Bioorganic & Medicinal Chemistry Letters 26 (2016) 5160–5163

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Phenylindenone isomers as divergent modulators of p38α MAP kinase

Andrea Cappelli [a,*], Chiara Nannicini [a], Alessia Chelini [a], Marco Paolino [a], Germano Giuliani [a], Maurizio Anzini [a], Antonio Giordani [b], Chiara Sabatini [b], Gianfranco Caselli [b], Laura Mennuni [b], Francesco Makovec [b], Gianluca Giorgi [a], Salvatore Vomero [a], Maria Cristina Menziani [c]

[a] Dipartimento di Biotecnologie, Chimica e Farmacia and European Research Centre for Drug Discovery and Development, Università degli Studi di Siena, Via A. Moro 2, 53100 Siena, Italy
[b] Rottapharm Biotech S.r.l., Via Valosa di Sopra 3, 20900 Monza, Italy
[c] Dipartimento di Scienze Chimiche e Geologiche, Università degli Studi di Modena e Reggio Emilia, Via Campi 103, 41125 Modena, Italy

ARTICLE INFO

Article history:
Received 12 August 2016
Revised 29 September 2016
Accepted 2 October 2016
Available online 4 October 2016

Keywords:
MAPK
Kinases
Synthesis
Docking

ABSTRACT

Two new fluorophenylindenone derivatives were designed as potential p38α MAPK modulators by preserving the key interactions of the vicinal pyridine/fluorophenyl pharmacophore with the enzyme protein. Interestingly, these two fluorophenylindenone isomers showed divergent activities, with compound **6** behaving as an inhibitor and **5** as a putative activator. These results were rationalized by docking studies and molecular dynamics simulations in terms of stabilization of DFG loop, by compound **5** in a conformation more accessible to phosphorylation.

Mitogen-activated protein kinases (MAPK) are protein kinases that can be classified in several distinct groups composed of sets of three evolutionarily conserved, sequentially acting kinases: MAPK, MAPK kinase (MKK), and MKK kinase (MAP3K).[1–3] The activation of MAP3K leads to phosphorylation and activation of an MKK, which in turn stimulates MAPK activity through dual phosphorylation on threonine and tyrosine residues located in the activation loop. Once activated, MAPK phosphorylate cytoplasmic and nuclear target substrates on serine or threonine residues.

p38 proteins are a class of MAPK playing a crucial role in important cellular processes (i.e. cell differentiation, apoptosis, and autophagy) and are responsive to stress stimuli such as heat and osmotic shock, ultraviolet irradiation, and inflammatory cytokines. The p38 MAPK family includes four different isoforms, namely p38α, p38β, p38γ, p38δ.[4] Among them, p38α has been reported to be largely expressed in monocytes and macrophages, whereas p38β is abundant in endothelial cells, p38γ in skeletal muscle, and p38δ in testes, pancreas, prostate, small intestine and in certain endocrine tissues.[5] Substrates of p38 MAPKs include a large number of different proteins and a significant portion of them is involved in the regulation of gene expression.[6] p38 has been considered an interesting target for the development of anti-inflammatory drugs.[7–10] Thus, over the past two decades, the development of small molecule p38α MAPK inhibitors has received an extraordinary level of attention both in the pharmaceutical industry and in the academia. Thus, the medicinal chemistry work has produced a large number of inhibitors (see Fig. 1 for some representative structures), and some of them have been co-crystallized with the enzyme in order to evaluate the drug-protein interactions in the complexes.[11]

The most explored chemical class of p38α MAPK inhibitors is characterized by the presence of a nitrogen-containing six member heterocyclic moiety (pyridine, pyrimidine, or equivalents) in a vicinal position to a lipophilic (most often a *p*-fluorophenyl) group. The development of this family of compounds has been stimulated by the interesting results obtained with compound **1** (SB203580) and its congeners by SmithKline Beecham.[12] The exploration of the chemical space around this structure has produced inhibitors showing potency in the nanomolar range and the discovery of 4-azaindole derivatives **4a**,**b**.[13]

In order to evaluate the effects of further core modifications on the interaction with p38α, fluorophenylindenone derivatives **5** and **6** (Fig. 1) were designed by replacing the imidazole heterocyclic core of **1** with the indenone scaffold. The central pharmacophore consisting in the vicinal pyridine/fluorophenyl system was

* Corresponding author. Tel.: +39 0577 234320.
E-mail address: andrea.cappelli@unisi.it (A. Cappelli).

http://dx.doi.org/10.1016/j.bmcl.2016.10.001
0960-894X/

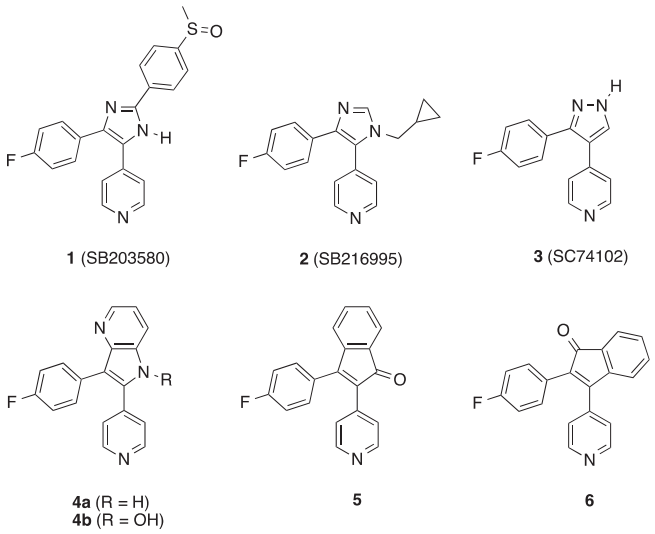

Figure 1. Comparison of the structures of the newly designed isomeric indenone derivatives **5** and **6** with those of some selected p38α MAP kinase inhibitors.

conserved so that the pyridine nitrogen could behave as the hydrogen bond acceptor for the NH of Met-109 and the 4-fluorophenyl moiety could occupy the hydrophobic back pocket. On the other hand, the indenone moiety of **5** and **6** could find different accommodations in the phosphate/sugar region of the binding site.

Compounds **5** and **6** were synthesized as described in the Supporting information, characterized by crystallography [CCDC 1487925 (**5**), 1487926 (**6**), see Supporting information], and tested for their potential modulatory activity on p38α MAPK enzyme at Life Technologies Ltd[14] [SelectScreen Kinase Profiling, Protein Serine/Threonine Kinase MAPK14 (p38α) direct Z-LITE kinase assays platform, for the experimental details see http://www.lifetechnologies.com/selectscreen] by using two different ATP concentrations, namely 500 μM (close to the K_m value) and 100 μM.[15]

In the enzymatic studies, indenone derivative **6** showed significant inhibitory properties, with a potency in the micromolar range that appeared to be dependent by the concentration of ATP used in the enzymatic assay (Fig. 2).

In particular, with the ATP concentration close to the K_m value the concentration–effect curve for compound **6** is slightly shifted to right compared with that observed with a lower ATP concentration. Accordingly the IC_{50} value in the presence of 500 μM ATP was slightly but significantly higher (1.35 μM vs 0.899 μM, for 500 and 100 μM ATP, respectively).

On the other hand, isomeric indenone **5** failed in showing an inhibitory activity at both the ATP concentrations tested. Surprisingly on the contrary, at the ATP concentration corresponding to the K_m value, compound **5** appeared to increase MAPK14 phosphorylation, thus behaving as a partial activator (activation effect around 40–50% at 10 μM, Fig. 3).

In order to verify this surprising result, compound **5** was re-sampled and re-assayed in the same test system and the results confirmed those obtained in the preliminary assay. In fact, the top three concentrations induced a significant increase of phosphorylation. This phenomenon could be caused by compound-dependent activation of the kinase, or by undetected assay interference of some sort. In order to rule out fluorescence interferences and development reaction interferences, control wells were used and compound **5** did not show any evidence of interference. On the other hand, the activation effect appeared to be almost negligible at 100 μM ATP, suggesting the importance of the ATP concentration in revealing the activation features of indenone derivative **5**.

Thus, the preliminary results of the enzymatic studies performed with compound **5** was surprising because this indenone isomer of the p38α MAPK inhibitor **6** appeared to behave as activator.

Owing to these intriguing results, docking studies and molecular dynamics simulations were performed in order to rationalize the apparently divergent behavior obtained with the isomeric indenone derivatives. Docking of compounds **5** and **6** to p38α MAPK was performed on the basis of the structural similarity of these compounds with the 4-azaindole inhibitor **4b** (type I inhibitor),[11] the crystal structure of which, in complex with p38α MAPK, has been resolved (PDB code :1OZ1, Fig. 4).[13]

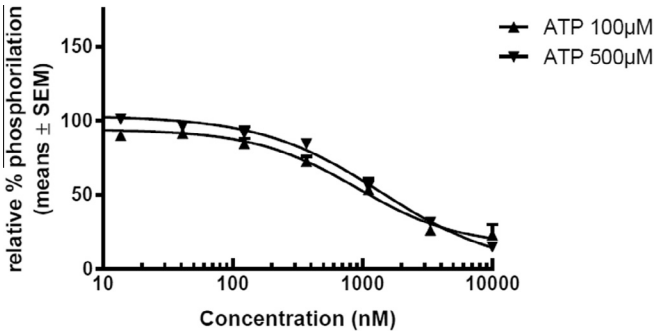

Figure 2. MAPK14 (p38α) phosphorylation in the presence of compound **6** and different ATP concentrations, 100 μM or 500 μM (close to the K_m value). Data are expressed as mean ± SEM.

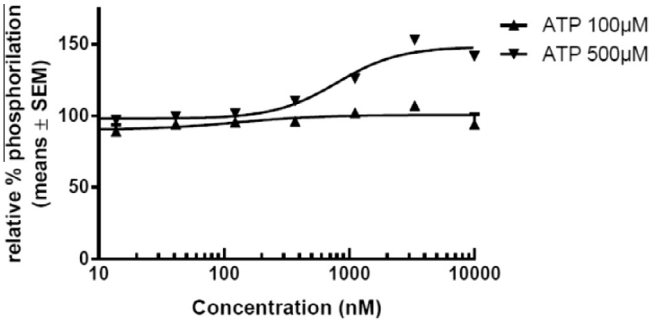

Figure 3. MAPK14 (p38α) phosphorylation in the presence of compound **5** and different ATP concentrations, 100 μM or 500 μM (close to the K_m value). Data are expressed as mean ± SEM.

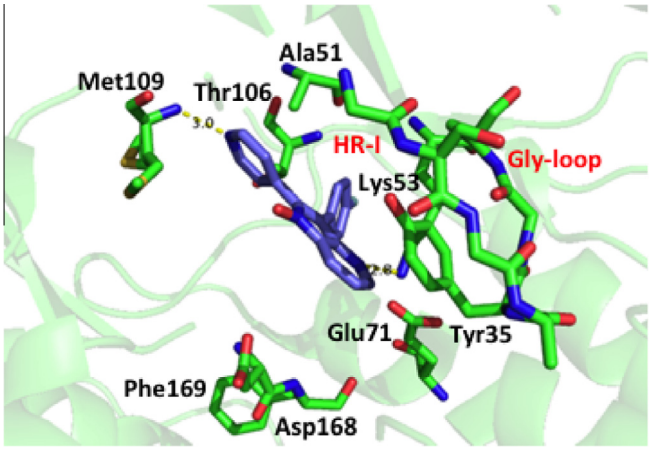

Figure 4. 4-Azaindole inhibitor **4b** bound to p38α MAPK (PDB code: 1OZ1).[13]