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Microwave-Assisted Synthesis of Pyridophenoxazinones, a Class of Antiproliferative Compounds

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This work is dedicated to Prof. Romualdo Caputo on the occasion of his 75th birthday

Microwave (MW) irradiation allows to prepare a mini-library of pyridophenoxazinone derivatives, that are crucial intermediates in the synthesis of new antiproliferative compounds, active for human lymphoma/leukemia. The optimized procedure, consisting of a continuous input power of 50 W (80 °C) and a reaction time of 30 min, leads in very satisfactory yields to both regioisomers 5*H*-pyrido[2,3-*a*]phenoxazin-5-ones and isomeric 5*H*-pyrido[3,2-*a*]phenoxazin-5-ones, starting from readily available quinoline-5,8-dione and substituted 2-aminophenols (APhOHs). Broad functional group tolerance was observed. The availability of derivatives carrying a carboxyl group at the C-9 and C-10 positions enables the synthesis of a new family of 5*H*-pyridophenoxazin-5-one conjugates that were shown to exhibit good anti-cancer activity.

Pyridophenoxazinones (PPXZs) are four-annulated iminoquinone planar systems (Figure 1) that satisfy the structural requirements of typical DNA-intercalating agents.[1] The DNA/PPXZ complexes are stabilized by π-π stacking interactions between the aromatic bases guanine-cytosine and the electron-poor iminoquinone system.[2,3] In addition, the iminoquinone moiety exploits the well-known ability of the quinones to generate reactive oxygen species (ROS) by reversible oxidation-reduction cycles.[4,5] To sum up, these features make the PPXZ-based compounds attractive tools that perform their antitumoral effect through different and concurrent mechanisms.[6]

Continuing our search for new potential anticancer drugs derived from PPXZ,[7] on the basis of our previous studies of molecular dynamics, compounds as **12** and **13** are expected to inhibit topoisomerases I and II, enzymatic systems with major modulating effects on DNA replication.

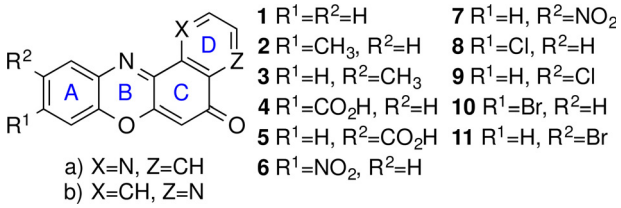

Figure 1. 5*H*-Pyrido[2,3-*a*]phenoxazin-5-one (a) and 5*H*-pyrido[3,2-*a*]phenoxazin-5-one (b) derivatives.

Indeed, molecular modelling suggests, that it is possible to have an efficient inhibition of topoisomerases I and II[6b,8] if a flexible hydrophilic moiety (such as polar amino acids, sugars, diamines, etc.) is linked to the PPXZ ring.

In this paper we wish to report the synthesis of some PPXZ derivatives carrying a carboxyl group at the C-9 and C-10 position at the A ring (**4** and **5**, Figure 1) and their conjugate derivatives (**12** and **13**, Figure 2).

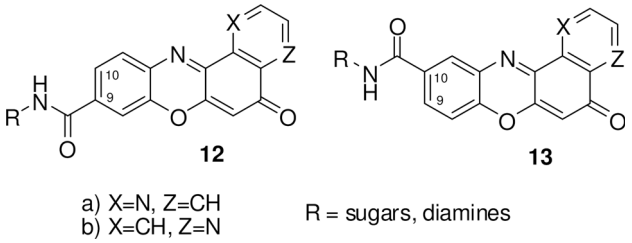

Figure 2. 5*H*-Pyrido[2,3-*a*]phenoxazin-5-one (a) and 5*H*-pyrido[3,2-*a*]phenoxazin-5-one (b) carboxyamide derivatives.

The rational production of new substituted phenazine and phenoxazinone scaffolds is highly desirable[9] and has been mainly prevented by the absence of general methodologies for the production of diversely substituted phenazines and phenoxazines.

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The conventional synthetic method to prepare PPXZ derivatives as **1–11**, which involves the reaction of an equimolar amount of quinoline-5,8-dione (QQ) and of substituted 2-aminophenols (APhOHs) in refluxing glacial acetic acid for 24 h, is not very effective owing to rather harsh reaction conditions and produces the poorest yields. Several side products, like phenoxazines (**14**), aminophenoxazin-3-ones (**15**), triphenodioxazines (**16**), are reported[7a,e] to accompany the desired PPXZs (Figure 3). A large amount of black, intractable polymeric

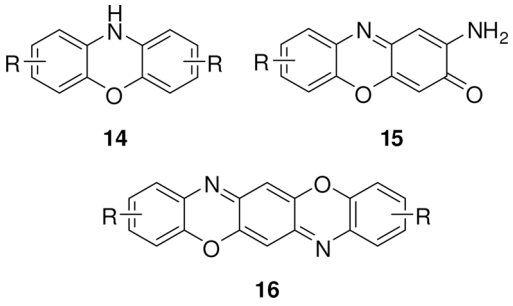


Figure 3. Side products obtained during the synthesis of PPXZs.

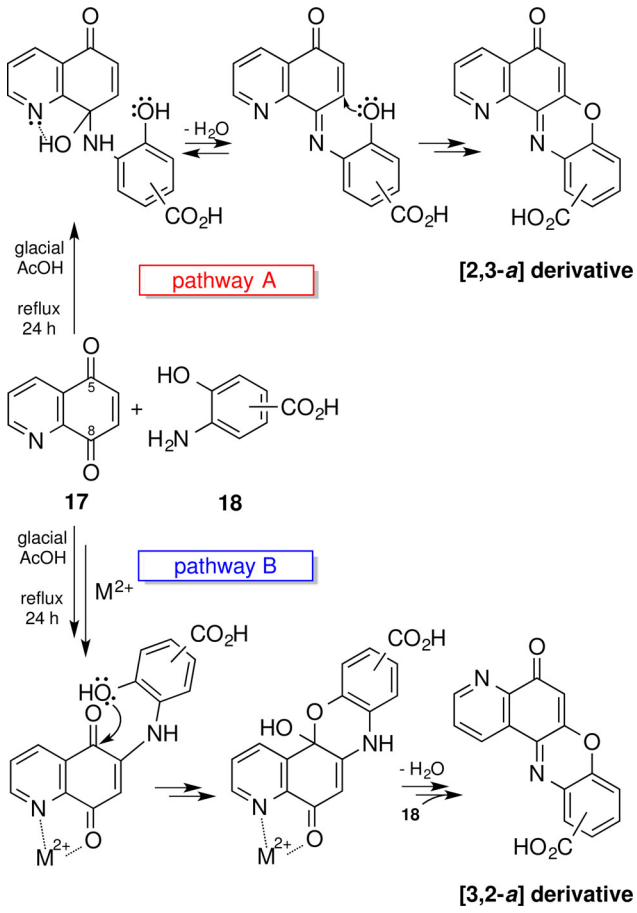
materials are also formed, thus decreasing significantly the yields of PPXZs and also encumbering the subsequent purification procedures of the final reaction mixtures.

Under the conditions mentioned above, QQ **17** (path A, Scheme 1) reacts with the substituted benzoic acids (**21** and **22**, Table 1) affording the two regioisomeric [2,3-*a*] PPXZ derivatives with yields of 2 and 3 %, respectively.

Previous theoretical studies[7a] and synthetic experiments suggested that the reaction yielding the [2,3-*a*] PPXZ system, starts firstly with the attack of the nucleophilic amino group of 2-APhOH on the C-8 carbonyl group of QQ **17** and the reaction proceeds towards the formation of the [2,3-*a*] PPXZ system through a dehydration step followed by a *intra*-molecular Michael addition of the aminophenol hydroxyl group to the α,β-unsatureted quinone system (path A, Scheme 1).

The [3,2-*a*] PPXZs formation is much less favoured than the corresponding [2,3-*a*] one, therefore to obtain the former derivative the C-8 carbonyl group of QQ **17** must become unavailable for the initial nucleophilic attack on the amino group, and this is achieved by adding some M^{2+} salt such as $Co(CH_3CO_2)_2$ to the reaction mixture. The result is the chelation by the metal ion of both nitrogen and C-8 oxygen atoms in QQ **17**, thus favouring a Michael addition of the nucleophilic amino group at C-6 carbon atom that is alpha to C-5 carbonyl group. Quinone reduction takes place and is followed by reoxidation by oxygen or by the quinone present in situ. Subsequently, an intramolecular attack of the hydroxyl group of aminophenol to the carbonyl carbon in the C-5 position yields a hemiketal, which undergoes a substitution reaction by the amino group of second molecule of aminophenol (**18**) affording, after rearrangement, the [3,2-*a*] PPXZ compound (path B, Scheme 1).

Our first attempts to prepare compounds like **4a,b** and **5a,b**, exploiting the above procedures, starting from QQ and



Scheme 1. Synthesis of substituted 5*H*-pyrido[2,3-*a*]phenoxazin-5-ones and of isomeric 5*H*-pyrido[3,2-*a*]phenoxazin-5-ones.

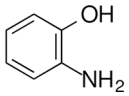
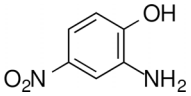
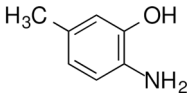
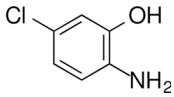
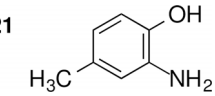
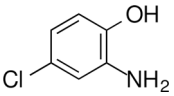
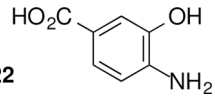
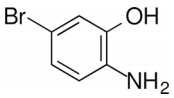
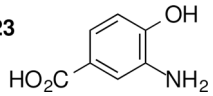
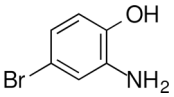
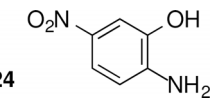
amino phenols carrying a carboxyl group, were totally unsuccessful and frustrating (cfr. Table 1). Several modifications of the experimental conditions, aimed at overcoming the problems encountered, were examined with no significant improvements.

Under these circumstances, we considered it worthwhile to report a new microwave (MW) assisted[10] simple and convenient synthetic procedure to obtain pyridophenoxazinones with high yields, very short reaction times and, most importantly, a drastically diminished production of black polymeric residues.

Therefore, a representative reaction where QQ **17** was combined with 2-aminophenol **19**, was carried out in microwave reactor DISCOUVER type from CEM company by varying the volumes of the solution (between 1 and 5 mL), and the ratio of substrates, microwave time (from 5 to 120 min) as well as power (80 °C internal probe, in the range 50–300 W). A continuous input power of 50 W and a reaction time of 30 min were found to give a very satisfactory yield (Table 1). The highest pressure achieved inside the probe was 16 PSI (1100 mbar).

The optimized procedure was then applied to miscellaneous 4- and 5-substitued 2-aminophenols (**20**-**29**) dissolved either in plain glacial acetic acid or in glacial acetic acid con-

Table 1. Comparison of yields and reaction times under classical (A and B) and microwave (A + MW and B + MW) assisted conditions for the synthesis of miscellaneous 5*H*-pyridophenoxazinones **1 a** and **1 b.**

2-PhOHs	Pathway[a]	[2,3-*a*] (%)	[3,2-*a*] (%)	2-PhOHs	Pathway[a]	[2,3-*a*] (%)	[3,2-*a*] (%)
19 	A	12	3	**25** 	A	4	2
	B	1	35		B	>1	15
	A + MW	78	8		A + MW	65	3
	B + MW	4	88		B + MW	7	71
20 	A	7	4	**26** 	A	13	>1
	B	3	30		B	1	26
	A + MW	59	10		A + MW	78	14
	B + MW	4	65		B + MW	4	87
21 	A	5	2	**27** 	A	11	1
	B	4	20		B	2	28
	A + MW	41	7		A + MW	71	21
	B + MW	3	54		B + MW	8	88
22 	A	2	0	**28** 	A	10	1
	B	0	>1		B	1	13
	A + MW	50	0		A + MW	58	15
	B + MW	1	50		B + MW	3	60
23 	A	3	0	**29** 	A	9	>1
	B	0	1		B	1	23
	A + MW	60	2		A + MW	70	13
	B + MW	>1	50		B + MW	16	70
24 	A	4	2				
	B	>1	8				
	A + MW	54	1				
	B + MW	1	55				

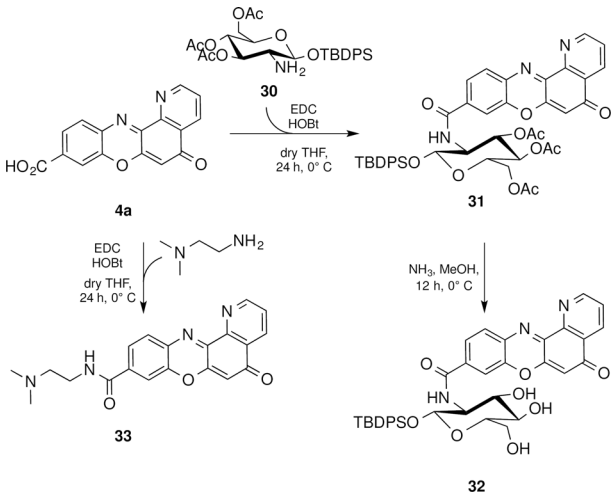
[a] Pathway A: QQ and 2-APhOH (1:1), glacial acetic acid, 24 h, reflux; Pathway B: QQ, 2-APhOH, and Co^{2+} (1:1:1), glacial acetic acid, 24 h, reflux; Pathway A + MW: QQ and 2-APhOH (1:1), glacial acetic acid, 80 °C (50 W), 30 min; Pathway B + MW: QQ, 2-APhOH, and Co^{2+} (1:1:1), glacial acetic acid, 80 °C (50 W), 30 min.

taining cobalt acetate in a stoppered glass tube. The glacial acetic acid was used as reaction medium because it represents a good compromise between the necessity of the carbonyl activation and the over-deactivation of the amine nucleophilicity.

The results reported in Table 1 (A *vs* A + MW and B *vs* B + MW) show clearly how the use of microwave heating leads to significant increases of the yields in all the cases investigated. In particular, it is noteworthy that APhOHs **22** and **23** gave the corresponding desired products (**4 a,b** and **5 a,b**) that could not be obtained at all by the classical synthetic procedures. All the evidence suggests that, under MW irradiation, the reaction proceeds by the same route followed under the conventional heating and that the MW probably helps the crucial step of dehydration (Scheme 1).

With the availability of such compounds we explored the coupling of PPXZ derivative **4 a** under standard conditions with properly protected sugar derivative **30.**[11,12] After removal of the acetyl protecting groups the final product **32** was obtained with overall yields of 63 %. Likewise the coupling of the derivative **4 a** was performed under the above conditions with the N^1,N^1-dimethylethane-1,2-diamine obtaining the product **33** in 81 % yields (Scheme 2). Both compounds, **32** and **33**, are currently under biological evaluation on human liquid- and solid-tumour cell lines.

In conclusion, we have proved that the use of microwave heating in the synthesis of the pyridophenoxazinone derivatives represents a sound innovation from a preparative point of view. To our knowledge this reaction represents a perfect starting point to expand the chemistry of iminoquinones as lead compounds for anticancer drugs. Finally, these results in addition to the ease of use, safety and rapid heating confirm microwaves as an attractive tool for synthetic chemistry as compared to conventional methods.



Scheme 2. Synthesis of new conjugate derivatives **32** and **33.**

Supporting Information available: Experimental procedures and characterization data of 26 compounds, ^{1}H and ^{13}C NMR.

Acknowledgements

All authors discussed the results and commented on the manuscript.

Keywords: Pyridophenoxazinones · Microwave Chemistry · Antiproliferative Compounds · Heterocycles

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