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ORIGINAL ARTICLE

Fluorescence Study of Imidazoquinoxalines

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Abstract The fluorescence properties of eleven novel derivatives based on the imidazo[1,2-*a*]quinoxaline structures have been studied. The absorption and emission spectra of these compounds have been recorded in dimethylsulfoxide solution. The phenyl substituting group on position 1 gives them particular properties thanks to the diverse hydroxy or methoxy decorating moieties, especially when they are multiplied or mixed. The investigated fluorescence auto-quenching revealed that the decreasing fluorescence intensity correlated only with the chemical structures of the aromatic compounds.

Keywords Imidazoquinoxaline · Imiquimod · Fluorescence · Absorption

Introduction

Heterocyclic compounds are known to have fluorescent properties and are therefore of interest in many fields such as molecular probes for biochemical research [1, 2], fluorescent whitening agents [3, 4], photoconducting material [5] and electroluminescent devices [6]. Because of their high quantum yield, quinolines are fluorescent compounds [7] used for example as probes in chemosensors. [8] Quinoxaline derivatives are used for example as base for probes in the field of bio-imaging. [9.]

Imiquimod, the international nonproprietary name of 1-isobutyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (Scheme 1) is the analogue of our entire synthetized molecules. [10–13] This compound was already the subject of experimental and theoretical fluorescent studies. [14, 15] Its relatively high fluorescence intensity results from an extended π-conjugated system.

In the course of our studies concerning the synthesis of imidazo[1,2-*a*]quinoxaline derivatives as potential anticancer compounds, we were confronted to the peculiar fluorescence response of our compounds incubated with cancerous cells. [16] In fact, a cytometric study using propidium iodide revealed fluorescence more important than predicted (data not shown). Thus, in order to determine whether the signal increase is due to our organic molecules, we were consequently interested by a fluorescence study of our compounds. We present herein briefly the synthesis of our molecules and their absorption and emission spectra.

Experimental Part

Chemistry

The synthetic pathway and the structures of the imidazo[1,2-*a*]quinoxaline derivatives used in this study are given respectively in Schemes 2 and 3. Compounds were synthesized thanks to a route we previously described [11, 13, 16]. Briefly, the carbonylimidazole dimer **2** results from the condensation of the 2-imidazole carboxylic acid **1** in presence of thionyl chloride. Addition of the *o*-fluoroaniline on the dimer **2** gives the intermediate **3**. Cyclisation is

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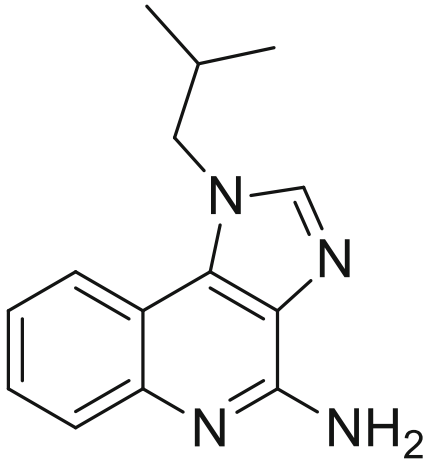


Scheme 1 Chemical structures of Imiquimod

allowed by using sodium hydroxide in dimethylacetamide. Treatment of compound **4** with phosphorus oxychloride and *N,N*-diethylaniline gives the chlorinated compound **5**. Then, an amino group substitutes the chlorine. To obtain the $NHCH_3$ residue **6**, methylamine in ethanol is grafted under microwave asssitance. To give the NH_2 residue, ammonia is used in acetonitrile also under microwave asssitance. The bromination of the intermediate **6** by *N*-bromosuccinimide led to compound **7**. Appropriate aryl boronic acids are introduced in position 1 via a Suzuki-Miyaura cross-coupling reaction to furnish compounds **8**. Some intermediates were submitted to boron tribromide to give the hydroxylated derivatives **9**.

Methods

Absorption spectra were recorded on a UV Visible spectrophotometer (Cary 400, Varian) from 250 to 400 nm. Fluorescence spectra were recorded on a Fluorescence spectrophotometer (Cary Eclipse, Varian) from 300 to 600 nm.

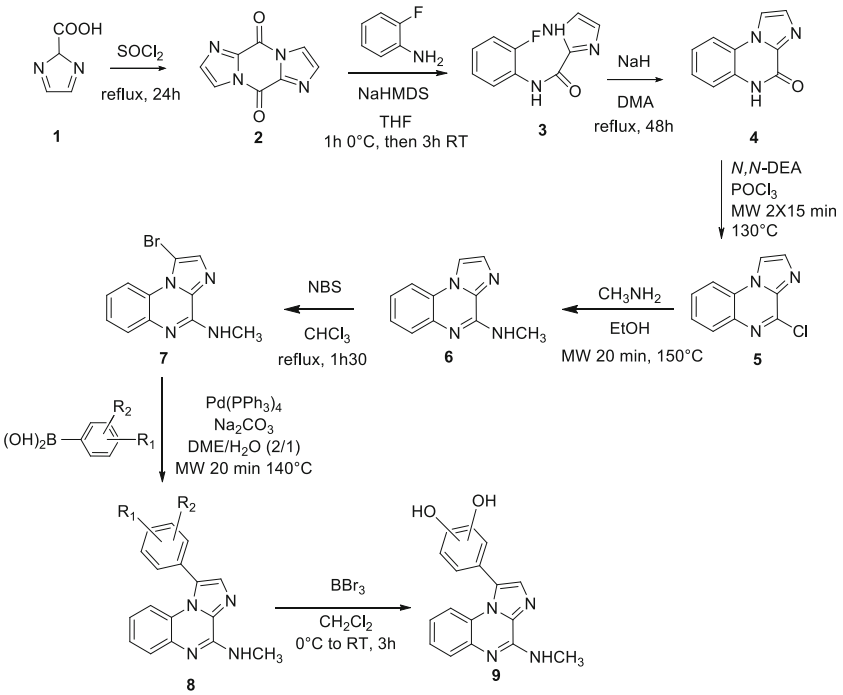
Solutions at 1.10^{-2} M of each compound were prepared in DMSO. These working standard solutions were then diluted with DMSO to obtain 1.10^{-4} M as final concentrations.

Results and Discussion

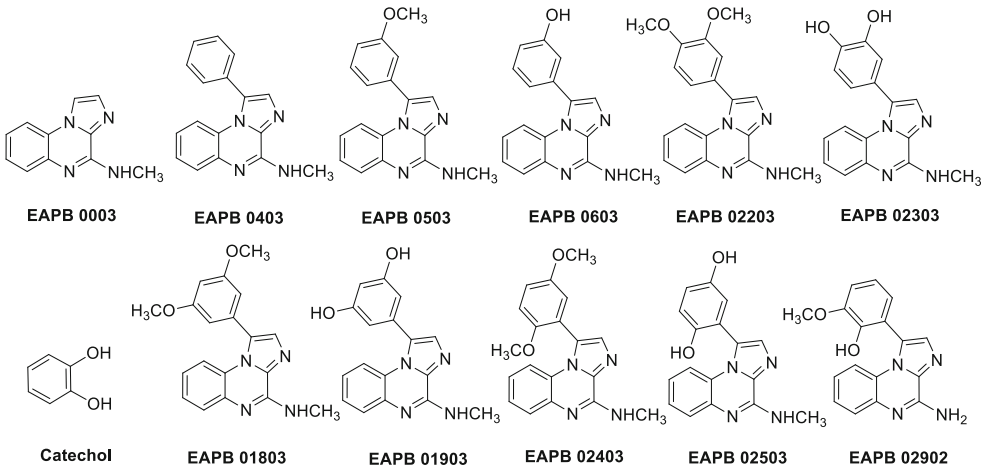
In a first study, UV spectra of all substituted compounds at 1.10^{-4} M were recorded (Fig. 1). Higher concentrations caused absorbance saturation for some molecules. Lower concentrations gave no analyzable results for some compounds. Spectra show similar profiles with maxima around 270 nm which is characteristic of the phenol moiety, more particularly here of the catechol part, then a decrease until 300 nm. Successive waves with secondary maxima could be observed around 320 nm. It seems to be a characteristic of the imidazo[1,2-*a*]quinoxaline, as depicted by compound EAPB 0003. No absorbance was detected after 360 nm in the visible region.

A slight tone could be done for compounds EAPB 02503 and EAPB 02902, which first maxima are closer than 260 nm. Nevertheless, they also display second maxima at 270 nm.

Scheme 2 General synthetic pathway



Scheme 3 Compounds structures



Maxima at 270 nm for compounds EAPB 02203 and EAPB 02303 should be nuanced. Even if they absorb in this region, the maxima are less marked than the other compounds and correspond to a steady state. The decrease is evident after 290 nm.

The substitute-devoid compound EAPB 0403 absorbs slightly less than the mono-hydroxylated compound EAPB 0603. Absorbance of the mono-methoxy derivative EAPB 0503 is quite divided by two between 260 nm and 330 nm.

Whereas absorbance for compounds EAPB 02403 and EAPB 02503 are equivalent, it is not the case for the others. Compounds with two methoxy groups, EAPB 01803 and EAPB 02203, absorb more than their respective di-hydroxy derivatives EAPB 01903 and EAPB 02303. Despite being less intense, compound EAPB 2902 shows a similar profile than compound EAPB 2503.

According to the UV spectra results, fluorescence spectra were recorded by excitation at 270 nm (Fig. 2) for all compounds at 1.10^{-4} M. As already observed for the absorbance study, compounds EAPB 0403, EAPB 01813 and EAPB 0603 show higher fluorescence intensities than compounds EAPB 0503, EAPB 02403 and EAPB 01903. They display the same maximum at around 395 nm. Fluorescence spectra of compounds EAPB 02203 and 02303 are shifted to the higher wavelengths. The maximum is around 420 nm for EAPB 02203 and around 445 nm for EAPB 02303. The fluorescence intensity is divided by three between those two compounds. In a general way, the di-hydroxy compounds show lower fluorescence intensities than their respective di-methoxy derivatives.

Compounds EAPB 02503 and EAPB 02902 show a very low fluorescence intensity when excited at 270 nm or 260 nm (first maxima in their absorbance spectra).

The fluorescence intensities are clearly different for these compounds of the same chemical family tested at the same concentration. Those results suggest a kind of auto-quenching phenomenon for EAPB 02303, EAPB 02902 and EAPB

Fig. 1 UV spectra

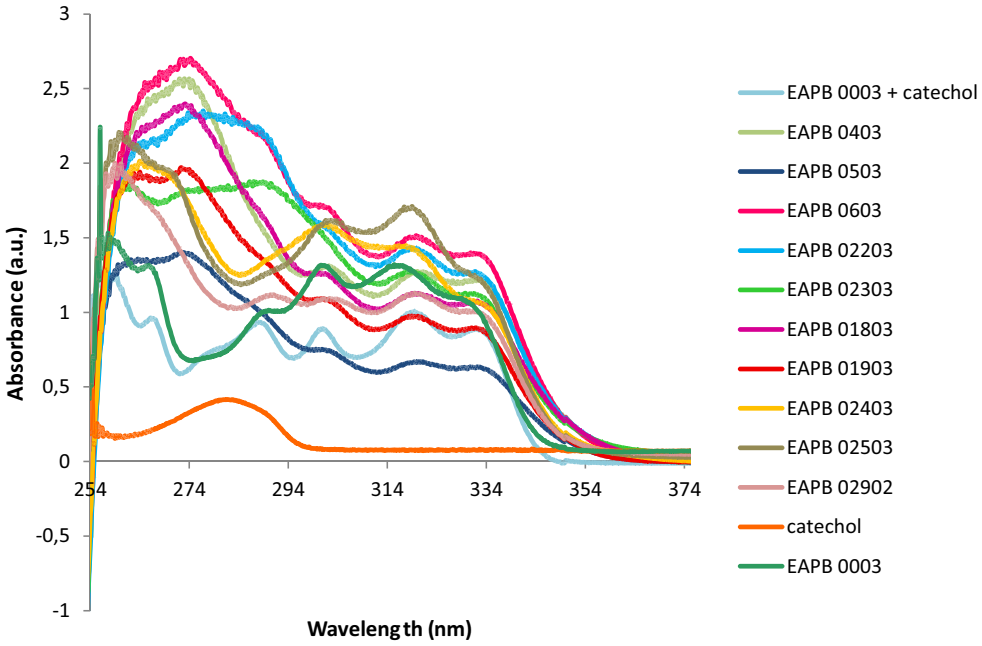


Fig. 2 Fluorescence spectra

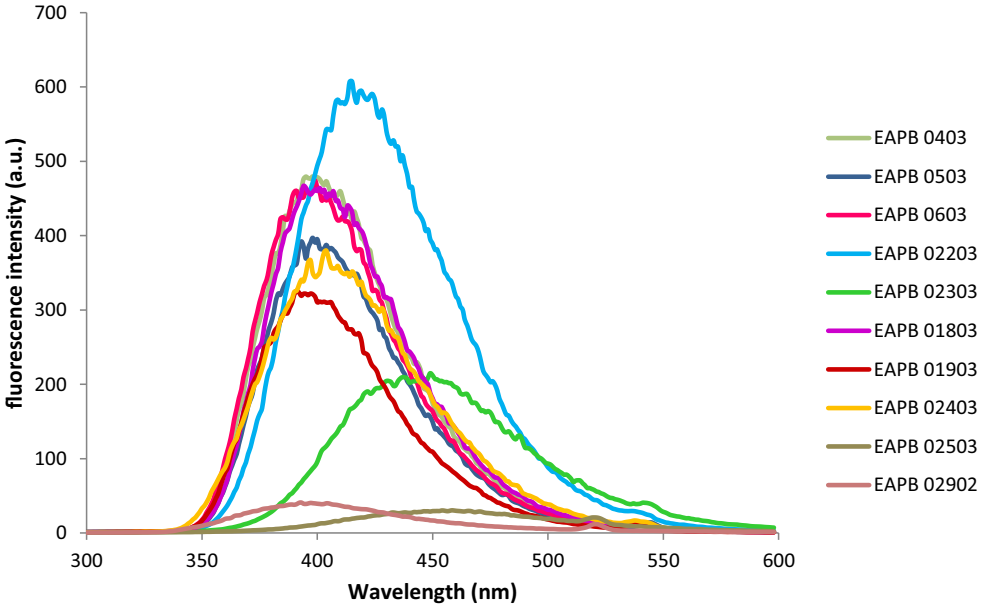


Fig. 3 Fluorescence Spectra of EAPB 02902

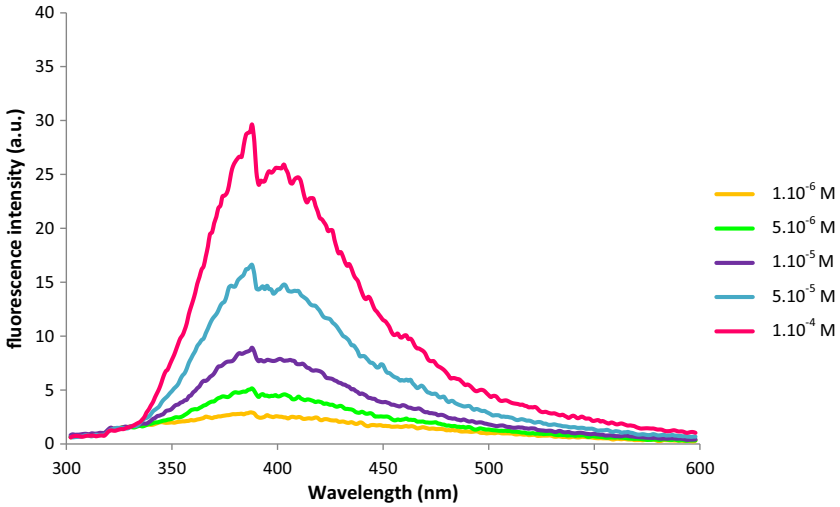
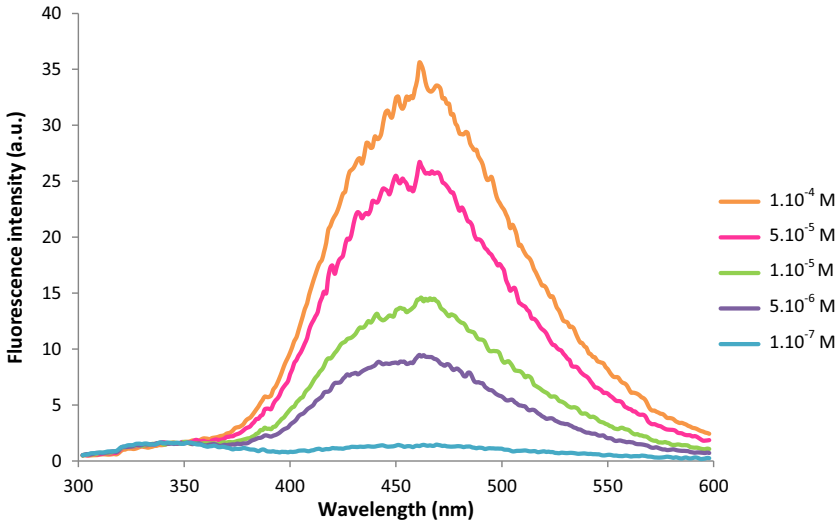


Fig. 4 Fluorescence Spectra of EAPB 02503



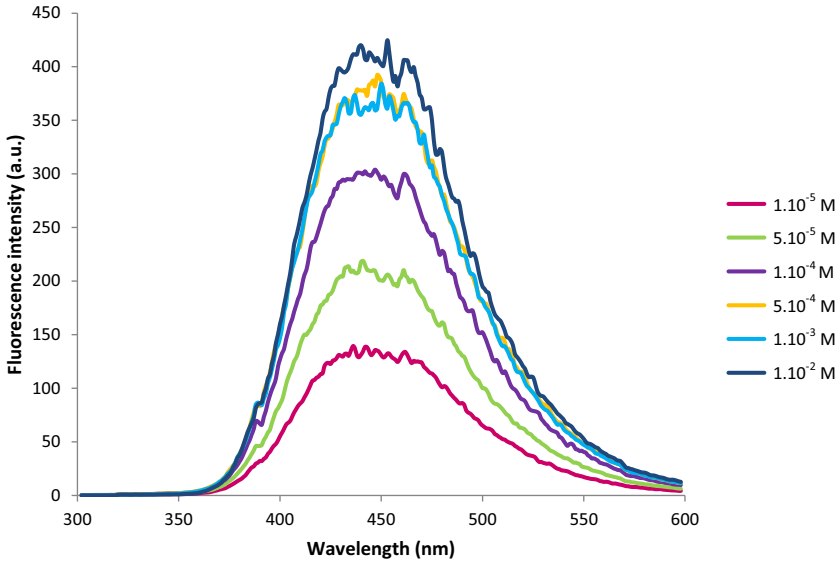


Fig. 5 Fluorescence Spectra of EAPB 02303

02503. Catechols are known to behave as quencher, thanks to their ability to absorb photon energy, resulting in their oxidation [17–19]. Fluorescence quenching is easily characterized by a decrease of fluorescence with increase substrate concentration. To explore the quenching properties of some of our compounds, concentrations range in dimethylsulfoxide have been prepared for EAPB 02902, EAPB 02503 and EAPB 02303, ranging respectively from 1.10^{-6} M to 1.10^{-4} M, 1.10^{-7} M to 1.10^{-4} M and 1.10^{-5} M to 1.10^{-2} M. Their fluorescence spectra are recorded and depicted respectively on Fig. 3, Fig. 4 and Fig. 5. The limit of detection for EAPB 02902 and EAPB 02503 correspond to 1.10^{-7} M. We observe on these spectra that fluorescence intensities increase with the concentration. So, there is no quenching of our compounds in the concentrations used for biological experiments.

Conclusion

UV and fluorescence spectra of eleven compounds based on the imidazo[1,2-*a*]quinoxaline structure are presented here. Even if all of these compounds refer to the same chemical family, differences were observed in their absorbance and emission responses, particularly the intensities. In our conditions, no auto-quenching fluorescence was observed for these compounds. The observed decreasing fluorescence intensity is only correlated to the particular structures and polyhydroxy substitutions of the aromatic studied compounds.

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