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Efficient microwave combinatorial synthesis of novel indolic arylpiperazine derivatives as serotoninergic ligands

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ABSTRACT

An easy and convenient microwave-assisted synthesis of a small library of indolic arylpiperazine derivatives is described. Parallel and mixed pool combinatorial methods are reported and compared. The described reactions are nucleophilic substitutions of several aromatic piperazines in presence of K_2CO_3. Good yields and short reaction times are the main aspect of these procedures. Binding assays shed additional light on the influence of the LCAPs on the 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors affinity and allowed to disclose three interesting compounds as 5-HT_{2C}, mixed 5-HT_{2A}/5-HT_{2C} and 5-HT_{1A}/5-HT_{2C} ligands (**4i**, **4l** and **4d**, respectively), with potential antiepileptic, anxiolytic or atypical antipsychotic agent therapeutical profiles.

1. Introduction

Over the last decade, microwave-assisted chemistry has become an important tool that can accelerate drug discovery within industry and academia. Shifting the focus from screening mixtures to a single compound libraries, it proved to be a powerful technique for medicinal chemists for either hit identification or lead refinement [1]. Long-chain arylpiperazines (LCAPs) are a class of molecules of considerable pharmaceutical interest. They bind to many classes of G-protein-coupled receptors (serotoninergic, dopaminergic, adrenergic) and produce a variety of pharmacological responses. In the past years our outmost attention was focused on 5-HT_{1A} receptor [2], since its role in the pathology of such mental disorders as anxiety or depression has been well-established [3]. In particular, 5-HT_{1A} receptor, belonging to the superfamily of G-protein-coupled receptors and negatively coupled to adenylyl cyclase, was found in high concentration in the limbic system, where it is thought to play a role in emotional processes and represents a major target for research and drug development due to its implication in the pathophysiology and treatment of major neuropsychiatric disorders, including depression, schizophrenia and anxiety. Moreover, 5-HT_{2A} receptor is known to play a key role in the action of psychedelics as well as being a therapeutic target for the treatment of schizophrenia [4]; finally 5-HT_{2C} receptor is considered to be an attractive target for the design of novel drugs for treatment of CNS-related diseases such as obesity, obsessive compulsive disorder, and sexual dysfunction, even if few 5-HT_{2C} selective agonists are known so far [5].

Structure-activity relationship (SAR) studies, performed with numerous generations of arylpiperazine derivatives, showed that CNS activity and receptor affinity and selectivity depend on the N-1-aryl substituent, the terminal fragment and the alkyl spacer length. To get direct and easy access to this class of molecules, we developed and optimized a microwave-assisted synthesis of a 12-members arylpiperazine small library characterized by an indolic nucleus. The indole substructure is a basic element for a number of biologically active natural and synthetic products. In fact, until today, there have been more than 400 drugs and 3000 patents in which the indole motif has been present; the range of applications for these therapeutically relevant compounds includes anti-inflammatory drugs, protein kinase C inhibitors, 5-HT agonists and antagonists, melatonin agonists and glucocorticoid receptor modulators [6].

In order to explore its influence on the serotoninergic activity, 5-hydroxy-1H-indole-2-carboxylic acid has been linked to some of

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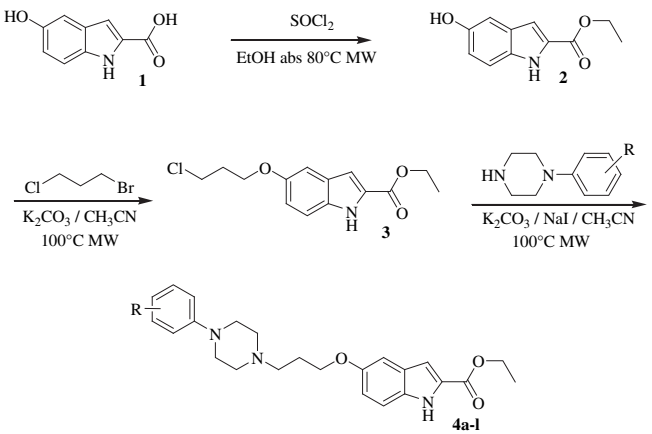
the most thoroughly studied aryl-substituted piperazines via a three methylene spacing unit. In recent years our interest has been devoted to the application of microwave in the syntheses of pharmacologically active peptidomimetic [7] or heterocyclic derivatives [8]. In this paper we describe the application of this technology for the fast generation of a small library of indolic arylpiperazines in parallel and mixed pool combinatorial methods. The application of microwave energy to organic compounds in order to perform synthetic reactions at highly accelerated rates has become a well-known technique [9]. Microwave technology, indeed, has become very popular among synthetic organic chemists both to improve classical organic reactions, shortening reaction times and/or improving yields, as well as to promote novel efficient synthetic routes. The developed procedure, particularly, in the last step, allows the obtaining of the complete arylpiperazines library with a single reaction and purification step and this can facilitate the quick screening of a greater number of synthetic analogues in the identification of a lead structure.

2. Chemistry

The synthesis of compounds **4a–l** is summarized in Scheme 1.

5-Hydroxy-1H-indole-2-carboxylic acid **1** was converted to the corresponding ethyl ester **2** by treatment with thionyl chloride in ethanol under reflux; the reaction with 1-bromo-3-chloropropane, in presence of K_2CO_3 in acetonitrile, gave the corresponding chloro-alkyl indolic derivative **3**. These reactions were carried out using a microwave oven (ETHOS 1600, Milestone®) especially designed for organic synthesis placing reagents and solvents in a sealed reactor specific for high pressure reactions. The subsequent condensation of compound **3** with the opportune substituted arylpiperazine, performed in acetonitrile in presence of K_2CO_3 and NaI, was carried out in parallel using sealed tubes fitted in the 36 positions of a multiPREP rotor (Milestone®); the remaining tubes were filled with the same amount of the reaction solvent (acetonitrile). The synthetic procedure was performed following a microwave program which was composed by appropriate ramping and holding steps. The temperature of the stirred reaction mixture was monitored by an IR probe and rotation of the rotor, irradiation time and power were monitored with the "easyWAVE" software package. The reactions provided the final compounds **4a–l**.

The piperazine coupling reaction was performed at 100 °C with 300 W, in acetonitrile for a total time of 1 h. This condition was found to be the optimized one because higher temperatures, times or power gave no increase in the obtained yields or resulted in decomposition of the reagents. The aryl-substituted piperazines (**a–l**) have been selected in order to consider different steric, hydrophobic and electronic features useful to develop a small library of indolic arylpiperazine derivatives with different physicochemical properties. The structures of the employed arylpiperazines are depicted in Fig. 1.



Scheme 1. Synthetic procedure for the synthesis of **4a–l**.

The main advantage of this synthetic route is that a short irradiation time of the reaction mixtures provided the compounds **4a–l** as the major products.

As starting point for a combinatorial mixed pool procedure, a small amount of pure previously obtained compounds **4a–l** was mixed and several analytical RP-HPLC elution gradients were evaluated to optimize a complete separation. The best condition for analytical determination was found to be the following one, carried out by two solvent systems: A: 0.05% TFA (v/v) in acetonitrile; B: 0.05% TFA in H_2O (linear gradient from 20% to 40% A over 60 min, UV detection at 254 nm, flow rate 1 mL/min). The obtained chromatographic profile showed sufficient resolution between the compounds to undergo preparative purification (Fig. 2).

On the basis of the obtained results, a mixed pool synthetic procedure was developed as a one-pot reaction: the chloro-alkyl ethyl ester **3** was reacted with the twelve arylpiperazines **a–l** in acetonitrile in presence of K_2CO_3 and NaI, heating the mixture by microwave irradiation with 300 W at 100 °C, for a total event time of 90 min. The reaction mixture was filtered to remove the potassium carbonate, subjected to an extraction with brine and was purified by preparative RP-HPLC applying the same gradient used for the analytical determinations. The crude analytical HPLC chromatographic profile was similar to the previously obtained (Fig. 3) with sufficient separations of the final compounds. High-reaction-temperature conditions achieved by microwave irradiation in acetonitrile allowed the synthesis to be carried out in one-pot to obtain the desired compounds **4a–l**.

3. Results and discussion

Results relative to the synthesis of the compounds **4a–l>** are summarized in Table 1 and show that there is not a significant difference between yields obtained in parallel or mixed pool method; a small reduction in yields is evidenced in mixed pool synthesis, but it can be addressed to the more complexity of the crude mixture in which the piperazine reactivity plays a dominant role.

On the basis of these results it's possible to state that the application of microwave irradiation and the simultaneous presence of K_2CO_3 and NaI improve the yields and significantly reduce reaction times in the synthesis of arylpiperazine derivatives, either in parallel or mixed pool combinatorial methods and this procedure could be applied in future for the generation of larger libraries of arilpiperazine derivatives to be evaluated for their biological properties.

The twelve piperazine derivatives (**4a–l**) were evaluated for their activity and selectivity towards 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors and some of the molecules showed affinity in nanomolar range towards these receptors (Table 2). Moreover, the multi-receptor profiles of promising derivatives were also evaluated in terms of binding affinities for dopaminergic (D_1, D_2) and adrenergic (α_1, α_2) receptors (Table 3).

The outstanding 5-HT_{1A} receptor affinity of compound **4g** (K_i = 35.8 nM), that showed a good nanomolar affinity on the 5-HT_{1A} receptor in conjunction with a good selectivity on the 5-HT_{2A} and 5-HT_{2C} receptors, is of particular interest; compound **4i** (K_i = 50.4 nM) showed the most interesting selectivity profile with a good affinity towards 5-HT_{2C} receptor, whereas compound