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# Microwave-assisted synthesis and structure–activity relationships of neuroactive pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine derivatives

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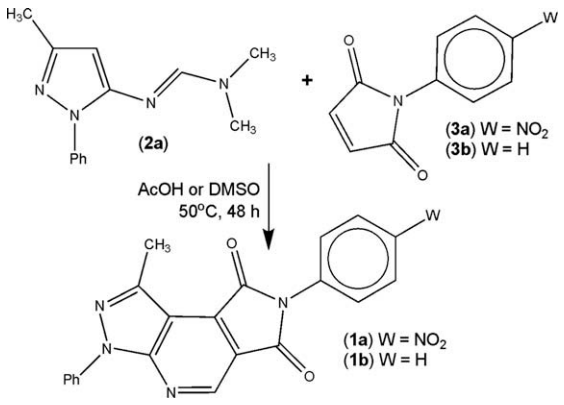
ABSTRACT

We described herein the optimization of the synthetic methodology exploited to obtain the pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine sedative prototype **1a** and novel analogues designed by successive molecular simplifications. By applying microwave irradiation during the hetero Diels-Alder key-step to obtain the heterotricyclic scaffold, under solvent-free conditions, we were able to obtain the desired compounds in drastically shorter times and better yields. Additionally, in vivo evaluation of the sedative effects of these heterocyclic derivatives showed that **1a** and the novel structurally-related analogue **1e** were the most efficient compounds to impair the locomotor activity in mice at the dose of 10 μmol/kg.



Among the most frequently used therapeutic classes, the CNS-acting drugs correspond to ca. 15% of the total, with worldwide sales of around U$ 118 billion in 2008.[1] In this context, many efforts to develop novel sedative/hypnotic drugs, presenting increased potency and reduced side effects, have been made in order to assure the availability of suitable alternatives for the treatment of sleep-disorders.[2] Previous works from our research group have described the discovery of two novel sedative and analgesic agents (**1a**) and (**1b**),[3] belonging to the pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine series, which displayed their CNS actions as muscarinic $M_1$ receptor agonists.[4] Although these heterotricyclic compounds presented remarkable neuroactive profile, further preclinical studies were strongly limited by the synthetic approach used to construct them. The functionalized pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine derivatives (**1a**) and (**1b**) were prepared in very low yields (20–35%), after long reaction time (48 h), through hetero Diels–Alder cycloaddition between the formamidine (**2a**) and the corresponding *N*-phenylmaleimide (**3**), under conventional heating,[3] using AcOH or DMSO as solvent (Scheme 1).

Hetero Diels–Alder reactions between pyrazolyl-2-azadienes and nitroalkenes,[5] propiolates or maleimides[6] using conventional heating are known to be difficult due to the hard conditions required and either do not occur or take a long time to produce the desired product in low yields.[3,7]




**Scheme 1.** Synthesis of heterotricyclic derivatives (**1**) exploiting hetero Diels–Alder reaction at the key-step, by using conventional heating.

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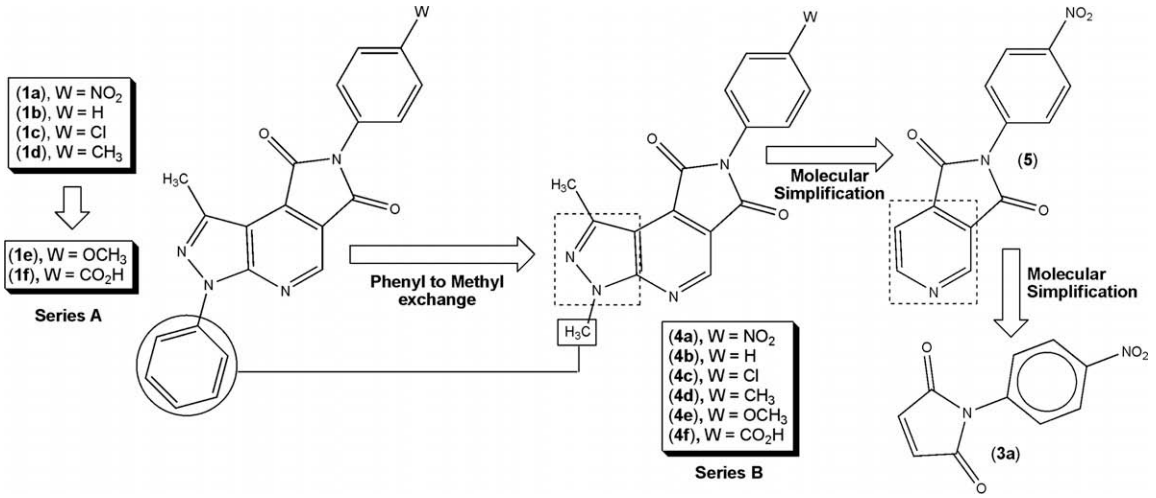



**Figure 1.** Design concept of pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine derivatives of series A (**1**), B (**4**), and their simplified analogues (**5**) and (**3a**).

Alternatively, the use of microwave irradiation under solvent-free conditions has proved to dramatically improve the process for obtaining new heterocyclic scaffolds exploiting Diels–Alder reaction as the key-step.[5]

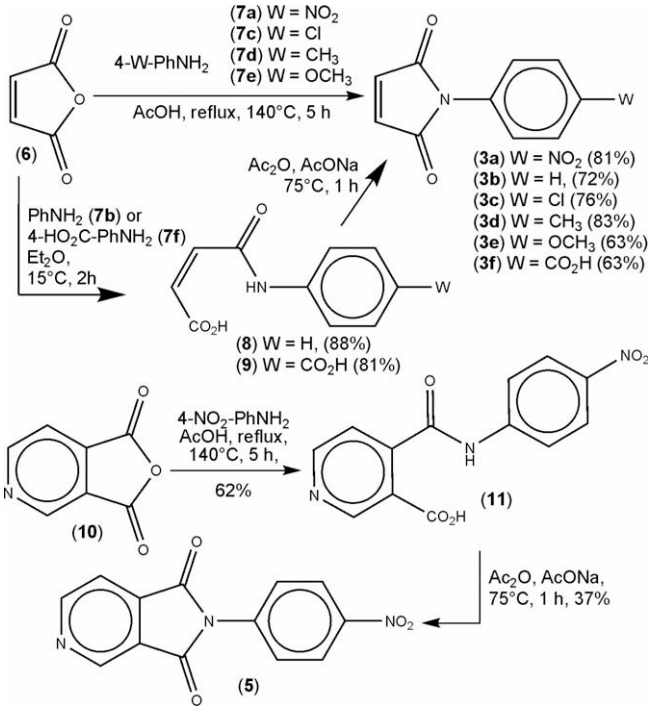
Considering this panorama, we described herein the optimization of the synthetic route to obtain the pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine derivative (**1a**) and some previously described analogues (**1b–1d**) using microwave-assisted synthesis for the hetero Diels–Alder step under solvent-free conditions. Thus, we propose the enlargement of the congeneric series by the introduction of the isosteric carboxyl group and the electron-donating methoxy substituent at the W position (series A, Fig. 1).

Moreover, we exploited the developed methodology to synthesize a new series of simplified heterocyclic analogues (**4a–4f**) designed by changing the pyrazole-attached *N*-phenyl by a *N*-methyl group in order to investigate the stereoelectronic and the lipophilic influences at this position on the sedative profile of pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine derivatives (Fig. 1).

On the other hand, the exploitation of successive molecular simplifications on the structure of sedative prototype (**1a**) led us to the design of the 3-pyridinylphthalimide (**5**) and 4-nitrophenylmaleimide (**3a**) derivatives, as a result of the suppression of the pyrazole and the pyridine rings (Fig. 1). The comparative evaluation of the sedative profile displayed by these heterocyclic derivatives on the locomotor activity in mice[8] provided a better understanding of structure–activity relationships associated with their CNS actions.

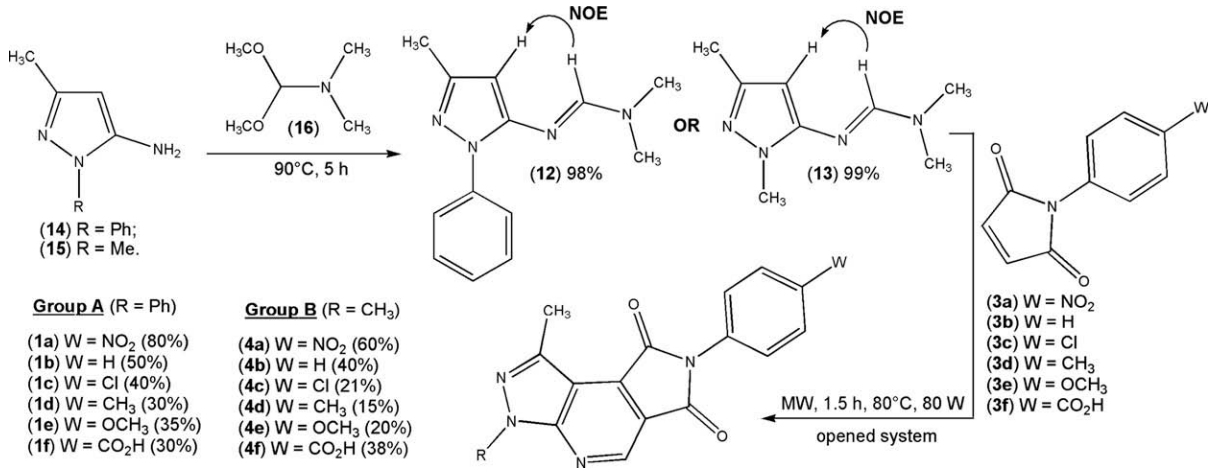
Initially, the six functionalized *N*-phenylmaleimides (**3a–3f**) were prepared starting from maleic anhydride (**6**) and the corresponding *para*-substituted anilines (**7**).

In order to obtain the desired *N*-phenylmaleimides (**3**), the different behavior of this reaction, determined by distinct solubility and nucleophilicity of the exploited anilines, was taken into consideration. Thus, only the *N*-phenylmaleimides (**3a–3d**) were prepared, in yields ranging from 63% to 83%, by refluxing a mixture of (**6**) and (**7**) in acetic acid[9] (Scheme 2). On the other hand, the attempt to prepare the unsubstituted *N*-phenylmaleimide (**3b**) and the 4-carboxyphenylmaleimide (**3f**) under the same conditions led to the formation of the carboxy-amide intermediates (**8**) or (**9**), respectively, accompanied by other undesired subproducts. To avoid this problem, another two-step methodology was selected to prepare these two *N*-phenylmaleimides, using ethyl ether as solvent in the first step, followed by cyclization of the obtained carboxy-amide derivatives (**8**) and (**9**) after treatment with sodium acetate in acetic anhydride[10] (Scheme 2). Azaphthalimide derivative (**5**) was prepared in 23% overall yield (2 steps) through the initial condensation of 3,4-pyridinedicarboxylic anhydride (**10**) and *para*-nitroaniline (**7a**) in acetic acid at reflux, followed by cyclization of the carboxy-amide (**11**) with AcONa in acetic anhydride (Scheme 2).




**Scheme 2.** Synthesis of the functionalized *N*-phenyl maleimides (**3a**–**3f**) and *N*-phenyl azaphthlimide (**5**).

The azadienes (**2a**) and (**2b**) were prepared as described previously,[3] in almost quantitative yields through the condensation of the corresponding pyrazolamines (**12**) or (**13**) and DMF dimethylacetal (**14**) (Scheme 3). The relative configuration (*E*) at imine double bond of azadienes (**12**) and (**13**) was determined by $^1$H NMR, through the irradiation of the C-4 attached pyrazole hydrogen and the evidence of a NOE effect at imine hydrogen (Scheme 3). This configuration is important to assure the formation of hetero Diels–Alder adduct presenting antiperiplanar orientation between the *N*,*N*-dimethylamino group and the vicinal hydrogen able to




**Scheme 3.** Synthesis of pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine derivatives (**1a**–**1f**) and (**4a**–**4f**) exploiting hetero Diels–Alder reaction under microwave irradiation in solvent-free conditions.

produce the desired heterocyclic derivatives (**1**) and (**4**) after elimination and oxidative aromatization steps.[7]

Next, in order to optimize the hetero Diels-Alder key-step we proceeded to the investigation of cycloaddition between (**2a**) and (**3a**) under microwave irradiation and solvent-free conditions, starting with 80 W, at 50 °C for 30 min.

The microwave tube used was exposed to atmospheric oxygen throughout the experiments to favor the oxidative aromatization step after Diels–Alder adduct formation.[7] Besides these initial conditions, the temperature was systematically increased from 50 to 60, 70, 80 and 90 °C, but (**1a**) was obtained in highest yield (35%) at 80 °C. When the temperature was 90 °C and/or the power was changed to 90 W, degradation of (**4**) was detected by TLC. After setting the power to 80 W, the temperature at 80 °C and extending the time of the reaction to 60, 90 and 120 min, (**1a**) was obtained in 50%, 80% and 75% yields, respectively, after work-up by washing with warm MeOH (ca. 60 °C). All other heterocyclic derivatives of series A (**1b–1f**) and B (**4a–4f**) were synthesized following the same methodology (80 W, 80 °C, 90 min., Scheme 3) and their structures are in agreement with analytical and spectral data.

The sedation produced by derivatives (**1a–1f**), (**4a–4f**) and simplified analogues (**5**) and (**3a**) was investigated using locomotor activity test in mice[8] (Table 1). The intraperitoneal administration of a screening dose of 10 μmol/kg for all these compounds revealed that *para*-nitro derivative (**1a)** and the novel *para*-methoxy derivative (**1e**), both from series A, presented the highest sedative activity, being able to reduce the number of movements/minute of treated animals from 214.4 ± 18.9 to 87.6 ± 16.2* and 108.6 ± 14.9* mov/min., respectively. These values are statistically equivalent to the reference drug, midazolam (Table 1). Even though the nitro and the methoxy groups at the W position (Fig. 1) exhibit different electronic characteristics, compounds (**1a**) and (**1e**) did not present significant differences in sedative activity probably due to the capability of these two groups, potential pharmacophoric points, to be recognized as H-bond acceptors with complementary residues of occasional target bioreceptors. This interaction seems to be essential for the effect investigated, once the corresponding unsubstituted compound (**1b**) and the two *para*-substituted derivatives (**1c**) and (**1d**) were not able to affect significantly the motor activity in animals. An exception to this behavior was observed in carboxyl derivative (**1f**), which did not impair the locomotor activity in animals, probably due to pharmacokinetic factors resulting from its ionization in vivo. As evidenced for compound (**1a**), the CNS effects of (**1e**) were reversed by the pre-treatment of animals with atropine, indicating that it could also act as a muscarinic agonist.[4]

**Table 1**
Effects of midazolam, pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine derivatives (**1a–1f**), (**4a–4f**) and the simplified analogues **5** and **3a** on the locomotor activity in mice[8]

| Compound | Series | Motor activity[a] (mov/min) |
|---|---|---|
| Control | — | 214.4 ± 18.9 |
| Midazolam[b] | — | 79.7 ± 25.1* |
| **1a** | A | 87.6 ± 16.2* |
| **1b** | A | 266.6 ± 30.6 |
| **1c** | A | 154.3 ± 24.3 |
| **1d** | A | 161.7 ± 29.2 |
| **1e** | A | 108.6 ± 14.9* |
| **1f** | A | 174.2 ± 21.8 |
| **4a** | B | 141.0 ± 25.8 |
| **4b** | B | 208.6 ± 11.4 |
| **4c** | B | 133.4 ± 16.3 |
| **4d** | B | 187.4 ± 23.0 |
| **4e** | B | 150.6 ± 15.7 |
| **4f** | B | 128.3 ± 20.6 |
| **5** | — | 189.0 ± 16.76 |
| **3a** | — | 148.0 ± 13.85 |

[a] All derivatives were administered ip (10 μmol/kg) and motor activity was determined during 40 min after injection. Data are expressed as means of the movements per minute ± SEM.
[b] Administered at the dose of 2 mg/kg.
* $P$ <0.05 relative to the control group (DMSO) (statistic test: ANOVA followed by Dunn's test).

In addition, the compounds of series B, presenting a methyl group instead of a phenyl subunit attached to the N-1 position of the pyrazole ring, were able to display no significant sedative activity. Only derivatives **4c** (133.4 ± 16.3 mov/min) and **4f** (128.3 ± 20.6 mov/min) presented slight ability to reduce the locomotor activity in mice. These results highlight the possible influence of the phenyl group bonded to the pyrazole ring of the pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine system to potentiate the sedative profile of these heterotricyclic derivatives. This behavior was confirmed through the comparative evaluation of the sedative activity of four nitro derivatives presenting sequential molecular simplifications—**1a** (87.6 ± 16.2* mov/min), **4a** (141.0 ± 25.8 mov/min), **5** (189.0 ± 16.76 mov/min) and **3a** (148.0 ± 13.85 mov/min)—which have indicated that all subunits contained in the scaffold of series A seem to be important for the observed remarkable effects of compound (**1a**) on the locomotor activity test.

As concluding remarks, the optimization of the experimental conditions exploited to access heterocyclic derivatives presenting pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine scaffold, through microwave-assisted hetero Diels–Alder reaction, permitted their synthesis in higher yields and shorter times in comparison with the conventional heating strategy previously described. The molecular

simplification study confirmed that almost all the subunits present in the structure of derivatives of series A are crucial to promote the sedative effect, as it was observed for derivative **1a** and the novel analogue **1e**. Taking together, these results make the multigram synthesis of prototype **1a** and its analogues possible, enabling the next steps towards the development of these heterotricyclic derivatives into innovative sedative agents.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.038.

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