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PII: S0960-894X(16)31029-0
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.10.001
Reference: BMCL 24304

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 12 August 2016
Revised Date: 29 September 2016
Accepted Date: 2 October 2016

Please cite this article as: Cappelli, A., Nannicini, C., Chelini, A., Paolino, M., Giuliani, G., Anzini, M., Giordani, A., Sabatini, C., Caselli, G., Mennuni, L., Makovec, F., Giorgi, G., Vomero, S., Menziani, M.C., Phenylindenone Isomers as Divergent Modulators of p38α MAP Kinase, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.10.001

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Phenylindenone Isomers as Divergent Modulators of p38α MAP Kinase

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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.

Keywords: MAPK, kinases, synthesis, docking

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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 Figure 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]

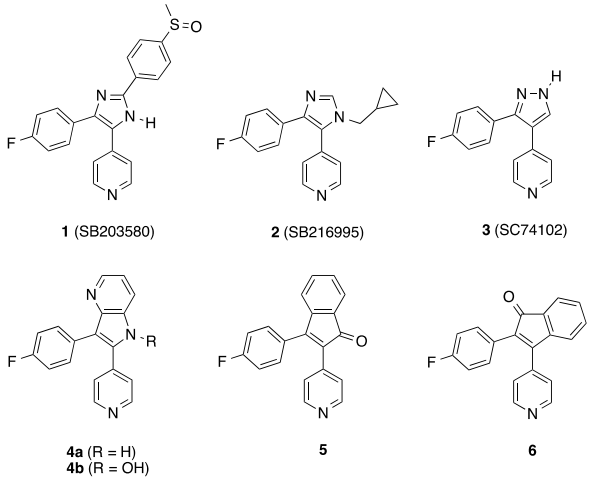

Figure 1. Comparison of the structures of the newly designed isomeric indenone derivatives **5** and **6** with those of some selected p38α MAP kinase inhibitors.

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 develop-

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ment 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** (Figure 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 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 (Figure 2).

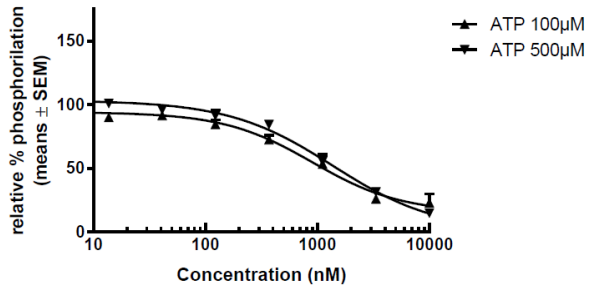

Figure 2. MAPK14 (p38α) phosphorylation in the presence of compound **6** and different ATP concentrations, 100 μM or 500 μM (close to the K_m value). Data are expressed as mean ± sem.

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, Figure 3).

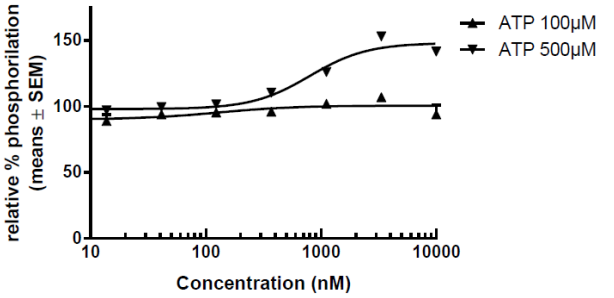

Figure 3. MAPK14 (p38α) phosphorylation in the presence of compound **5** and different ATP concentrations, 100 μM or 500 μM (close to the K_m value). Data are expressed as mean ± sem.

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, Figure 4).[13]

The X-ray crystal structure of the complex showed that the pyridine nitrogen of the 4-azaindole inhibitor **4b** behaved as the hydrogen bond acceptor for the NH of Met109, the 4-fluorophenyl moiety occupied the hydrophobic back pocket called hydrophobic region I (HR-I), and the 4-azaindole moiety was capable of direct hydrogen bonding the terminal nitrogen of Lys53. The hydrogen bond with Lys53 was claimed to be required for the

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regioselective positioning of the pyridine and the 4-fluorophenyl groups.[16]

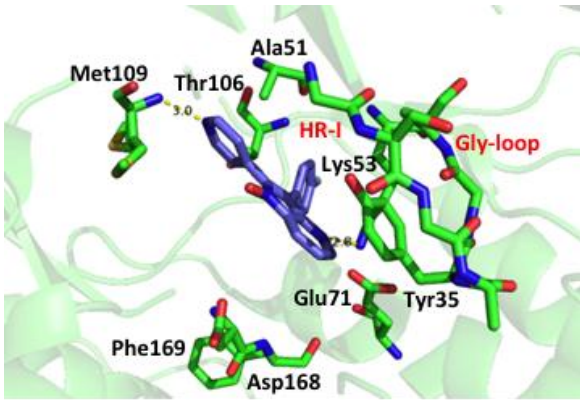

Figure 4. 4-Azaindole inhibitor **4b** bound to p38α MAPK (PDB code: 1OZ1).[13]

Therefore, in the docking of compounds **5** and **6** we assumed that the ligands target the ATP binding site of the enzyme in its active form. The active form of the enzyme is characterized by an open conformation of the activation loop (DFG-loop), which is usually referred to as DFG "in" on the basis of the position of the Phe169 residue belonging to the conserved triad Asp168-Phe169-Gly170 at the beginning of the activation loop.[17-19]

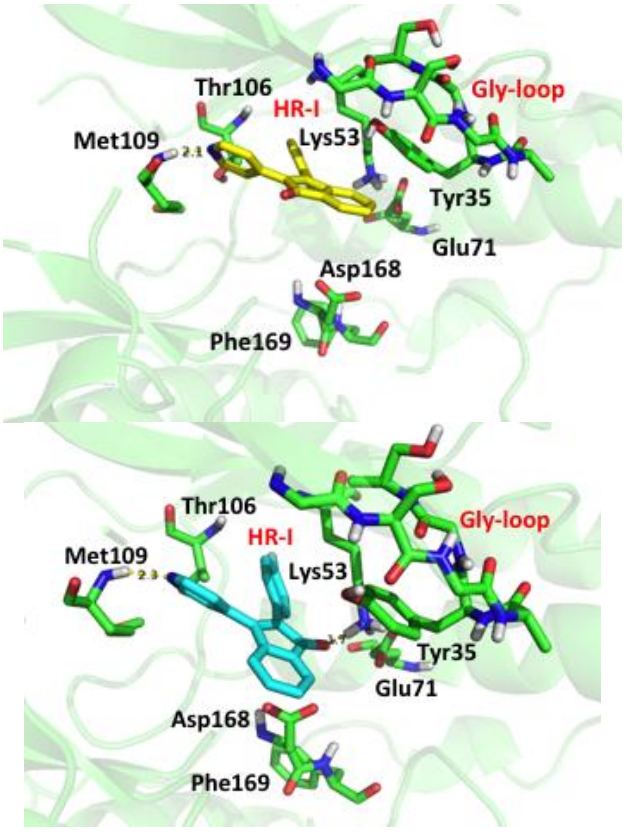

Figure 5. Docking of compound **5** (top) and **6** (bottom) to p38α. DFG loop is in the "in" conformation (binding mode of type I inhibitors).

As expected, fluorophenylindenone derivatives **5** and **6** (Figure 5) maintained the key interactions of type I inhibitors. In particular, a hydrogen bond is established between pyridine nitrogen and the amide proton of Met109, and significant hydrophobic interactions involve the 4-fluorophenyl group with hydrophobic region I. HR-I is located at the back of the ATP binding site, and is defined by Ala51, Lys53, Leu75, Ile84, Leu104, Thr106 and Leu167.[11]

Most importantly, compound **6** also accepts a hydrogen bond from Lys53 (Figure 5). On the contrary, in compound **5** the oxygen acceptor of H-bond is located at the opposite side, preventing the achievement of this interaction (Figure 5), which is necessary to engage the inhibitor in loco.[13,16] This may trigger the different behaviour of the two ligands in the enzymatic essay. In fact, a marked conformational change upon binding of compound **5** is observed for Arg173, which moves away from Tyr35 of the Gly–loop (aa res. 31-37) and establishes a network of strong electrostatic interactions with Asp168 and Glu178 of the DFG loop. Consequently, the binding site is more accessible, being the gateway of the binding pocket around 3 Å larger, the DFG motif is stabilized in its active DFG-in conformation and the phosphorylation sites more accessible (Figure 6).

In conclusion, two new fluorophenylindenone derivatives (**5** and **6**) were designed from p38α MAPK inhibitor **1** by replacing its imidazole heterocyclic core with indenone scaffold and conserving the central pharmacophore (i. e. the vicinal pyridine/fluorophenyl system). In our working hypothesis, the pyridine nitrogen of **5** and **6** could behave as the hydrogen bond acceptor for the NH of Met-109, the 4-fluorophenyl moiety could occupy the hydrophobic back pocket, and the indenone moiety could find different accommodations in the phosphate/sugar region of the binding site. The fluorophenylindenone isomers **5** and **6** were synthesized, characterized by X-ray crystallography, and tested for their potential modulatory activity on p38α MAPK enzyme by using two different ATP concentrations. In these studies, compound **6** behaved as an inhibitor, whereas isomeric indenone **5**, at the ATP concentration corresponding to the K_m value, appeared to behave as an activator. This unprecedented result was rationalized by docking studies, which showed that compounds **5** and **6** retained the typical interactions of type I inhibitors, as expected. The main difference was the absence of the hydrogen bond interaction from Lys53 in the complexes of activator **5** that produced a remarkable conformational change resulting in the stabilization of DFG loop in a conformation more accessible to phosphorylation.

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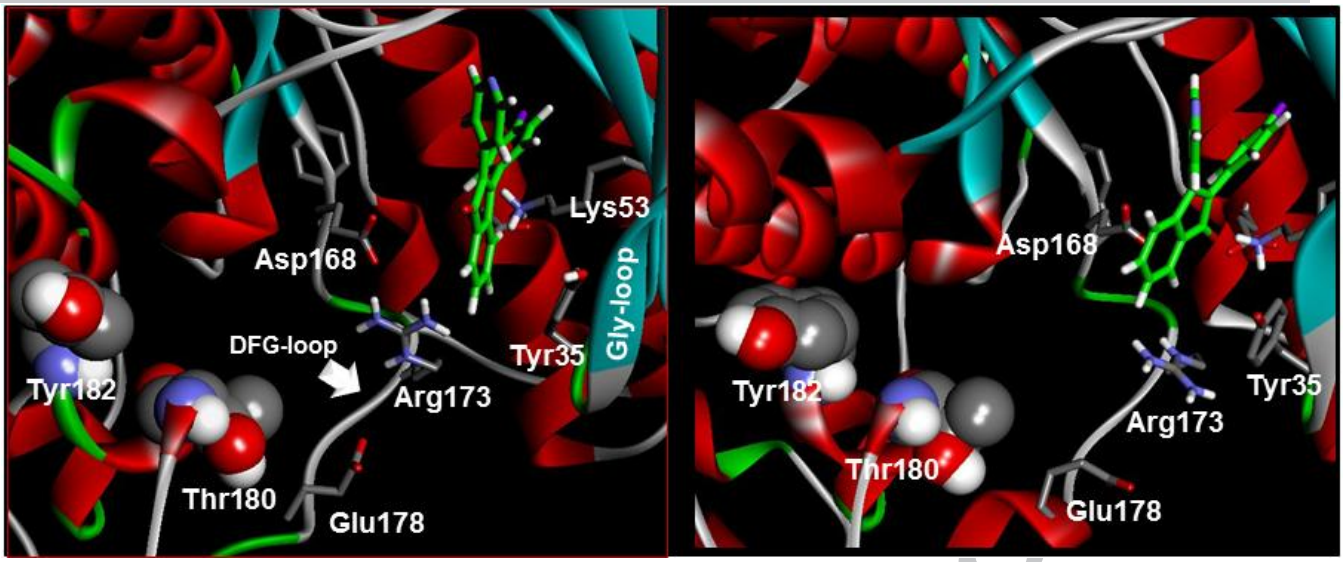

Figure 6. 3D structure of complexes between the compound **5** (left) and **6** (right) and p38α MAPK, as resulted from the molecular dynamics simulation.

Acknowledgment

The authors are grateful to Dr. Francesco Berrettini (CIADS, Università di Siena) for the X-ray data collection and to Prof. Alessandro Donati (Università di Siena) for the NMR experiments performed with compound **6**.

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Supporting Information

Experimental details for the synthesis and the characterization of target compounds and their intermediates. The experimental procedures used in molecular modeling studies.

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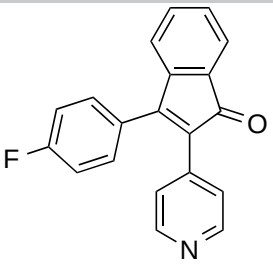

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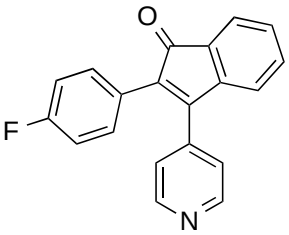

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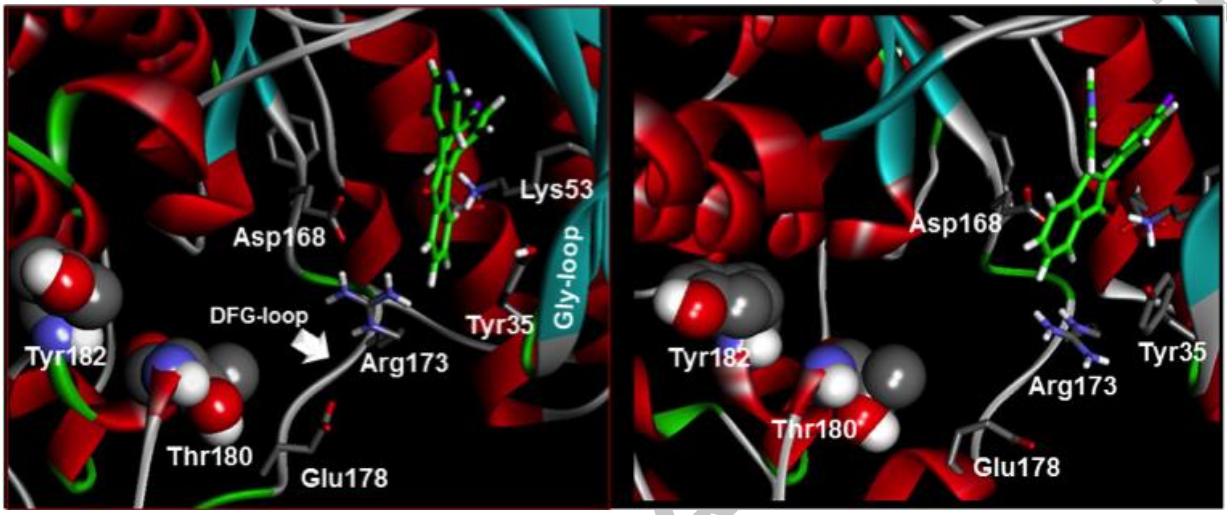

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