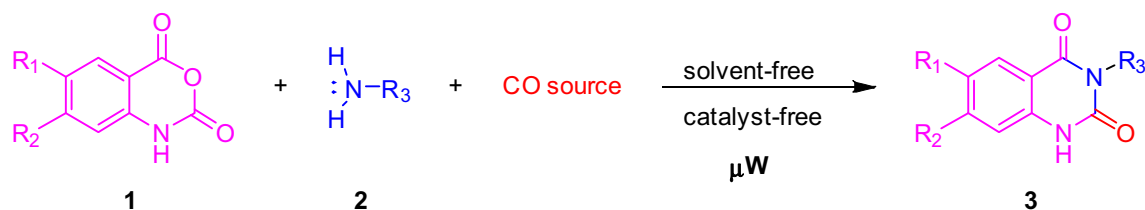
In continuation of our research interest, we report the synthesis of 3-substituted quinazolin-2,4(1*H*, 3*H*)-diones **3** by a multi-component solvent- and the catalyst-free reaction of the isatoic anhydrides **1**, primary amines **2** and a carbonyl source under microwave irradiation (Scheme 1).

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 R¹ and/or R²-positions are substituted (**3k-m**) compared with no substituted compound (**3a**). When R³ is tolyl (**3h-j**), the yield follows the reactivity *meta* > *para* > *ortho*; the same behavior was observed when R³ is pyridine (**3c-d**).

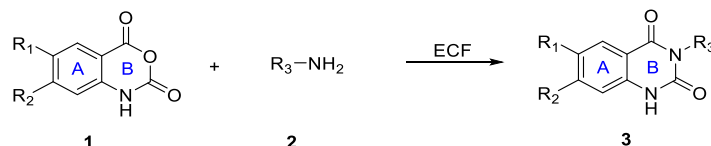
3.2 Biological activities

The enzymatic inhibition against α -glucosidase and α -amylase of the synthesized quinazolin-2,4(1*H*,3*H*)-diones **3a-m** is shown in Table 2 and were evaluated using acarbose as a reference standard. Among the analogs made, seven quinazolin-2,4(1*H*,3*H*)-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 than acarbose at the concentration of 100 $\mu\text{g mL}^{-1}$ 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.



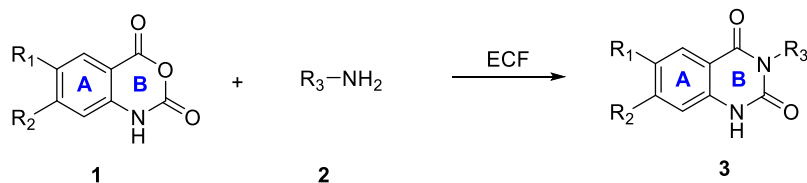
	R ₁	R ₂	R ₃	yield (%)
3a	H	H	C ₆ H ₅	51
3b	H	H	C ₆ H ₅ CH ₂	50
3c	H	H	2-C ₅ H ₃ N	35
3d	H	H	3-C ₅ H ₃ N	60
3e	H	H	CH ₃ CH ₂ CH ₂	46
3f	H	H	C ₅ H ₉	45
3g	H	H	C ₆ H ₁₁	65
3h	H	H	2-CH ₃ C ₆ H ₅	41
3i	H	H	3-CH ₃ C ₆ H ₅	64
3j	H	H	4-CH ₃ C ₆ H ₅	53
3k	Me	H	C ₆ H ₅	30
3l	F	H	C ₆ H ₅	32

as a regioisomeric mixture (6- and 7-substitution). The enhanced activity was observed when R₃ substituent was *c*-hexyl, propyl or pyridyl group, in comparison with the phenyl group. When R₃ 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 R₁ and/or R₂ positions by halogens (**3m**) displayed the highest enzymatic inhibition. These results suggest that the changes in R₁ and/or R₂ substituent on ring A of the quinazolin-2,4(1*H*,3*H*)-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 $\mu\text{g mL}^{-1}$. An increase in enzymatic inhibitory activity was observed when saturated substituents as *c*-hexyl (2.60-fold) or propyl (1.04-fold) are in R₃, compared with the phenyl group. If R₃ substituent is a tolyl group, the enzymatic inhibition followed the

reactivity *ortho* > *meta* > *para*. However, the substitution of quinazolin-2,4(1*H*,3*H*)-diones in R₁ and/or R₂ positions of the ring A resulted in being inactive against α -amylase inhibition. These suggest that saturated substituents in R₃ of the quinazolin-2,4(1*H*,3*H*)-diones could lead to obtain inhibitors against α -amylase.

In the antioxidant activity (AA), all the synthetic quinazolin-2,4-diones behave as reducing agents over the DPPH[•] 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 R³ is a tolyl (**3h-j**), the DPPH[•] 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 \pm 0.920 %) at 10 $\mu\text{g mL}^{-1}$ being 1.00- and 1.21-fold better than **3f** and **3i**, respectively. However, all quinazolin-2,4-diones synthesized are inactive (IC₅₀ >1000 $\mu\text{g mL}^{-1}$) compared with vitamin C (IC₅₀= 8.79 $\mu\text{g mL}^{-1}$) or gallic acid (IC₅₀= 4.08 $\mu\text{g mL}^{-1}$) by DPPH assay.

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

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

^a % of activity obtained at 100 $\mu\text{g mL}^{-1}$; ^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 evaluate 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 CC₅₀ 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 R₃ position. When R₃ 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 R₃ position and it was 3646-, 293-, 705- and 16764-fold more cytotoxic than **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 \cdots H-N of Arg600 (1.62 Å, 133.7°), N-H \cdots 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 \cdots H-N of Arg600 (1.92 Å, 141.3°) and N-H \cdots 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

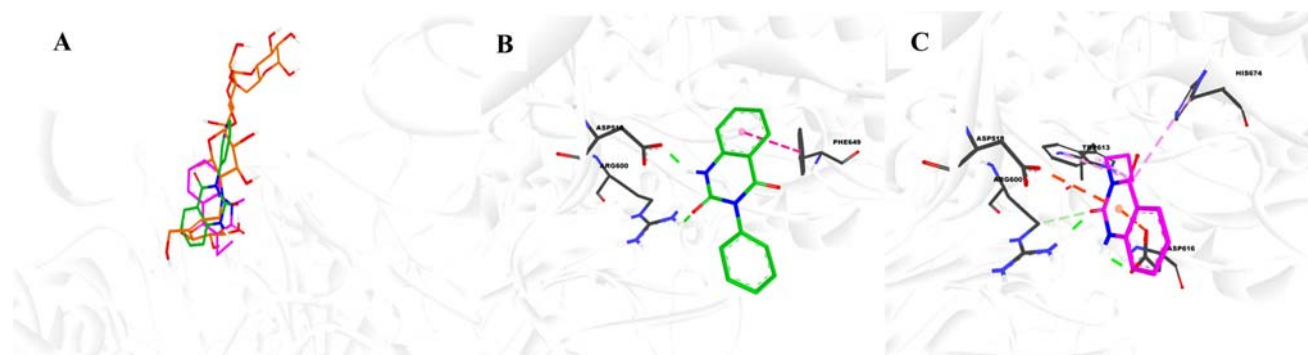


Figure 1. Molecular docking of acarbose drug (orange color), compounds **3d** (green color), and **3e** (pink color) in the active site of α -glucosidase. Putative binding interactions of the compounds **3d** (Figure B) and **3e** (Figure C). Hydrogen, electrostatic and hydrophobic bonds are shown as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

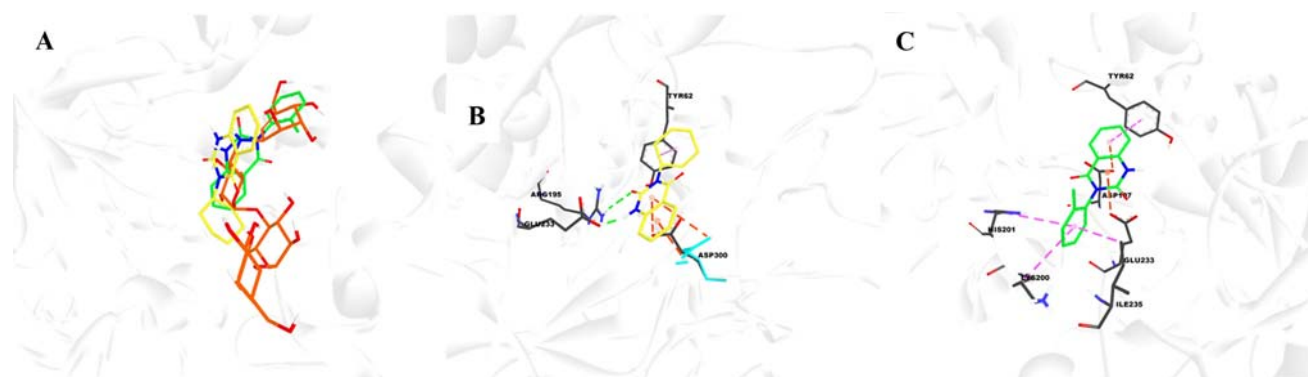


Figure 2. Molecular docking of acarbose drug (orange color), compounds **3g** (yellow color) and **3h** (green color) in the active site of α -amylase. Putative binding interactions of the compounds **3g** (Figure B) and **3h** (Figure C). Hydrogen, electrostatic and hydrophobic bonds are shown as green,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

acarboses, 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 α A upon binding in the active site residues. In this sense, the compound **3g** that exhibited the highest *in vitro* activity against α A is bound into receptor site *via* hydrophilic binding by H-bond between O \cdots H-N of Arg195 (2.48 Å, 160.2°) and N-H \cdots 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 α A mainly displayed H-bond on oxygen and/or nitrogen of the ring B, while that rings A and R₃ 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.

Acknowledgements

We gratefully acknowledge support for this project by Consejo Nacional de Ciencia y Tecnología (SEP-CONACyT, GRANT No CB-2012-178266-Q) and Programa de Fomento y Apoyo a Proyectos de Investigación (PRO-FAPI2014/206) of the Universidad Autónoma de Sinaloa.

References

- Smith A L, Thomson C G and Leeson P D 1996 An efficient solid phase synthetic route to 1,3-disubstituted 2,4(1*H*,3*H*)-Quinazoliniones suitable for combinatorial synthesis *Bioorg. Med. Chem. Lett.* **6** 1483
- Hassan M A, Younes A M M, Taha M M and Abdel-Monsef A-B H 2011 Synthesis and reactions of 3-aminotetrachloroquinazolin-2,4-dione *Eur. J. Chem.* **2** 514
- Hassan M A, Seleem M A, Younes A M M, Taha M M and Abdel-Monsef A-B H 2013 Synthesis and spectral characterization of some heterocyclic nitrogen compounds *Eur. J. Chem.* **4** 121
- Park Choo H-Y, Kim M, Lee S K, Woong Kim S, Kwon Chung I, Choo H-Y P, Kim M, Lee S K, Kim S W and Chung I K 2002 Solid-phase combinatorial synthesis and cytotoxicity of 3-aryl-2,4-quinazoliniones *Bioorg. Med. Chem.* **10** 517
- Li Z, Huang H, Sun H, Jiang H, Liu H, Zhaoguang L, He H, Hongbin S, Hualiang J and Hong L 2008 Microwave-assisted efficient and convenient synthesis of 2,4(1*H*,3*H*)-quinazoliniones and 2-thioxoquinazolines *J. Comb. Chem.* **10** 484
- Yalysheva N Z and Granik V G 1984 Unexpected formation of 2,4-quinazolinione in the reaction of α -cyano- β -dimethylaminocrotonamide with ethyl anthranilate *Chem. Heterocycl. Compd.* **20** 1186
- Gao J, He L-N, Miao C-X and Chanfreaux S 2010 Chemical fixation of CO₂: efficient synthesis of quinazoline-2,4(1*H*, 3*H*)-diones catalyzed by guanidines under solvent-free conditions *Tetrahedron* **66** 4063
- Willis M C, Snell R H, Fletcher A J and Woodward R L 2006 Tandem palladium-catalyzed urea arylation-intramolecular ester amidation: regioselective synthesis of 3-alkylated 2,4-quinazoliniones *Org. Lett.* **8** 5089
- Beylin V, Boyles D C, Curran T T, Macikenas D, Parlett R V and Vrieze D 2007 The preparation of two, preclinical amino-quinazoliniones as antibacterial agents *Org. Proc Res. Dev.* **11** 441
- Davidson J S 1984 The preparation of 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3*H*)-one and its rearrangement to 3-amino-2,4(1*H*,3*H*)-quinazolinione *Monatsh. Chem.* **115** 565
- Ryu C, Shin K, Seo J and Kim H 2002 6-Arylamino-5, 8-quinazoliniones as potent inhibitors of endothelium-dependent vasorelaxation *Eur. J. Med. Chem.* **37** 77
- Havera H J 1979 Derivatives of 1,3-disubstituted 2,4(1*H*,3*H*)-quinazoliniones as possible peripheral vasodilators or antihypertensive agents *J. Med. Chem.* **22** 1548
- Usifoh C O and Scriba G K E 2000 Synthesis and anticonvulsant activity of acetylenic quinazolinone derivatives *Arch. Pharm.* **333** 261
- Fujimori H and Cobb D P 1965 Central nervous system depressant activity of ma1337, 3-(3-(4-m-chlorophenyl-1-piperazyl)propyl)-2,4(1*H*,3*H*)-quinazolinione hydrochloride *J. Pharmacol. Exp. Ther.* **148** 151
- Hayao S, Havera H J, Strycker W G, Leipzig T J, Kulp R A and Hartzler H E 1965 New sedative and hypotensive 3-substituted 2,4(1*H*,3*H*)-quinazoliniones *J. Med. Chem.* **8** 807
- Tran T P, Ellsworth E L, Stier M A, Domagala J M, Showalter H D, Gracheck S J, Shapiro M A, Joannides T E and Singh R 2004 Synthesis and structural-activity relationships of 3-hydroxyquinazoline-2,4-dione antibacterial agents *Bioorgan. Med. Chem. Lett.* **14** 4405
- Enciso E, Sarmiento-Sánchez J I, López-Moreno H S, Ochoa-Terán A, Osuna-Martínez U and Beltrán-López E 2016 Synthesis of new quinazolin-2,4-diones as anti-Leishmania mexicana agents *Mol. Divers.* **20** 821
- Ryu C-K, Shim J-Y, Yi Y-J, Choi I H, Chae M J, Han J-Y and Jung O-J 2004 Synthesis and antifungal activity of 5,8-quinazolinione derivatives modified at positions 6 and 7 *Arch. Pharm. Res.* **27** 990
- Elansary A K, Kadry H H, Ahmed E M and Sonousi A S M 2012 Design, synthesis, and biological activity of certain quinazolinione derivatives as potent phosphodiesterase4 inhibitors *Med. Chem. Res.* **21** 3557
- Kirincich S J, Xiang J, Green N, Tam S, Yang H Y, Shim J, Shen M W H H, Clark J D and McKew J C 2009 Benzhydrylquinazoliniones: Novel cytosolic phospholipase A2 α inhibitors with improved physicochemical properties *Bioorg. Med. Chem.* **17** 4383
- Lansdon E B, Liu Q, Leavitt S A, Balakrishnan M, Perry J K, Lancaster-Moyer K, Kutty N, Liu X, Squires N H, Watkins W J and Kirschberg T A 2011 Structural and binding analysis of pyrimidinol carboxylic acid and N-hydroxy quinazolinione HIV-1 RNase H inhibitors *Antimicrob. Agents Chemother.* **55** 2905
- Muhammad M, Kevin R M, Arkady M, Xilin Z, Kalyan C, Robert J K, Karl D, Malik M, Marks K R, Mustaev A, Zhao X, Chavda K, Kerns R J and Drlica K 2011 Fluoroquinolone and quinazolinione activities against wild-type and gyrase mutant strains of *Mycobacterium smegmatis* *Antimicrob. Agents Chemother.* **55** 2335
- Kakuta H, Tanatani A, Nagasawa K and Hashimoto Y 2003 Specific nonpeptide inhibitors of puromycin-

- sensitive aminopeptidase with a 2,4(1*H*,3*H*)-quinazolin-2-one skeleton *Chem. Pharm. Bull.* **51** 1273
24. Park Choo H-Y, Kim M, Lee S K, Woong Kim S and Kwon Chung I 2002 Solid-phase combinatorial synthesis and cytotoxicity of 3-aryl-2,4-quinazolin-2-one derivatives *Bioorgan. Med. Chem.* **10** 517
 25. DeFossa E, Kadereit D, Klabunde T, Burger H-J, Herling A, Wendt K-U, Von Roedern E and Schoenafinger K 2012 Urea- and urethane-substituted acylureas, process for their preparation and their use. Patent US7262220
 26. Clauss A, Glaess C, Marciniak G, Nave J-F and Vivet B 2010 Quinazolin-2-one derivatives, preparation thereof and various therapeutic uses thereof. Patent US8722659B2
 27. Moreland R J, Higgins S, Zhou A, VanStraten P, Cauthron R D, Brem M, McLarty B J, Kudo M and Canfield W M 2012 Species-specific differences in the processing of acid α -glucosidase are due to the amino acid identity at position 201 *Gene* **491** 25
 28. Zeng Y-F, Lü Z-R, Yan L, Oh S, Yang J-M, Lee J and Ye Z M 2012 Towards alpha-glucosidase folding induced by trifluoroethanol: Kinetics and computational prediction *Proc. Bio.* **47** 2284
 29. Sarmiento-Sánchez J I, Montes-Avila J, Ochoa-Terán A, Delgado-Vargas F, Wilson-Corral V, Díaz-Camacho S P, García-Páez F and Bastidas-Bastidas P 2014 Synthesis of 1*H*-benzoxazine-2,4-diones from heterocyclic anhydrides: evaluation of antioxidant and antimicrobial activities *Quim. Nova* **37** 1297
 30. Sarmiento-Sánchez J I, Ochoa-Terán A and Rivero I A 2014 Synthesis and antioxidant evaluation of enantiomerically pure bis-(1,2,3-triazolylmethyl)amino esters from modified α -amino acids *Sci. World J.* **2014** 1
 31. Montes-ávila J, Sarmiento-sánchez J I, Delgado-vargas F, Rivero I A, Díaz-camacho S P and Uribe-beltrán M 2016 Antioxidant activity and antimicrobial evaluation of 1-benzyl-1,2,3-triazole *Acta Univ.* **26** 63
 32. López H S, Enciso J E, Ochoa-Terán A, Velazquez J I and Sarmiento J I 2016 An easy one-step synthesis of imidazolin-2-ones from phthalic anhydrides and their antioxidant evaluation *Mendeleev Commun.* **26** 69
 33. Shestakov A S, Sidorenko O E, Bushmarinov I S, Shikhaliev K S and Antipin M Y 2009 3-aryl(alkyl)quinazolin-2,4(1*H*,3*H*)-diones and their alkyl derivatives *Russ. J. Org. Chem.* **45** 1691
 34. Koay N and Campeau L-C 2011 Efficient preparation of 3-substituted quinazolin-2-one derivatives directly from anthranilic acids and isocyanates *J. Heterocycl. Chem.* **48** 473
 35. Goldstein S, Dhainaut A, Tizot A, Fauchere J-L, Kucharczyk N, Hickman J, Pierre A, Tucker G and Kraus-Berthier L 2003 New compounds derived from Quinazolin-2-one. Patent US 2003/0199530 A1
 36. Mohammadi A A 2016 One-pot syntheses of some new 2,4-(1*H*,3*H*)-quinazolin-2-one derivatives in the absence of catalyst *J. Heterocycl. Chem.* **54** 2075
 37. Papadopoulos E P and Torres C D 1982 Convenient preparation of N-substituted 2-amino-4*H*-3,1-benzoxazin-4-ones and 3-substituted 2,4(1*H*,3*H*)-quinazolin-2-one derivatives *J. Heterocycl. Chem.* **19** 269
 38. Adisakwattana S, Ruengsamran T, Kampa P and Sompong W 2012 In vitro inhibitory effects of plant-based foods and their combinations on intestinal α -glucosidase and pancreatic α -amylase *BMC Complement. Altern. Med.* **12** 110
 39. Kwon Y-I, Apostolidis E and Shetty K 2008 In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension *Bioresour. Technol.* **99** 2981
 40. Clarke G, Ting K N, Wiart C and Fry J 2013 High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest *Antioxidants* **2** 1
 41. Michael A S, Thompson C G and Abramovitz M 1956 *Artemia salina* as a test organism for bioassay *Science* **123** 464
 42. Mughal E U, Javid A, Sadiq A, Murtaza S, Zafar M N, Khan B A, Sumrra S H, Tahir M N and Khan K M 2018 Synthesis, structure-activity relationship and molecular docking studies of 3-O-flavonol glycosides as cholinesterase inhibitors *Bioorgan. Med. Chem.* **26** 3696
 43. Ali H I, Tomita K, Akaho E, Kambara H, Miura S, Hayakawa H, Ashida N, Kawashima Y, Yamagishi T and Ikeya H 2007 Antitumor studies. Part 1: design, synthesis, antitumor activity, and AutoDock study of 2-deoxy-2-phenyl-5-deazaflavins and 2-deoxy-2-phenylflavin-5-oxides as a new class of antitumor agents *Bioorgan. Med. Chem.* **15** 242
 44. Méndez-Cuesta C A, Méndez-Lucio O and Castillo R 2012 Homology modeling, docking and molecular dynamics of the *Leishmania mexicana* arginase: a description of the catalytic site useful for drug design *J. Mol. Graph. Model.* **38** 50
 45. Tian W, Chen C, Lei X, Zhao J and Liang J 2018 CASTp 3.0: computed atlas of surface topography of proteins *Nucl. Acids Res.* **46** W363
 46. Rajabi S, Ramazani A, Hamidi M and Naji T 2015 *Artemia salina* as a model organism in toxicity assessment of nanoparticles *DARU J. Pharm. Sci.* **23** 20