Research Article

Chemical & Pharmaceutical Research

Synthesis, α-Glucosidase Enzymatic Inhibition and Docking Studies of some Fused Heterocycles

Marco Brito-Arias[1*], Paola L. Ramírez-Hernández[1], Esmeralda C. García-Barrera[1], Mario Perez-Venegas[2], Christian Montiel-Valenciana[1] and Leonor P. Rodríguez-Pascual[1]

[1]Departamento de Ciencias Básicas, Unidad Profesional Interdisciplinaria de Biotecnología del Instituto Politécnico Nacional (UPIBI-IPN) Avenida Acueducto s/n La Laguna Ticomán Ciudad de México, México.

[2]Departamento de Química Centro de Investigación y Estudios Avanzados (Cinvestav-IPN) Avenida Instituto Politécnico Nacional 2508 San Pedro Zacatenco Ciudad de México México.

[*]Correspondence:
Marco Brito-Arias, Departamento de Ciencias Básicas, Unidad Profesional Interdisciplinaria de Biotecnología del Instituto Politécnico Nacional (UPIBI-IPN), México, E-mail: mbrito@ipn.mx.

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ABSTRACT

In the search of heterocyclic ring systems potentially useful as α-glucosidase inhibitors we have synthesized a set of heterocycles bearing hydroacridone 4-5, hydroxanthone 6, quinazolone 8, benzoyl phtalimide 9 and isoquinolone 10-11. These compounds under study were subjected to enzyme inhibition and compared against reference inhibitor acarbose observing potent inhibition for benzoyl phtalimide 9, and isoquinolone dione 11. Based on their inhibition activity, both candidates were selected for docking analysis to determine their best posing, and the interactions involved between the ligand and the residues. A comparative analysis was established to determine the potential correlation between the interactions found, the inhibition observed for the candidates and the interactions observed for the reference inhibitor acarbose.

Keywords

Fused heterocycles, α-Glucosidase, Enzymatic inhibition, Molecular docking.

Introduction

The glycaemic control is considered a crucial step in the prevention and management of diabetes mellitus, and the search of inhibitors targeting the enzymes α-amylase and α-glucosidase responsible for the hydrolysis of starch and oligosaccharides present in the diet an important strategy in the modulation of the glycaemic profile. It is well known that α-glucosidase inhibitors slow down the process of digestion and absorption of carbohydrates by competitively blocking its activity, leading to the reduction of the glucose concentrations in blood [1].

In the search of active compounds useful as α-glucosidase inhibitors an intensive screening has been undertaken particularly from natural resources including microorganisms and medicinal plants. For instance a recent review reported the evaluation of 61 terpenes, 37 alkaloids, 49 quinones, 7 xanthones, 103 flavonoids and 37 phenols either with or without glycosidic moieties, providing important information about structural diversity which can be applied in drug discovery and lead modification [1-3].

On the other hand, progress in the search of synthetic α-glucosidase inhibitors specially having heterocyclic rings [4] such as pyrazole [5], coumarins [6], fused pyrimidines [7], benzo[d]oxazol [8], bisbenzimidazoles [9], oxadiazole [10], xanthones [11], 4-quinazolones [12-14], and quinoxaline [15] derivatives has been also described.

With the aim of looking for heterocyclic rings within the wide range of structural diversity in either natural or synthetic compounds displaying α-glucosidase inhibition we proceed to synthesize different fused heterocycles bearing quinolone, isoquinoline, xanthone, phtalimide and quinazolinone ring systems and those presenting α-glucosidase inhibition used as lead molecules for a structure-activity relationship program.

Results and Discussion

Chemistry

The heterocyclic rings used for the screening analysis were

hydroacridinone 4, hydroquinolinone 5, xanthone dione 6, hydroquinazolone 8, benzoyl phtalimide 9, benzoyl isoquinolone 10 and isoquinoline trione 11 (Figure 1).

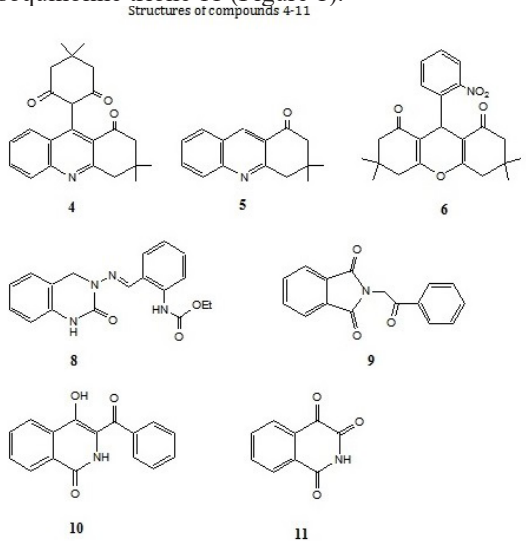


Figure 1: Fused rings evaluated as α-glucosidase inhibitors.

The synthesis of hydroacridinone 4 was carried out according to scheme I consisting in the reaction between 2-nitrobenzaldehyde 1 with 2 equivalents of dimedone 2 under basic condition to yield heterocycle 4 (Figure 2).

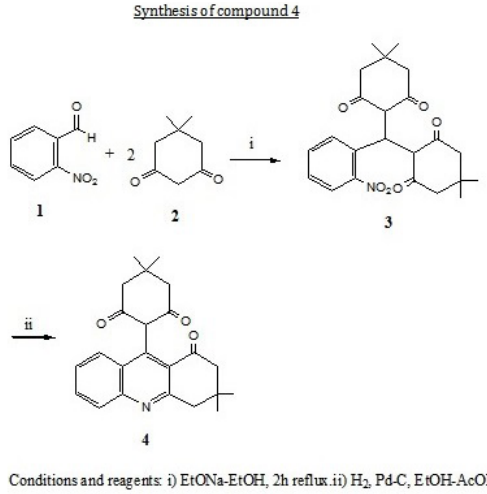
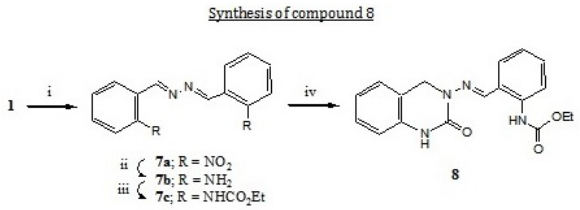


Figure 2: General scheme for the synthesis of compound 4.

The synthesis of heterocycle 5 was carried out from intermediate 3 [16] which under reducing condition afforded the acridinone 5, and its structure confirmed by X ray diffraction analysis [17]. The hydro xanthone 6 was prepared also from intermediate 3 which was submitted to base treatment with sodium methoxide under refluxing conditions as described in scheme II. The structure of this heterocycle was also confirmed by X-ray analysis [18].

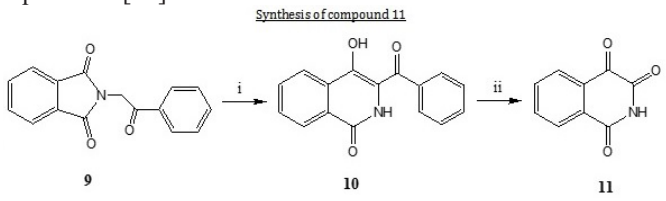
The synthesis of quinazolinone 8 was achieved by preparation of dimeric intermediates 7a-c and final ring formation of the carbamate intermediate by hydride nucleophilic addition to yield 8 as shown in Figure 3.



Reagents and conditions: i) $NH_2NH_2.HCl$, Py, 2 h, reflux. ii) H_2, Pd-C, EtOH. iii) $ClCO_2Et$, Py, 2h reflux.

Figure 3: General scheme for the synthesis of compound 8.

The fused heterocycles 9, 10 and 11 where prepared according to Figure 4 starting from benzoylphtalimide 9 which was prepared by reaction of bromoacetophenone with potassium phtalimide in DMF, and treated with sodium methoxide conditions to afford benzoyisoquinolone 10, and then converted to the isoquinoline trione 11 by treatment with acetic anhydride in nitric acid. This method is an alternative approach to a previous synthesis of isoquinoline-1,3,4-triones based on strong oxidative conditions of isoquinoline [19].



Conditions and reagents: i) MeONa-MeOH 2h, reflux. 2) Ac_2O-HNO_3 0°C 30 min.

Figure 4: General scheme for the preparation of compound 11.

In order to provide a plausible explanation about the formation of the isoquinolone dione 11 from 10 we propose a step sequence involving the initial enol protonation through a tautomerism equilibrium to form an enamine which under acidic medium is converted to an iminium salt. Addition of a water molecule is followed by a nucleophilic attack leading to isoquinolin trione formation as shown in Figure 5.

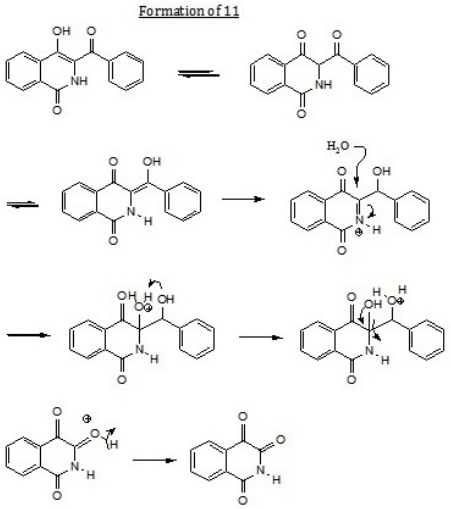


Figure 5: Proposed reaction mechanism for the formation of compound 11.

α-Glucosidase inhibition Assay

The α-Glucosidase assay used for the inhibitory determination was the colorimetric protocol based on the glycosidic bond cleavage of the p-nitrophenyl-α-glucopyranoside (p-NPG) and the resulting absorbance measured at 405 nm, in the presence of inhibitor.

Assay tubes containing 10µl of 3 mM p-nitrophenyl-α-d-glucopyranoside (p-NPG), 4µl of Saccharomyces cerevisiae α-glucosidase enzyme (9.67U/mL) in 50 mM phosphate buffer (pH 6.8) in a total volume of 500µl where mixed gently and then 10µl of compounds under evaluation at final concentration 24, 48, 96 and 120 µg/ml were added. Acarbose (Glucobay, Bayer Pharmaceuticals) and Dimethyl sulfoxide (DMSO) were used as positive and negative controls respectively. Tubes were incubated at 37oC during 3 min, followed by the addition of 500µl 0.1M sodium bicarbonate as stopping agent. Absorbance was measured at 405 nm in a Genesys 10 S Spectrophotometer (Thermo Fisher Scientific).

One unit of α-Glucosidase is the amount of enzyme that catalyzes the hydrolysis of 1.0 µmol of substrate (p-nitrophenol) per minute at 37°C. The resulting enzymatic activity was used to build the Lineweaver-Burk double reciprocal plot of substrate concentration vs velocity, and from the equation the Vmax and Km was determined. Based on the Vmax and Km values observed from the graphics we conclude that benzoylphtalimide 9 and isoquinolone dione 11 were competitive and mixed inhibitors respectively (Figure 6 and 7).

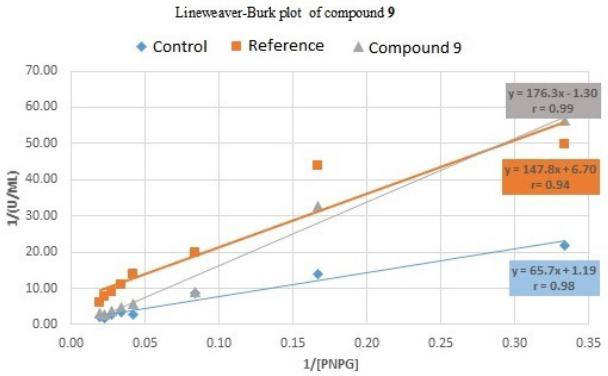


Figure 6: Lineweaver-Burk plot for the inhibition Saccharomyces cerevisiae α-glucosidase enzyme by compound benzoylphtalimide 9.

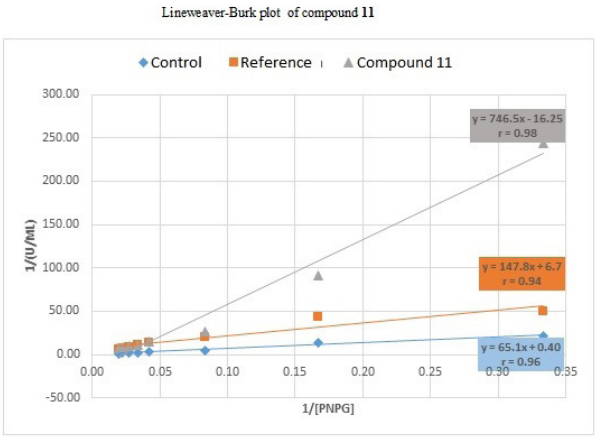


Figure 7: Lineweaver-Burk plot for the inhibition Saccharomyces cerevisiae α-glucosidase enzyme by compound isoquinolone dione 11.

Next, we proceed to determine the IC_{50} by plotting the activity against the inhibitor concentration and the Ki values which were calculated from the Lineweaver-Burk inhibition equation. The Ki calculated values of compound 9 were 2.80 mM and for compound 11 were of 5.56 mM. The IC_{50} values for compound 9 and 11 the highest inhibition potency in the range of inhibition of the reference acarbose (Table 1).

Compund	Inhibition mode	Ki (mM)	IC_{50} (mM)
9	Competitive inhibition	2.80	5.6
11	Mixed inhibition	5.56	3.5

Table 1: Inhibition mode and constant (Ki value) and IC_{50} value of compound 9 and compound 11 against Saccharomyces cerevisiae α-glucosidase.

The compounds tested probed to be effective inhibitors against *Saccharomyces cerevisiae* α-glucosidase, and compound 9 were the most potent with a competitive inhibition mode, explained by its structure and dockins studies. The mixed competitive inhibitor compound 11, was less effective in inhibition of α-glucosidase.

Docking studies

The glycoside hydrolase (GH), GluI operates via an inverting mechanism and the catalytic acid and base are not definitively known [20]. Although substrate-binding motif in the rat and mammalian homologs has been proposed no eukaryotic structures have been determined, and therefore we can only rely on two structures which have been solved of prokaryotic GH63: the *Escherichia coli* homolog YgjK (PDB code 3D3I), and the T. Thermophilus homolog TTHA0978 (PDB ID 2Z07). However neither of these structures is sufficiently similar to mammalian GluI to act as a realistic model at the atomic level.

Much of what we know about the characteristics of GluI has been learned from studying the *Saccharomyces cerevisiae* enzyme, Cwh41p. Cwh41p and human GluI share 24% overall identity and from 34 to 59% identity in the catalytically active C-terminal domain, and so similar structures are expected. Yeast and human GluI share similar substrate specificity, pH optimum, and inhibitor sensitivity. Thus, the yeast enzyme besides its affordability serves as a good experimental model to learn more about the structure, substrate specificity, and enzymatic mechanism of human GluI [20].

Therefore the protein chosen for the docking studies was *Saccharomyces cerevisiae* Cwh41p (Protein Data Bank PDB ID 4J5T) and the program we use for the docking analysis was Autodock vina [21] and the visualizer program Discovery [22] to identify the binding modes interacions ligand-residues (Figure 8 and 9).

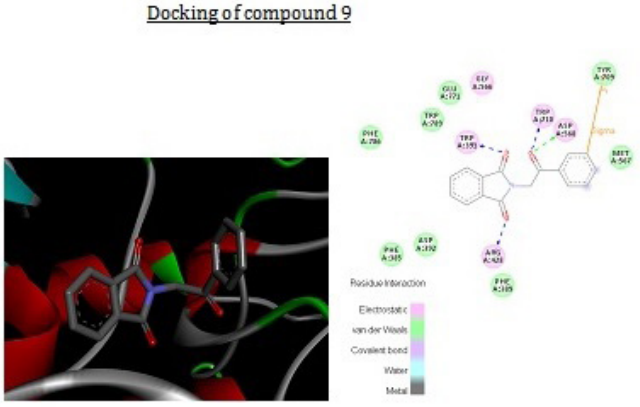


Figure 8: Binding mode for compounds benzoylphtalimide 9 in the active site of *Saccharomyces cerevisiae* α-glucosidase enzyme (PDB 4J5T).

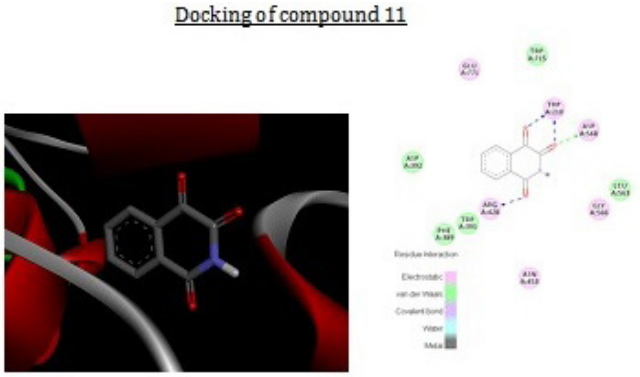


Figure 9: Binding mode for compound isoquinolone dione 11 in the active site of *Saccharomyces cerevisiae* α-glucosidase enzyme (PDB 4J5T).

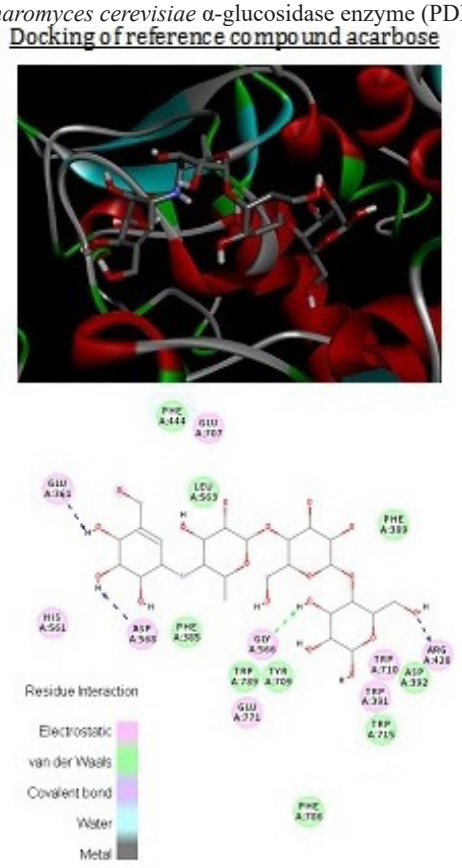


Figure 10: Binding mode for the reference inhibitor acarbose in the active site of Saccharomyces cerevisiae α-glucosidase enzyme (PDB 4J5T).

Acarbose was docked with α-glucosidase PDB ID 4J5T from *Saccharomyces cerevisiae* to establish the interactions for the best pose with affinity -8.0 kcal/mol and as a result it was found for the best conformation the residues establishing polar contacts were Arg 428, Asp 568, Glu 361 and Gly 566 as shown in Figure 10.

A graphical model was made to establish an interaction pattern between the amino acid residues present in the pocket and the 8 conformations obtained for ligand 9 and 11 having the highest inhibition, along with the reference inhibitor acarbose and the natural substrate maltose used as a control.

As a result we observed that the polar contacts with highest scores were Arg 428 with 8 for reference inhibitor acarbose and control maltose, and 6 for compound 11 and 4 for compound 9. Other residues establishing polar contacts are Asp 568, Trp 391 and Trp 710 having compound 9 the highest proportion of polar contacts with Trp 710 residue (Figure 11).

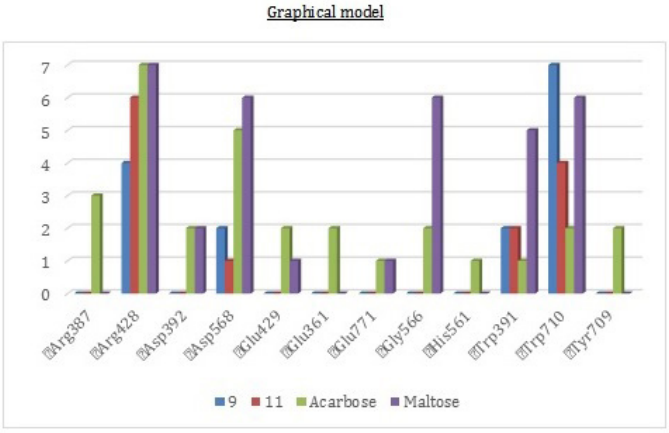


Figure 11: Graphical model describing the scores presented by the 8 conformations determined for compound 9, 11, acarbose and maltose.

Experimental

Solvents were purchase as analytical degree and all intermediates were purified by column chromatography using silica gel 60 and hexane-ethyl acetate at different gradients as elution system. Thin layer chromatography were carried out on pre-coated aluminum sheets silica gel with fluorescent indicator UV_{254} (Macherey-Nagel, Germany). ^{1}H and ^{13}C NMR were recorded on Varian Mercury 300 MHz using $CDCl_3$ or DMSO-d_6 as internal standard. High resolution mass spectra were performed by positive electrospray ionization micrOTOF-Q II 10392 analyzer.

Bis-(1E,1'E)-(2-nitrobenzyl)hydrazone (7a)

In a 50 ml round flask was dissolved 2-nitrobenzaldehyde 1 (0.5 g, 3.30 mmoles) in pyridine (7 mL) and hydrazine hydrochloride (0.7 g, 6.78 mmol) was added and the reaction heated under reflux during 4 hrs. The pyridine was evaporated under diminished pressure and the solid dissolved in CH_2Cl_2 30 mL, washed with water, dried over sodium sulfate and evaporated to yield 0.82 g

(83 %) of a yellow solid. ^{1}H NMR spectrum ($CDCl_3$): δ 7.65 (t, 2H, J = 6.9,), 7.75 (t, 2H, J = 6.9,), 8.09 (d, 2H, J = 7.5), 8.29 (d, 2H, J = 7.5), 9.13 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 124.80, 128.67, 129.60, 131.27, 133.60, 157.82, 158.51. HRMS (ESI-pos, m/z) C14H10N4O4 [M + H]+ Found 299.0778 Calculated 298.0702.

Bis-(1E,1'E)-(2-aminobenzyl)hydrazone (7b)

In a 50 mL round-bottom flask the nitro dimer 7a (0.4 g, 1.34 mmoles) was dissolved in ethanol (7 mL) and stirred until dissolution. Then palladium on activated carbon 10 % (30 mg) was added and the reaction stirred under hydrogen atmosphere during 6 h and filtered in a short column packed with celite using methanol as elution system. The product was concentrated on rotavapor to furnish 0.31 g (97%) of amino dimer 2. ^{1}H NMR ($CDCl_3$) δ 6.70 (d, 4H, J = 7.2), 7.19 (t, 2H, J = 7.6), 7.27 (dd, 2H), 8.72 (s, 2H). ^{13}CNMR ($CDCl_3$) δ 114.95, 115.44, 115.54, 131.90, 134.08, 149.49, 164.32. HRMS (ESI-pos, m/z) C14H14N4 [M+H]+ Found 239.1294, Calculated 239.1218.

Bis-(1E,1'E)-(2-ethylcarbamatebenzyl)hydrazone (7c)

Intermediate 7b (0.7 g, 2.93 mmoles) placed in a 50 mL round-bottom flask was dissolved in pyridine (7 mL), and with syringe added dropwise ethylchloroformate (0.55 mL, 5.87 mmol) and then heated to reflux during 2 hrs. The reaction was dissolved in CH_2Cl_2 (30 mL) and washed with HCl 1 N (30 mL), saturated solution of $NaHCO_3$ (30 mL), H_2O-brine (30 mL), dried over sodium sulfate and evaporated on rotavapor to yield 0.92 g (82%) of dimer 3 as a pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.38 (t, 6H, J = 7.2), 4.29 (q, 4H, J = 7.1), 7.07 (t, 2H, J = 7.0), 7.46 (m, 4H), 8.45 (d, 2H, J = 8.4), 8.67 (s, 2H), 11.17 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 14.66, 61.17, 118.41, 118.51, 121.92, 132.63, 134.14, 140.16, 154.00, 165.14. HRMS (ESI-pos, m/z) $C_{20}H_{22}N_4O_4$ [M + H]+ Found 383.1714 Calculated 383.1641.

3-(2-ethylcarbamatebenzylamine)-4-dihydroquinazolin-2-one (8)

In a round-bottom flask of 100 mL were dissolved carbamate dimer 7c (0.7 g, 1.83 mmoles) in ethanol (10 mL) and stirred until dissolution. The reaction is cooled under ice bath and sodium borohydride was added portion wise, heated cautiously and maintained during 4 h under refluxing conditions. The reaction was stopped by addition of citric acid solution (15 mL, 10%) and then adjusting the pH to neutral with 1N NaOH. The reaction was extracted with CH_2Cl_2 (2 x 30 mL) dried over sedum sulfate and evaporated on rotavapor to yield 0.32 g (51%) of quinazolin-2-one 4 as yellow precipitate which was crystallized by slow evaporation from ethanol solution. ^{1}H NMR (DMSO d-6) δ 1.22 (t, 3H, J = 7.2), 4.13 (q, 2H, J = 7.1), 4.92 (s, 1H), 6.86 (d, 1H, J = 7.8), 6.96 (t, 1H, J = 6.3), 7.10 (t, 1H, J = 7.5), 7.20 (m, 2H), 7.34 (t, 1H, J = 8.7), 7.57 (d, 1H, J = 8.1), 8.08 (s, 1H), 8.24 (d, 1H, J = 8.7), 9.92 (s, 1H), 11.3 (s, 1H). 13C NMR (DMSO d-$_6$) δ 14.79, 46.85, 60.86, 114.33, 116.78, 119.05, 121.49, 122.10, 122.56, 126.74, 128.68, 130.17, 132.21, 135.83, 137.97, 142.58, 149.45, 154.26.

Isoquinoline-1,3,4-trione 11

In a round bottom flask were placed 0.250 g (94.2 mmoles) of 10 and dissolved with a mixture of acetic acid-acetic anhydride 3mL (1:1 v/v) with stirring. The reaction was cooled in ice-water bath and nitric acid previously cooled was added dropwise during 30 min. The reaction was poured into a beaker containing ice to induce precipitation. The yellow solid was filtered and washed with cold water to yield 0.123 g (yield 74.5%) of compound 11. ^{1}HNMR of 11 in DMSO-d_6: δ 7.87 (m, 2H), 8.03 (dd, 1H), 8.11 (dd, 1H), 12.00 (s, NH). ^{13}CNMR DMSO-d_6 δ 127.1, 128.5, 130.2, 132.7, 134.4, 135.3, 157.9, 163.6, 175.9.

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