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Indole-Based Heterocyclic Inhibitors of p38α MAP Kinase: Designing a Conformationally Restricted Analogue

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Abstract—p38α Mitogen Activated Protein Kinase (MAP kinase) is an intracellular soluble serine threonine kinase. p38α kinase is activated in response to cellular stresses, growth factors and cytokines such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α). The central role of p38α activation in settings of both chronic and acute inflammation has led efforts to find inhibitors of this enzyme as possible therapies for diseases such as rheumatoid arthritis, where p38α activation is thought to play a causal role. Herein, we report structure–activity relationship studies on a series of indole-based heterocyclic inhibitors that led to the design and identification of a new class of p38α inhibitors.

Mitogen activated protein kinases (MAPK) are represented by four isoforms and while the role of the ubiquitously expressed isoform p38α in settings of inflammation is more clearly defined, the function of the other isoforms, p38β, p38γ, and p38δ is not so well understood. Hence, p38α has been the primary target of many drug discovery efforts over the last decade.

p38α MAP kinase is activated in response to cellular stresses, growth factors and cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α). Once activated, p38α activates other kinases which go on to phosphorylate heat shock proteins and transcription factors, further controlling the production of these cytokines. It is due to this ability of p38α to modulate several key pro-inflammatory and inflammatory cytokines that it has been implicated in a number of patho-physiological states associated with inflammation. Further support comes from the demonstration in variety of animal models of rheumatoid arthritis (RA) that an inhibitor of this enzyme has beneficial effects in arresting the progression of the disease. This has driven efforts to find inhibitors of this enzyme as possible therapies for diseases such as RA.[1–11] While chronic inflammation was the early focus for the development of p38α kinase inhibitors, there are several new reports and efforts that speak of the potential utility of such inhibitors in more acute inflammatory states. There is a growing body of evidence that elucidates the benefits of inhibiting p38α kinase in the treatment of diseases such as diabetes, cancer and acute myocardial infarctions.[12–18] Herein, we report studies that describe the design and structure activity relationship of a new class of p38α MAP kinase inhibitors.

Modification of an initial hit, identified by high throughput screening, led to the p38α inhibitor indole-5-carboxamide derivative **1**,[19] which had a modest activity against p38α with an IC_{50} of 4.36 μM.[20] These compounds also block LPS mediated IL-1 and TNF-α synthesis in hPBMC's and U937 cells. The most active molecules in this report are capable of blocking LPS mediated IL-1 and TNF-α synthesis in human whole blood. The concentrations at which the most potent molecules reported in this paper achieve the reversal of p38α mediated cellular events is approximately 10–15-fold higher than their potency against the kinase itself. The corresponding regioisomer, indole-6-

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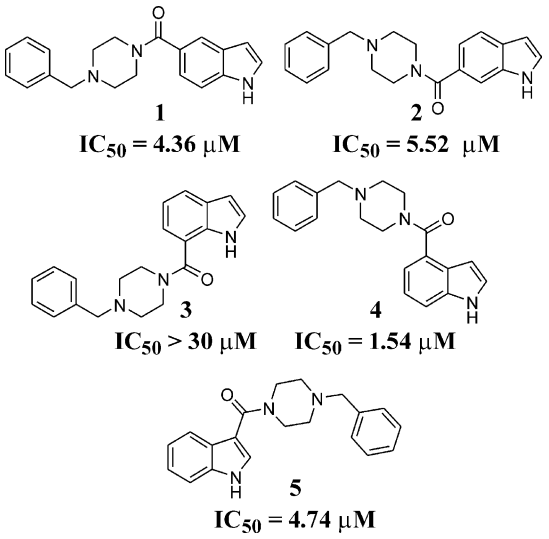
carboxamide **2**, was also a modest inhibitor of p38α (IC_{50} = 4.36 μM), however the indole-7-carboxamide **3**[21] was found not to be an inhibitor of p38α. It has been reported in the literature that the regioisomeric indole-4- and indole-3-carboxamides, **4**[22] and **5**, respectively,[23] are also modest inhibitors of p38α.

Structure–activity relationship study showed that both the benzyl group and the indole moiety are important for activity. Also, the amide linker is important for activity as substitution with alternate linkers including amine, amidine and sulfonamide, resulted in loss in p38α activity. This study also did not lead to analogues with substantially improved potency. Since the presence of the indole and the benzyl moities is important for activity, it suggests that the orientation of these moities may also be important for activity.



It may be that the inactivity of **3** is due to the presence of an intramolecular hydrogen bond (Fig. 1), that may affect its ability in adopting a binding conformation (Fig. 2).[27] In addition, this internal H-bond may interfere with the important interaction with the hinge region of p38α as exemplified in the proposed binding mode of compound **1**, where the carbonyl moiety of the amide interacts with the hinge region of p38α, an interaction generally cited as being present in most of the p38α inhibitors for which structural information is available, and the benzyl group occupies a hydrophobic pocket, adjacent to the ATP binding site. The location of the binding site and the binding mode was also influenced by the fact that **1** is an ATP competitive inhibitor of p38α (Fig. 2).

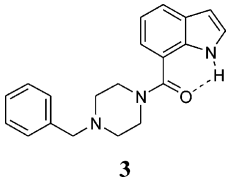


Figure 1.

Since the orientation of the benzyl group and the indole ring is crucial for activity, it followed that designing conformationally restricted analogues may lead to further improvement in potency.

To achieve this, the 6-substituted indole and the 2,5-dimethylpiperazine analogues were synthesized, as they would restrict the rotation of the indole–carbonyl bond and restrict the conformational flexibility of the piperazine ring. Both the presence of the substituent at the 6-position of the indole (**6**) and the replacement of the piperazine ring with the dimethypiperazine[24] ring (**7**) led to a modest increase in potency. This substitution on the indole ring led to a small but consistent increase in potency. This increase in potency could also be associated to the reduced conformational freedom of both compounds (Fig. 2), clearly suggesting that a molecule with the combined attributes of **6** and **7** could show more enhanced potency. Indeed compound (±)-**8** was found to be approximately 14-fold more active than **6**, with an IC_{50} of 0.07 μM, and over 60-fold more potent than **1**. Further, resolution of (±)-**8** resulted in the enantiomers (+)-**9**[21,25] and (−)-**10**,[21,26] where the 2*R*,5*S*-dimethylpiperazine analogue (+)-**9** (IC_{50} = 0.02 μM) shows greater than 40-fold improvement in potency over **6** and over 200-fold increase in potency over **1**. Whereas the 2*S*,5*R*-dimethypiperazine analogue (−)-**10**

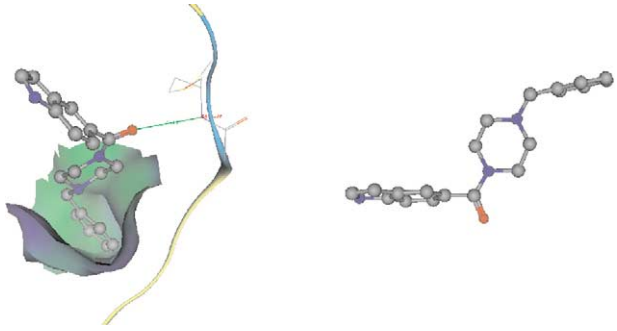


Figure 2. Proposed binding mode and conformation of **1**.

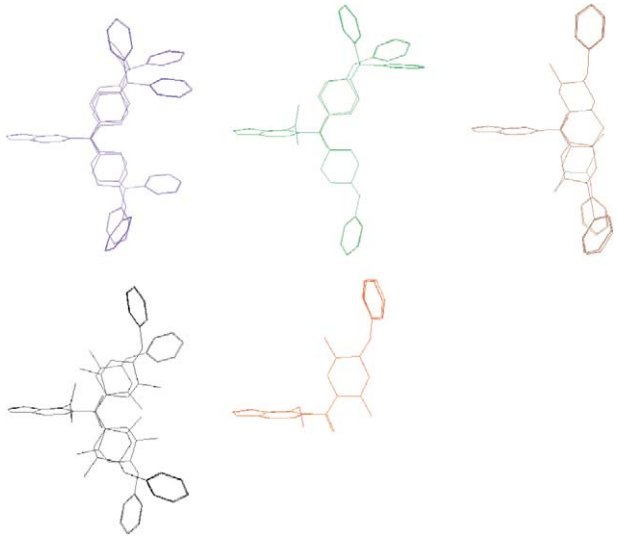
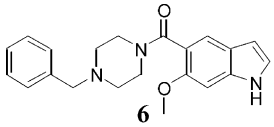


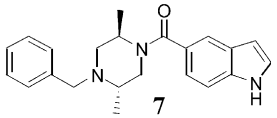
Figure 3. Calculated low energy conformations of compounds **1** (blue), **6** (green), **7** (brown), **9** (red) and **10** (black).

(IC_{50} = 1.65 μM) showed almost no improvement in activity in comparison to **6**.



6

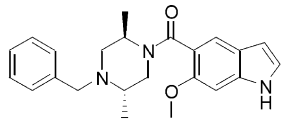
IC_{50}= 0.95 μM



7

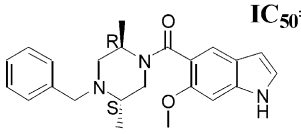
IC_{50}= 1.3 μM

Molecular modeling studies (Fig. 3),[27] of (+)-**9** and (−)-**10** supports the observation that the highly conformationally restricted analogue (+)-**9** should be more potent than (−)-**10**. Thus, in (+)-**9** the substitution pattern helps restrict the analogue and only populate the proposed active binding conformation, whereas this is not the case in (−)-**10**. Though it is obvious that the increase in potency of (±)-**8** is due to the entropic effect of a conformationally restricted analogue, at this time it is not possible to rule out any secondary contributions due to a hydrophobic interactions of the substituents on the piperazine moiety and/or the indole ring and the binding pocket of p38α.



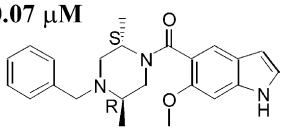
(+/-)-8

IC_{50}= 0.07 μM



9

IC_{50}= 0.02 μM



10

IC_{50}= 1.65 μM

In conclusion, following the discovery from high throughput screening, initial SAR studies were carried out on a novel series of p38α MAP kinase inhibitors. The results of these studies led to a preliminary identification of the important structural features of these compounds. The potency of these compounds were dramatically enhanced by the rational design of conformationally restricted analogues and led to the identification of a potent inhibitor of p38α MAP kinase.

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19. **Synthesis of 1**: 0.382 g of benzylpiperazine was dissolved in 10 mL DMF, to this was added 0.322 g of indole-5-carboxylic acid and 0.382 g of EDAC. To the reaction mixture was added 10 mg μL of dimethylaminopyridine. The reaction mixture was stirred at room temperature for 2 h. The contents of the reaction mixture were poured into 50 mL water and extracted with 3×30 mL chloroform. The choloroform layer was washed with water and 2×20 mL aq sodium carbonate (5%) solution, and brine. After drying over anhydrous sodium sulfate and filtration, solvent was removed to give a crude residue that was re-dissolved in the minimal amount of chloroform and chromatographed on silica gel, using chloroform/methanol 99/1. The fractions corresponding to the desired product were pooled and concentrated to give a white solid. 257 mg. This material was re-dissolved in the minimal amount of dichloromethane to this was added 2.5 mL 2 N HCl

in ether. The white solid that separated after standing for 50 min was filtered and washed with dry ether to give the HCl salt of 1250 mg. EIMS $(M+H)^+$: 320.

20. **Procedure for human p38α kinase assay**: Compounds were assayed in an in vitro method which measured the incorporation of radiolabeled ATP into a peptide substrate. Compounds were dissolved in DMSO and diluted into water to the desired concentrations. Compounds were mixed with the enzyme reagent, and the reactions are initiated by the addition of a 4X substrate cocktail containing 500 μg/mL biotin-peptide substrate and 0.2 mM ATP (+200 μCi/mL gamma-^{32}P-ATP). Final assay conditions were 25 mM MOPS, pH 7.0, 26.25 mM beta-glycerol phosphate, 80 mM KCl, 22 mM $MgCl_2$, 3 mM $MgSO_4$, 1 mg/mL gelatin, 0.625 mM EGTA, 1 mM DTT, 125 μg/mL peptide substrate, 50 μM ATP, and 36 nM p38α. After a 40-min incubation at room temperature, the reactions were stopped by the addition of 10 μL per reaction of 0.25 M phosphoric acid. A portion of each of the reactions was spotted onto the well of a SAM^2 ™ streptavidin filter plate (Promega Corporation, Madison WI, USA). The plates were washed 4× in 2M NaCl, 4× in 2 M NaCl with 1% phosphoric acid, 2× in water, and briefly in 95% ethanol. The plates were dried and liquid scintillation cocktail was added to the wells. Counts incorporated were determined on a scintillation counter. Relative enzyme activity at each compound concentration was calculated by subtracting background counts (counts measured in the absence of enzyme) from each result, and comparing the resulting counts to those obtained in the absence of inhibitor. The IC_{50} is the concentration of compound which reduced the incorporation of ATP into the substrate by 50%, when compared with the no-inhibitor control reactions.

21. Compounds **2** [EIMS $(M+H)^+$: 320], **3** [ESIMS $(M+H)^+$: 320], **4** [ESIMS $(M+H)^+$: 320] and **5** [ESIMS $(M+H)^+$: 320] **6** [EIMS M^+: 350], **7** [ESIMS $(M+H)^+$: 348], **8** [ESIMS $(M+H)^+$: 378], **9** [ESIMS $(M+H)^+$: 378], and **10** [ESIMS $(M+H)^+$: 378] were synthesized using appropriate modifications of the procedure described for the synthesis of **1**.

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25. **Synthesis of *trans*-2S,5R-dimethyl-4-benzyl-piperazine:** To a solution of racemic *trans*-2,5-dimethyl-4-benzylpiperazine (59 g, 0.29 mol) in methanol (150 mL) was added a solution of (+) tartaric acid (87 g, 0.58 mol) in methanol (250 mL) dropwsie over 5 min. Crystallization was effected by keeping the resulting mixture at 0 °C for 48–72 h. Scratching of the solution after 12–16 h facilitates the crystallization process. The mixture was filtered and washed with cold methanol and dried to give the tartaric acid salt (73.9 g) as white crystals. A single re-crystallization from methanol, cooling to room temperature afforded the salt as white crystals. (58 g) $[\alpha]_D = +47°$, (*c* 1.00, methanol). The enantiomerically pure salt was neutralized by dissolution in 350 mL, methylene chloride and treatment with 1 N NaOH, (3×100 mL) drying of the organic layer over anhydrous magnesium sulfate and filtration was followed by concentration on a rotary evaporator to give 18.6 g of the free base of *trans*-2*S*,5*R*-dimethyl-4-benzylpiperazine.

26. **Synthesis of *trans*-2R,5S-dimethyl-4-benzyl-piperazine:** To a solution of racemic *trans*-2,5-dimethyl-4-benzylpiperazine (59 g, 0.29 mol) in methanol (150 mL) was added a solution of (−) tartaric acid (87 g, 0.58 mol) in methanol (250 mL) dropwise over 5 min. Crystallization was effected by keeping the resulting mixture at 0° C for 48–72 h. Scratching of the solution after 12–16 h facilitates the crystallization process. The mixture was filtered and washed with cold methanol and dried to give the tartaric acid salt (68.4 g) as white crystals. A single re-crystallization from methanol, cooling to room temperature afforded the salt as white crystals. (53 g) $[\alpha]_D = -48.1°$ (*c* 1.00, methanol). The enantiomerically pure salt was neutralized by dissolution in 450 mL, methylene chloride and treatment with 1 N NaOH, (3×100 mL) drying of the organic layer over anhydrous magnesium sulfate and filtration was followed by concentration on a rotary evaporator to give 15.4 g of the free base of *trans*-2*R*,5*S*-dimethyl-4-benzylpiperazine.

27. **Computational methods:** The docking, conformation searching and energy calculation were done using the MOE software package. Systematic conformation searches were carried out in lower 7 kcal/mol energy windows and potential energies of resulting conformations were calculated using the MMFF94s force field. After removing the original ligand and water molecules, the ATP pocket of published crystal structure 1BL6 was used in the docking studies reported in this publication.