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Design, synthesis and biological evaluation of novel sigma ligands

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This resarch was focused on the rational design, synthesis and pharmacological evaluation of novel sigma1 and sigma2 ligands as potential drugs for CNS-related disorders and cancer diseases. With this aim, four different series including 21 arylalkenylamines, a library of 64 arylalkenylamides, 36 arylalkenylaminoalkylamines and 24 indolylalkylbenzylamines were designed taking into account either molecular modeling suggestions or SAR evaluations of known sigma ligands. The synthetic procedures developed allowed us to obtain the selected compounds of each series in high purity suitable for biological screening. Assayed compounds showed very promising results for both subtypes, with the highest affinity values in a subnanomolar and nanomolar range for sigma1 and sigma2 receptors respectively.

1 Introduction

Although sigma receptors were discovered about 30 years ago as a subtype of opioid receptors, the importance of their biological properties is not yet completely clarified [1]. It has been accepted that sigma receptors are a unique family of proteins different from opioid, N-methyl-D-aspartate (NMDA) and phencyclidine receptors [2]. Pharmacological studies have identified two distinct subtypes of sigma receptors, sigma 1 and sigma 2, expressed in various areas of the brain and in peripheral organs [3] The sigma1 subtype can be considered an important intracellular regulatory protein since it can be translocated, when activated, from the endoplasmic reticulum, where it is present under resting conditions [4]. Many transduction systems (glutamatergic, cholinergic and catecholaminergic) are sensitive to sigma1-mediated neuromodulation [5]. Both receptor structure and endogenous ligands of sigma1 receptor have not been yet identified. The sigma2 subtype has not yet been cloned [6]. Sigma2 receptor activation induces several biological effects including changes in cell morphology and apoptosis producing both transient and sustained increases in calcium ions [7].

The important roles played by sigma receptors in certain biological systems suggest that the development of high-selective sigma ligands may provide potential drugs for CNS-related disorders and cancer diseases, since both sigma receptor subtypes are distributed within the central nervous system (particularly sigma1) and over-expressed in several tumor cell lines (particularly sigma 2) [3]. Sigma1 receptor neuromodulation could be the target of therapeutic strategies in many neurodegenerative diseases. Sigma2 receptor antagonists have been demonstrated to limit the motor extrapyramidal side effects caused by typical antipsychotic agents and attenuate convulsions caused by cocaine. Interestingly, sigma2 agonists could be used as novel antineoplastic agents; furthermore, sigma2 selective ligands could be used as imaging agents for measuring the proliferative status of breast tumors *in vivo* by PET analysis [8].

There are published reports on several highly selective sigma1 ligands synthesized to gain SAR information for a more detailed understanding of the pharmacological function of the sigma1 protein. In contrast, the sigma 2 receptor suffers from a lower degree of knowledge, because of the lack of high-affinity ligands which generally display a poor selectivity profile particularly over sigma1 receptor [9].

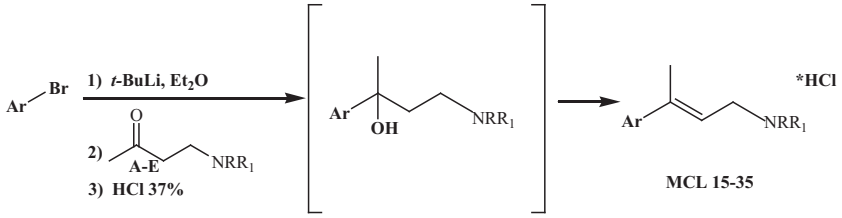

Fig. 1: General procedure for the synthesis of compounds **MCL 15-35** (Series I).

2 Design and synthesis of novel sigma ligands

In the last years, our research group designed and synthetised new arylalkyl- and arylalkenylamines (**MCL 1**-**14**) with different functionalities both on the aromatic moiety and the alkylamine chain [10]. Sigma1 selective pharmacophore models from literature were considered and synthetic methods suitable to prepare compounds with two independent points of diversity were investigated. Binding assays showed high sigma1 affinity and interesting selectivity for sigma1 compared to sigma2 receptor. A moderate affinity for sigma2 receptor was also observed for some compounds [10]. These promising results encouraged us to address our efforts to the rational design, synthesis and phamacological evaluation of novel potential ligands with affinity *versus* both sigma receptors subtypes. With this aim, our research was carried out following four different strategies.

2.1 First strategy. Design, synthesis and pharmacological evaluation of novel sigma1 selective and sigma2 ligands with arylalkenylamine structure

A molecular modeling study based on a selective sigma1 pharmacophore model was conducted in order to deeply investigate and rationalize the structure-receptor affinities of our sigma ligands [10]. Basing on this study, we designed a new series of 21 arylalkenylamines (**MCL 15**-**35**, Series I; Scheme 1), structurally related to our sigma ligands previously prepared and identified as hits compounds [10]. The designed compounds showing different functionalities both on the aromatic and the amine moiety, were prepared according to the convenient methodology described in our previous work, with suitable modifications (Scheme 1) [10]. Firstly, we prepared *via* aza-Michael the β-aminoketones **A**-**E**, that were purified either by fractional distillation or filtering through an alumina pad. Thereafter, the compounds were prepared by nucleophilic addition of the aromatic anion to the β-aminoketones intermediates, followed by direct dehydration by quenching the reaction with HCl 37%. An acid-base work-up combined with a subsequent flash chtomatography, for almost all compounds, allowed the isolation of the desired compounds in poor to good yields. The regio- and stereo-selectivity of the reactions were studied by ^{1}H-NMR and ^{1}H-^{1}H NOESY experiments. With the only exception of **MCL 24** and **MCL 34**, the (E) stereochemistry was assigned to the main reaction product.

Both the uncomplete dehydration of the alcoholic intermediate and the poor yields observed for some compounds, particularly for **MCL 24** and **MCL 34** (11% and 4%, respectively), led us to develop a more efficient dehydration method. Preliminary experiments were carried out on **MCL 24** and **MCL 34** and consisted in the isolation of the alcoholic intermediate, followed by its dehydration using concentrate H_2SO_4. As confirmed by ^{1}H-NMR and ^{1}H-^{1}H NOESY spectra, this procedure allowed us to obtain the desired compounds in higher yields (37% and 12% for **MCL 24** and **MCL 34,** respectively) and to increase the (E)-strereoselectivity. In order to gain more detailed structure-activity relationships (SAR) of sigma1 and sigma2 ligands, the binding properties of the novel compounds (**MCL 15-35**) were investigated. Limetedly to the assayed compounds, very promising results were obtained. Interestingly, some compounds displayed sigma1 binding values in nanomolar range (**MCL 27**, Ki = 2.30 nM; **MCL 19**, Ki = 14.2 nM)

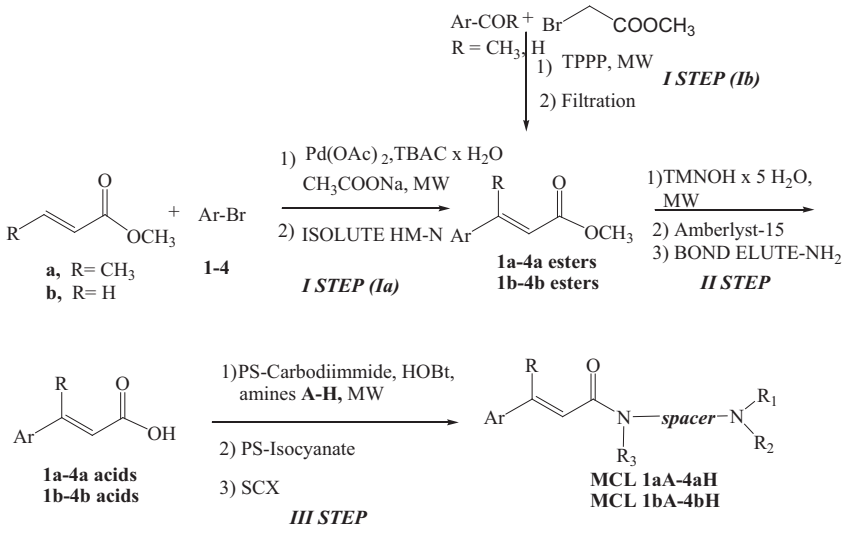

Fig. 2: General procedure for the synthesis of compounds **MCL 1aA-4aH, 1bA-4bH** (Series II).

or subnanomolar range (**MCL 22**, Ki = 0.86 nM; **MCL 21**, Ki 0.97 nM). Moreover, a moderate sigma2 affinity was evidenced for some compounds (**MCL 21**, Ki = 35.1 nM; **MCL 27**, Ki = 95 nM).

2.2 Second strategy. Design, synthesis and pharmacological evaluation of a drug discovery library of novel potential sigma2 ligands with arylalkenylamide structure

Basing on the chemical features of some reference compounds selected from known selective sigma2 ligands and our arylalkenylamines [10], a new scaffold based on (N-alkylamino-substituted)arylalkenylamide structure was drawn [11]. Successively, taking into account the synthetic feasibility and the commercial availability of the starting materials, we designed a drug discovery library of 64 (N-alkylaminoalkyl-substituted)arylalkenylamides (**MCL 1aA-4aH**, **MCL 1bA-4bH**, Series II; Scheme 2) [11].

A three-step synthetic route was identified to achieve the library compounds. The first step was the preparation of the key intermediates α,β-unsaturated esters (**1a**-**4a**, **1b**-**4b**), followed by hydrolysis and amidation reactions (Figure 2). An efficient and convenient protocol based on PASPS (Polymer Assisted Solution Phase Synthesis) and MAOS (Microwave Assisted Organic Synthesis) techniques was developed. To further accelerate the library preparation, the reaction work-up was simplified by employing SPE (Solid Phase Extraction) (Figure 2). Firstly, we developed two efficient, easy-to-use and stereo-selective protocols based on Heck and Wittig reactions (1a, 1b; I step, Figure 2). Although both methods were suitable for our key intermediates preparation, Heck reaction was preferred because it is less expensive than the other one [12, 13]. Following this synthetic approach, we obtained all the designed compounds in high purity (equal or greater than 80%) suitable for biological screening. *In vitro* studies, are to date in progress on a randomly selected library subset of 20 compounds. 8 compounds so far screened showed satisfactory affinity values for sigma1 receptors (**MCL 4bB**, Ki = 32.7 nM; **MCL 2bG**, Ki = 60.9 nM), whereas no compounds showed sigma2 affinity (Ki $>$ 1000 nM). The preparation of the same 20 compounds pool, selected for sigma assays, was scaled-up. Heck and hydrolysis reactions were performed according to the optimized procedures, whereas a protocol based on parallel synthesis combined with peptide coupling agents was developed for the amidation reaction. Further binding assays towards various receptors (NMDA, μ- and κ-opioid, dopaminergic, adrenergic and serotoninergic receptors) are in progress.

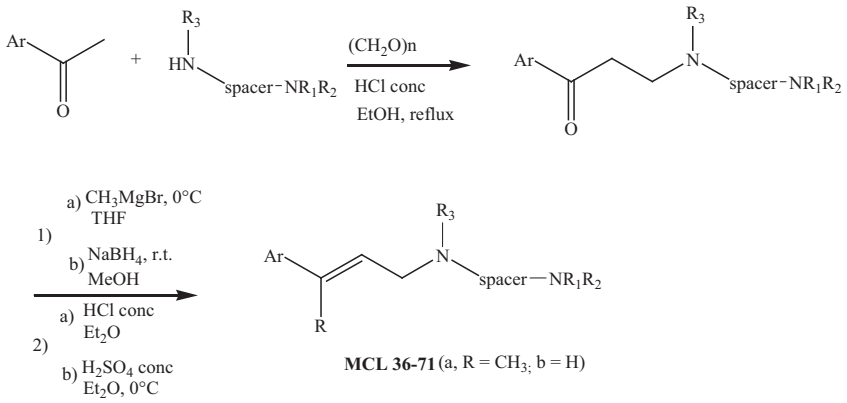

Fig. 3: General procedure for the synthesis of compounds **MCL 36-71** (Series III).

2.3 Third strategy. Disegn, synthesis and pharmacological evaluation of novel sigma2 ligands with arylalkenylaminoalkylamine structure

Molecular modeling studies were performed in order to identify a sigma2 pharmacophore model. Although the model is not well defined yet, the structural features essential for sigma2 receptor binding were hypothesized. Taking into account both molecular modeling suggestions and SAR evaluation of our sigma ligands, we designed a new series of 36 compounds (**MCL 36-71**, Series III; Figure 3) with arylalkenylaminoalkylamine structure, related to both series I and II compounds. The designed compounds showed a further tertiary amine site and a more centered one, compared to Serie I and Series II compounds, respectively. Moreover, a bulk substituent at the distal basic nitrogen and a different substituent at the olefinic bond (R = CH_3 or H) were present in each compound.

In Scheme 3 is reported the general synthetic procedure, which has been applied so far to the compounds **MCL 60** (R = CH_3) and its homologue **MCL 61.** Firstly, we synthesized the β-aminoketone intermediate *via* Mannich reaction. The reaction of the β-aminoketone with CH_3Mg Br, followed by dehydration of the alcoholic intermediate in the presence of concentrate HCl 37% (Figure 3) gave compound **MCL 60**. **MCL 61** was synthesized by treating the β-aminoketone with $NaBH_4$ and using concentrate H_2SO_4 as dehydrating agent. Both compounds were obtained in good yields and purity suitable for biological screening (in progress).

2.4 Fourth strategy. Design, synthesis and pharmacological evaluation of novel selective sigma2 ligands with indolylalkylbenzylamine structure

In the attempt to develop new sigma2 ligands, we designed a series of 24 indolylalkylbenzylamines (**MCL 1a-l, MCL 2a-l**, Serie IV; Scheme 4), based on the chemical structure of a known selective sigma2 ligand Siramesine (LU 28-179) and its derivatives [14, 15]. All compounds were synthesized as indicated in Figure 4, starting from the indolyl-propanoic or –butanoic acid, which were reduced with $LiAlH_4$ to the corresponding indolylalkyl alcohols. By heating the intermediate alcohols with 4-fluorobenzene in the presence of K_2CO_3, CuI and ZnO, the corresponding N-fluorophenyl derivatives were obtained. By treatment of the intermediates with methanesulfonyl chloride, the methane sulfonate esters were synthesized, from which final compounds were prepared by reaction with substituted N-benzylamines.

After traditional purification methods, all compounds were obtained in purity and quantity suitable for binding assays towards sigma1 and sigma2 receptors [14]. The binding results suggested that the presence of a butylene bridge may produce in compounds **MCL 2a-l** a favourable distance for sigma2 receptor binding. The best affinity values were observed for **MCL 2l**, which showed a nanomolar sigma2 affinity

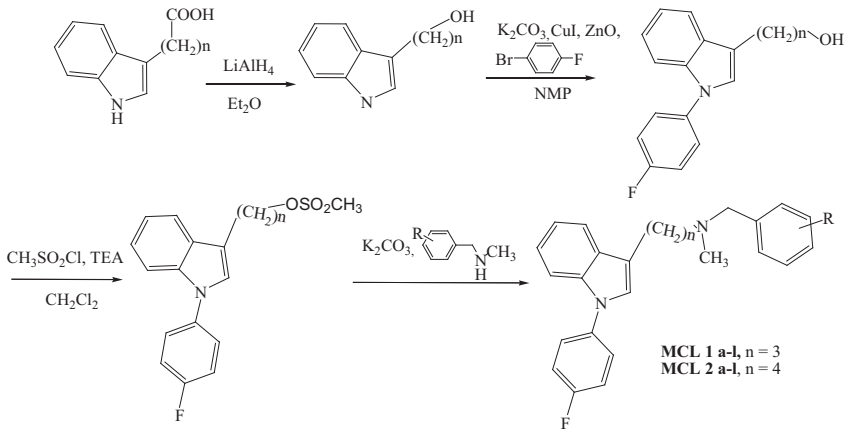

Fig. 4: General procedure for the synthesis of compounds **MCL 1a-l, MCL 2a-l** (Series IV).

(Ki = 5.9 nM) and an appreciable sigma2/sigma1 selectivity (Ki sigma1/Ki sigma2 = 22). These results will be useful tools to gain more insights into the molecular features responsible for sigma2 receptor binding as well as to identify a hit compound for further SAR studies.

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References

[1] W. R. Martin, C. G. Eades, J. A.Thompson, R. E. Huppler, P. E. Gilbert, Journal of Pharmacology and Experimental Therapeutics, **197,** 517 (1976).
[2] R. Quirion, R. Chicheportiche, P. C. Contreras, K. M. Johnson, D. Lodge, S. W. Tam, J. H. Woods, S. R. Zukin, Trends in Pharmacological Sciences, **10**, 444 (1987).
[3] J. M. Walker, W. D. Bowen, F. O. Walker, R. R. Matsumoto, B. de Costa, K. C. Rice, Pharmacological Reviews, **42**, 335 (1990).
[4] Y. Itzhak (Academic Press, San Diego, CA 1994).
[5] T. Hayashi, T. P. Su, CNS Drugs, **18**, 269 (2004).
[6] F. F. Moebius, J. Striessnig, H. Glossmann, Trends in Pharmacological Sciences, **18**, 67 (1997).
[7] W. D. Bowen, Pharmaceutica Acta Helvetiae, **74,** 211 (2000).
[8] F. Berardi, S. Ferorelli, C. Abate, N. A. Colabufo, M. Contino, R. Perrone, V. Tortorella, Journal of Medicinal Chemistry, **47**, 2308 **(**2004).
[9] R. A. Glennon, S. Y. Ablordeppey, A. M. Ismaiel, M. B. El-Ashmawy, K. Burke Howie, Journal of Medicinal Chemistry, **37**, 1214 (1994).
[10] S. Collina, G. Loddo, M. Urbano, L. Linati, A. Calligari, F. Ortuso, S. Alcaro, C. Laggner, T. Langer, O. Prezzavento, G. Ronsisvalle, and O. Azzolina, Bioorganic & Medicinal Chemistry, **15**, 771 (2007).
[11] M. Urbano, S. Collina, D. Rossi, A. Carnevale Baraglia, S. Alcaro, A. Artese, O. Azzolina, Letters in Drug Design & Discovery, (2007), (in press).
[12] S. Collina, D. Rossi, M. Urbano, A. Carnevale Baraglia, O. Azzolina, Letters in Organic Chemistry, **4**, 384 (2007).
[13] S. Collina, M. Urbano, A. Magnani, G. Loddo, O. Azzolina, Letters in Organic Chemistry, **3**, 16 (2006).
[14] M. G. Mamolo, D. Zampieri, C. Zanette, C. Florio, S. Collina, M. Urbano, O. Azzolina, L. Vio, European Journal of Medicinal Chemistry, (2007), (in press).
[15] M. S. Ostenfeld, et al., Cancer Research, **19**, 8975 (2005).