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Synthesis of potential chemotherapic quinoxalinone derivatives by biocatalysis or microwave-assisted Hinsberg reaction

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Abstract

In recent years, great efforts have been dedicated to the design of compounds acting as selective inhibitors of the HIV-1 reverse transcriptase (RT). Due to the promissory results previously attained with some quinoxaline derivatives, we aimed to improve the specific standard Hinsberg synthetic pathway by means of biocatalysis or microwave (MW) irradiation. Both techniques rendered the products in very good yields. However, employing the microwave-assisted organic synthesis (MAOS), in the absence of solvent, the same reactions may be completed in minutes. Some of these quinoxalinone derivatives exhibited good inhibitor activity against some human tumoral cells and the lymphoma related to HIV-1.

Keywords: Quinoxaline derivatives; Biocatalysis; Microwave irradiation; Hinsberg reaction; AIDS

Since of 2005, the World Health Organization (WHO) estimated that worldwide more than 40 million people were infected with the human immunodeficiency virus (HIV). In particular South America is facing a rapidly growing number of HIV infections which require an effective antiviral therapy. Ideally, anti-HIV drugs should be highly selective, have good oral availability, and favorable pharmacokinetics.

Moreover, large-scale production, at low costs to make them accessible for patients in developing countries would constitute a crucial advantageous feature.

Several derivatives of quinoxaline (e.g., HBY-097 and S-2720) display interesting activity against HIV as non-nucleosidic inhibitors of the reverse transcriptase (RT).[1,2] The present study describes the synthesis and characterization of a family of quinoxalinone derivatives.

The Hinsberg reaction,[3] although known for more than a century, is still the most useful method for the preparation of such type of compounds. By heating *ortho*-phenylenediamines with α-ketoacids, quinoxalinones were obtained, though yields were never above 50–65%. Furthermore, it was necessary to vary the conditions of the reactions (e.g., temperature, reaction solvent, catalysis) for each particular synthesis.[4]

Computational studies indicated that this reaction loses efficiency due to several factors.[5] Among them, when many intermediates coexisted, the reaction reached the equilibrium. These findings were in agreement with the experimental observations (e.g., chromatography),[5] and were characterized by very low yields and required a laborious work to isolate the quinoxaline. Consequently, this complex reaction displays a pronounced yield decrease as the amount of reactants increases, leading to even lower performances in larger scales. Even though a few reports describing the microwave-assisted synthesis of quinoxalines are available in the literature,[6,7] this is the first work presenting the preparation of benzoquinoxalines using this technology.

Herein, six quinoxalinones (**3a**–**i**) and three benzoquinoxalinones (**4a**–**c**) were obtained in good yields, by

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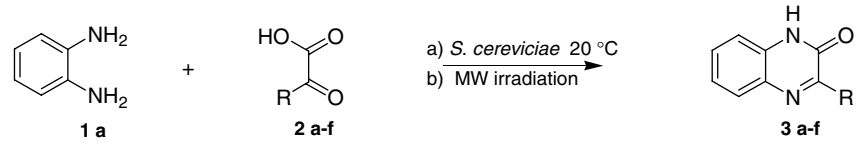
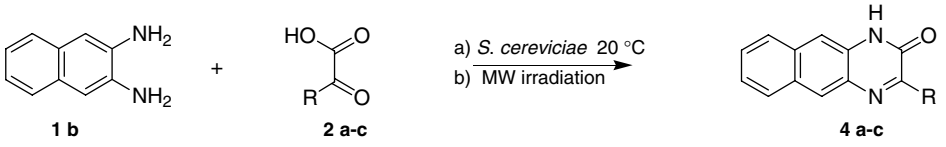


R= -CH_3 (**a**); -C_2H_5 (**b**); -$(CH_2)_2$-CO_2H (**c**); -CH_2-CO_2H (**d**); -CH_2-C_6H_5 (**e**); -OH (**f**)



R= -CH_3 (**a**); -C_2H_5 (**b**); -$(CH_2)_2$-CO_2H (**c**)

Scheme 1. Synthesis of quinoxalinone and benzoquinoxalinone derivatives by biocatalysis or microwave-assisted Hinsberg reaction.

reacting *ortho*-phenylenediamine or 2,3-diaminenaphthalene with a variety of α-ketoacids (Scheme 1) through enzymatic catalysis or microwave irradiation. Data are reported in Table 1.

Synthesis via enzymatic catalysis involves enormously complex biochemical reactions compared to the classical organic synthesis. Reactions depicted in this Letter will have probably a strong empirical aspect. Nevertheless, enzymes were used due to their usefulness as efficient catalysts to synthesize some quinoxalinone derivatives (see Table 1). Microorganism-mediated processes, are being used as means for oxidative–reductive reactions, condensations, acylations, and cyclizations. Reaction rates were enhanced by a factor of 10^8–10^{10} when compared to regular organic synthetic pathways. Moreover, since enzymes perform at pH 7 at room temperature and are completely degradable, the biocatalysis is more environmentally friendly. These facts minimize undesired decompositions, isomerizations, racemizations, or rearrangements. Also, the manipulation of the bacterial activity to increase the expression of the desired enzymes, as well as the inhibition of other enzymes related to undesired secondary reaction products constitutes a very important additional tool. In the context of the synthesis of these heterocycles by using enzymatic means, our group had previously published preliminary assays.[8]

Compounds **3a**–**f** and **4a**–**c** were prepared by mixing the starting materials in a sucrose solution. *Saccharomyces cereviciae* was added and the mixture was stirred.[9] Products were crystallized from the appropriate solvent. Melting points were compared with those reported in the literature, and the structures were confirmed by IR and ^{1}H NMR spectroscopy.[10]

The other technique employed was the microwave irradiation. Microwave radiation is converted into heat with high efficiency, so that superheating becomes possible at ambient pressure. Great acceleration in the reaction time can be achieved, and the assay that takes several hours under conventional conditions, can be completed over the course of minutes. In addition, unmodified home microwave units are suitable in many cases for the development of the reaction.

Microwave-assisted synthesis was carried out in one-pot reaction, where *ortho*-phenylenediamine or 2,3-diaminenaphthalene was mixed with the α-ketoacid without solvent, and irradiated with a domestic oven applying a potency of 650–750 W.[11] These solvent free reactions prove that it is possible to prepare easily isolable compounds in acceptably good yield, and in a short time reaction. Furthermore, the method could be efficiently applied to greater scales with a minimal risk of by-products generation.

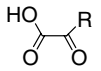
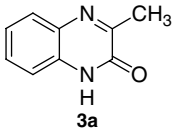
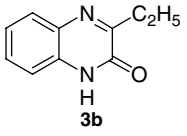
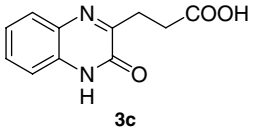
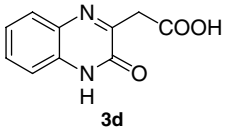
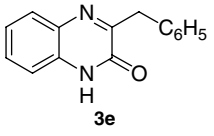
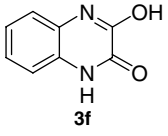
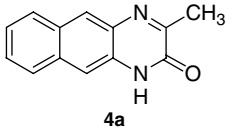
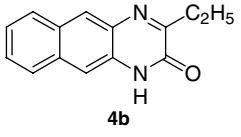
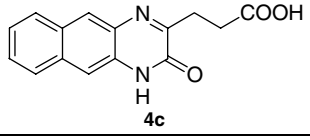
Reaction times and yields of compounds **3a**–**f** prepared by microwave irradiation are presented in Table 1.

Finally, comparing the findings obtained by both methodologies, it could be stated that even though the biocatalytic reaction presents an interesting and broad field of application, and that the yields are slightly higher, it demands a more tedious work-up to purify the products. However, biocatalysis is a method to be taken into consideration in the case of more complex reactions. On the other hand, the use of microwaves as the heating source results in an easier and cleaner technique, leading to very good yields in relatively short reaction times. So, having into account the facility and versatility of the MW chemistry applied to the synthesis of quinoxalinones and benzoquinoxalinones, as well as the good yields achieved and the easy work-up of the reactions, this technique is preferable with respect to the use of enzymatic catalysis.

The studies against HIV were developed for compound **3e** and the methyl ester of **4c**, and no activity was observed, as compared to efavirenz, though it is worth mentioning that the assayed concentrations up to the order 10^{-6} M were not cytotoxic.

On the other hand, potential antitumoral activity of compounds **4a** and **4b** has been investigated in the National Cancer Institute (USA). Compound **4a** displayed in vitro activity against a non-small lung cell line (EKVX) and human kidney cells (CL A498), while **4b** displayed in vitro activity against central nervous system (CNS) human cells.[12] However, the cytotoxicity shown in both

Table 1
Synthesis of quinoxalinone derivatives by biocatalysis and MW irradiation

Entry	Substrate 1	Substrate 2 	Products 3, 4	Mp (°C) (lit.)	Biocatalysis		MW irradiation[a]	
					Time (h)	Yield[b] (%)	Time (min)	Yield[b] (%)
1	**1a**	–CH_3 **2a**	 **3a**	243–245 (244–245)[13]	72	95	8.5	88
2	**1a**	–C_2H_5 **2b**	 **3b**	263–264	72	95	3.5	90
3	**1a**	–$(CH_2)_2COOH$ **2c**	 **3c**	256–258 (257–258)[14]	72	95	5	91
4	**1a**	–CH_2COOH **2d**	 **3d**	211–213	72	95	11	90
5	**1**[a]	–$CH_2C_6H_5$ **2e**	 **3e**	198–200 (199–200)[5]	72	95	13	86
6	**1**[a]	–OH **2f**	 **3f**	>320 (410)[15]	72	92	6	90
7	**1b**	–CH_3 **2a**	 **4a**	291–292 (293–295)[16]	72	93	5	80
8	**1b**	–C_2H_5 **2b**	 **4b**	263–264	72	93	5	70
9	**1b**	–$(CH_2)_2COOH$ **2c**	 **4c**	262–263 (261–263)[16]	72	90–95	3	90

Comparison of reaction times and yields between both methods.
[a] Using household microwave.
[b] Yields refer to pure products.

cases was higher than the minimal required to continue the screening. **4c** was evaluated by the Biological Evaluation Committee (BEC). Findings indicated a higher activity against AIDS-related lymphoma than the reference

compound employed by the NCI.[12] Nevertheless, it did not reach the lower toxicity level to perform in vivo studies.

Acknowledgment

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References and notes

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9. *Typical procedure for the biocatalytic method* (compounds **1**–**9**): Saccharose (10% P/V in distilled water) and *Saccharomyces cereviciae* (10 g) were mixed in a 1-l flask and stirred for 1 h at room temperature. Then, 100 mg of accurately measured (1 mmol) *ortho*-phenylenediamine or 2,3-diaminonaphthalene, and different α-keto-acids in excess (1.4 mmol) were added to the mixture. Stirring was continued for 48 h at room temperature. After the reaction was completed, the mixture was centrifuged and the acid pellet was isolated and re-suspended in methanol. This mixture was stirred for another 24 h and centrifuged. The upper layer was separated and the solvent, evaporated. The solid obtained was washed to eliminate the saccharose residue and render the final heterocyclic compound. Products were crystallized from the appropriate solvent. Melting points were compared with those reported in the literature, and their structure was confirmed by IR and ^{1}H NMR spectroscopy.
10. *Spectra data for products*: 3-Methylquinoxalin-2(1*H*)-one (entry 1, **3a**): white needles (anhydrous ethanol); spectral properties agree with those described by Nishio.[13] *3-Ethylquinoxalin-2(1H)-one* (entry 2, **3b**): white crystals (ethanol); ^{1}H NMR (DMSO-d_6): δ 1.50 (t, 3H, CH_3), 3.06 (q, 2H, CH_2), 7.60 (t, 1H, aromatics), 7.69 (t, 1H, aromatics), 12.50 (s, 1H, NH); IR: (cm^{-1}), 1640, 1670, 3405; UV (methanol): λ_{max} nm: 270, 285; Anal. Calcd for $C_{10}H_{10}N_2O$: C, 68.95; H, 5.78; N, 16.08; found: C, 68,86; H, 5.97; N, 16.30. *3-[3-Quinoxalin-2(1H)-one] propanoic* acid (entry 3, **3c**): white crystals (ethanol); spectra data agree again with those reported by Rodrigo.[14] *2-[3-Quinozalin-2(1H)-one] acetic acid* (entry 4, **3d**): white crystals (ethanol); ^{1}H NMR (DMSO-d_6): δ 2.39 (s, 2H, CH_2), 7.82 (m, 2H, aromatics), 7.48 (t, 1H, aromatics), 7.69 (d, 1H, aromatics), 12.4 (s, 1H, COOH); IR (cm^{-1}): 166, 1608, 3167–3435; Anal. Calcd for $C_{10}H_8N_2O_3$: C, 58.82; H, 3,95; N, 13,72; found: C, 58.91; H, 4.09; N, 13.88. *3-Benzylquinoxalin-2(1H)-one* (entry 5, **3e**): white needles (ethanol); spectra data was previously reported by us[5] and agree again with the results obtained in this work. *3-Hydroxyquinoxalin-2(1H)-one* (entry 6, **3f**): white powder (ethanol) in part volatile without decomposition, reported by Motylevski.[15] *3-Methylbenzo[g]quinoxalin-2(1H)-one* (entry 7, **4a**): yellow crystals (methanol/water) reported by Rodrigo.[16] *3-Ethylbenzo[g]quinoxalin-2(1H)-one* (entry 8, **4b**): yellow crystals (methanol/water); ^{1}H NMR (DMSO-d_6): δ 1.45 (t, 3H, CH_3), 3,04 (q, 2H, CH_2), 7.63 (t, 1H, aromatics), 7.69 (t, 1H, aromatics), 7.72 (t, 1H, aromatics), 7.82 (s, 1H, aromatics), 8.11 (d, 1H, aromatics), 8.23 (d, 1H, aromatics), 8.54 (s, 1H, aromatics), 12.47 (s, 1H, NH); IR (cm^{-1}): 1640, 1670, 3405; UV (methanol): λ_{max} nm: 272, 282; Anal. Calcd for $C_{14}H_{12}N_2O$: C, 74,98; H, 5.39; N, 12.49; found: C, 75.05; H, 5,46, N, 12,60. *3-{3-Benzo[g]quinoxalin-2(1H)-one} propanoic acid* (entry 9, **4c**): yellow crystals (methanol/water) synthesized by Rodrigo.[16] There was agreement with spectroscopic data determined in this work.
11. *Typical procedure for microwave irradiation*: Reactions were performed in the absence of solvent. In every case equimolecular amounts of both reactants (2 mmol) were mixed and subjected to microwave irradiation under the reaction conditions described in Table 1 for each compound. After the completion of the reaction (TLC), the mixture was diluted with MeOH, EtOH or CH_2Cl_2 (20 ml) and concentrated under reduced pressure to give always a solid substance. Compounds already known were crystallized from the appropriate solvent. Melting points were compared with those reported in the literature, and their structure was confirmed by IR and ^{1}H NMR spectroscopy.
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