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The Design, Synthesis and Activity of Non-ATP Competitive Inhibitors of pp60$^{c\text{-}src}$ Tyrosine Kinase. Part 2: Hydroxyindole Derivatives

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Abstract—As part of a continuing effort to identify novel scaffolds that inhibit the pp60$^{c\text{-}src}$ protein tyrosine kinase, a series of hydroxyindole amides was rationally designed and synthesized. The most potent derivative was found to bind non-competitively with respect to ATP.

Protein tyrosine kinases (PTKs) are enzymes that catalyze the transfer of the γ-phosphate of ATP to the hydroxyl group of tyrosine in proteins. pp60$^{c\text{-}src}$ is the parent of the *'src'* sub-class of PTKs known as the non-receptor PTKs. pp60$^{c\text{-}src}$ binds to various growth factor receptor PTKs, as well as the focal adhesion associated PTK. Formation of these signaling complexes results in synergistic activation of the associated PTKs, triggering enhanced cell growth and motility, respectively.[1] Elevated pp60$^{c\text{-}src}$ activity has also been associated with increased metastatic potential and VEGF mRNA expression.[2,3] Inhibition of pp60$^{c\text{-}src}$ in tumor cells overexpressing growth factor receptor PTKs induces growth arrest, triggers apoptosis, and reverses the transformed phenotype.[4] Importantly, many tumor cells appear to require elevated pp60$^{c\text{-}src}$ activity whereas, in normal cells, other members of the *src* family of PTKs are able to compensate for pp60$^{c\text{-}src}$ inhibition.[5,6] These and other reports suggest that inhibition of pp60$^{c\text{-}src}$ might provide the following four benefits for cancer therapy: (1) inhibition of uncontrolled tumor cell growth caused by autocrine growth factor loops; (2) inhibition of metastasis due to triggering apoptosis upon breaking free from the cell matrix; (3) inhibition of tumor angiogenesis via reduced VEGF levels; and (4) low toxicity.

In the preceding communication,[5] the structure-based design of a series of pp60$^{c\text{-}src}$ inhibitors utilizing a naphthalene scaffold was described. These compounds were designed to bind in the peptide substrate site because of the potential for greater selectivity and efficacy in a cellular environment relative to the alternative ATP substrate site. The current report presents an extension of these design concepts to a series of pp60$^{c\text{-}src}$ inhibitors based upon an indole scaffold. Once again the crystal structure of the autoinhibited insulin receptor PTK (IRTK) was used to carry out qualitative molecular modeling studies, except in this case an indole ring was superimposed upon the IRTK Tyr 1162. This superimposition indicated that the indole NH can mimic the Tyr 1162 NH, that a carbonyl should be placed at the 2-position, and a hydroxyl group at the 5-position to mimic the Tyr 1162 carbonyl and OH, respectively (Scheme 1). The conceptual cyclization of Tyr 1162 to the smaller five-membered ring of an indole illustrated in Scheme 1, relative to a six-membered ring in the case of the naphthalene scaffold,[5] results in a movement of the optimal positioning of the OH from carbon 6 in the naphthalene scaffold to carbon *5* in the indole scaffold (also see the modeling results described later).

The indole amide derivatives containing hydroxy phenyl/benzyl side chains **2d–f**, **2j–l** (Table 1), respectively, were selected based upon the increase in pp60$^{c\text{-}src}$ inhibitor potency observed for the analogous naphthalene-based hydroxy phenyl amides reported in the previous paper.[5] The corresponding methyl ethers **2a–c**,**g–i**,**v** are precursors

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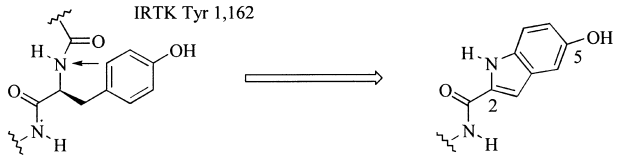


Scheme 1.

in the synthesis. The additional analogues shown in Table 1 were prepared to begin expanding the range of side chains beyond the hydroxy/methoxy groups that have now been extensively probed with both the indole and naphthalene scaffolds.

The indole amides containing only hydroxy or methoxy side chains were synthesized as illustrated in Scheme 2.

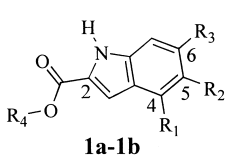
The 2-indolecarboxylic acid derivative, the methoxyphenyl amine (1.1 equiv, Aldrich, Lancaster or Fluka), and the coupling reagent PyBOP[7] (1 equiv, Fluka) were dissolved in anhydrous DMF. The solution was cooled to 0 °C under argon and then diisopropylethylamine (DIEA, 3 equiv) was added. The reaction was stirred at 0 °C for 1 min followed by 1 h at rt. After work up the residue was purified by silica gel chromatography.

The methyl ethers were cleaved with boron tribromide (1 M in DCM, Aldrich) when desired. The indole amide methyl ether was suspended in dry DCM and cooled to −78 °C under argon. One equivalent of BBr_3 was added for each heteroatom in the starting material plus one excess equivalent. The resulting dark red solution was stirred at −78 °C for 30 min and then at rt for 1–2 h. The reaction was quenched with water (10 min) before work up.

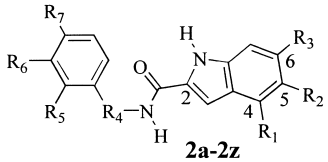
Using this synthetic route, the series of 5-hydroxyindole amide inhibitors **2a**–**m**,**y**,**z** were prepared from 5-hydroxy-2-indolecarboxylic acid. The 4- and 6-hydroxyindole amides (**2x**,**u**, respectively) were synthesized from methyl 4-methoxy-2-indolecarboxylate and methyl 6-methoxy-2-indolecarboxylate, respectively. The 5,6-dihydroxyindole amide **2t** was prepared from ethyl 5,6-dimethoxyindole-2-carboxylate. Sonication of the esters in 1 N NaOH for l h provided the corresponding carboxylic acids for coupling. The des-hydroxy indole amides **2v**,**w** were synthesized from indole-2-carboxylic acid. All of the indole starting materials were commercially available (Aldrich or Lancaster).

The fluoro inhibitors **2r**,**s** were likewise prepared from the corresponding fluorophenyl amines (Aldrich). The

Table 1. pp60[c-src] Inhibitory activity of hydroxymdole derivatives[a–c]



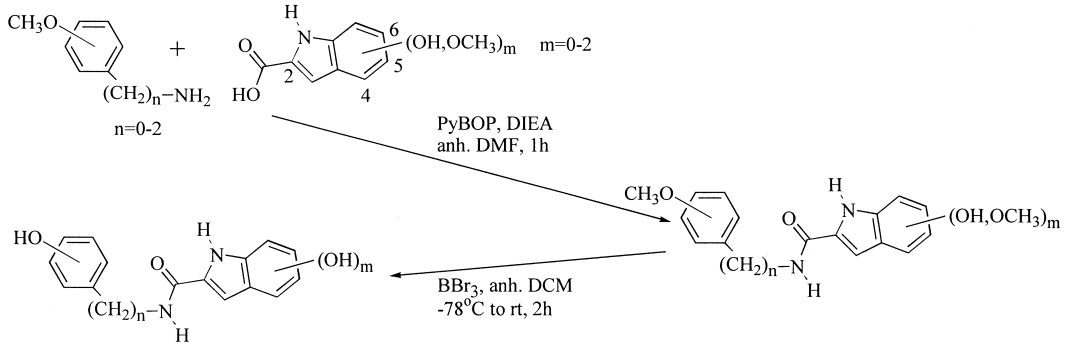
1a-1b



2a-2z

Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	% Inhibition at 100 μM (SD)
1a	H	OH	H	CH_3	N/A	N/A	N/A	40 (±5) (at 500 μM)
1b	H	OH	OH	CH_2CH_3	N/A	N/A	N/A	28 (±3)
2a	H	OH	H	—	OCH_3	H	H	3 (±1)
2b	H	OH	H	—	H	OCH_3	H	21 (±2)
2c	H	OH	H	—	H	H	OCH_3	39 (±9)
2d	H	OH	H	—	OH	H	H	43 (±1)
2e	H	OH	H	—	H	OH	H	30 (±6)
2f	H	OH	H	—	H	H	OH	45 (±3)
2g	H	OH	H	CH_2	OCH_3	H	H	21 (±5)
2h	H	OH	H	CH_2	H	OCH_3	H	7 (±6)
2i	H	OH	H	CH_2	H	H	OCH_3	18 (±4)
2j	H	OH	H	CH_2	OH	H	H	24 (±3)
2k	H	OH	H	CH_2	H	OH	H	74 (±2) (IC_{50} = 38 μM)
2l	H	OH	H	CH_2	H	H	OH	54 (±2)
2m	H	OH	H	CH_2CH_2	H	H	OH	21 (±7)
2n	H	OH	H	CH_2	H	H	CO_2H	Not active
2o	H	OH	H	CH_2	H	H	CO_2CH_3	11 (±4)
2p	H	OH	H	—	H	H	CH_2CO_2H	7 (±6)
2q	H	OH	H	—	H	H	$CH_2CO_2CH_3$	32 (±7)
2r	H	OH	H	—	H	F	H	21 (±7)
2s	H	OH	H	CH_2	H	F	H	57 (±6)
2t	H	OH	OH	CH_2	H	OH	H	26 (±2)
2u	H	H	OH	CH_2	H	OH	H	56 (±6)
2v	H	H	H	CH_2	H	H	OCH_3	4 (±4)
2w	H	H	H	CH_2	H	H	OH	36 (±4)
2x	OH	H	H	CH_2	H	OH	H	60 (±3)
2y	H	OH	H	$CH(CH_3)R$	H	OH	H	15 (±3)
2z	H	OH	H	$CH(CH_3)S$	H	OH	H	13 (±7)

[a]All compounds were tested as described in the preceding paper.[5]
[b]All compounds were characterized by proton NMR, FAB(+) MS and are pure by TLC.
[c]N/A, not applicable.



Scheme 2.

inhibitors containing esters or carboxylic acids on the amide side chain, **2n–q**, were prepared from the corresponding amino carboxylic acids (Aldrich). The side chain carboxylic acid was first protected as a methyl ester (anhyd MeOH pre-saturated with HCl, reflux, 1 day), followed by PyBOP coupling (as above), then saponification back to the carboxylic acid when desired.

The methyl ester **1a** was prepared by refluxing a solution of the carboxylic acid overnight in anhydrous methanol pre-saturated with HCl gas. The ethyl ester **1b** was prepared by BBr_3 deprotection of ethyl 5,6-dimethoxyindole-2-carboxylate as above. All of the inhibitors listed in Table 1 were purified by silica gel chromatography.

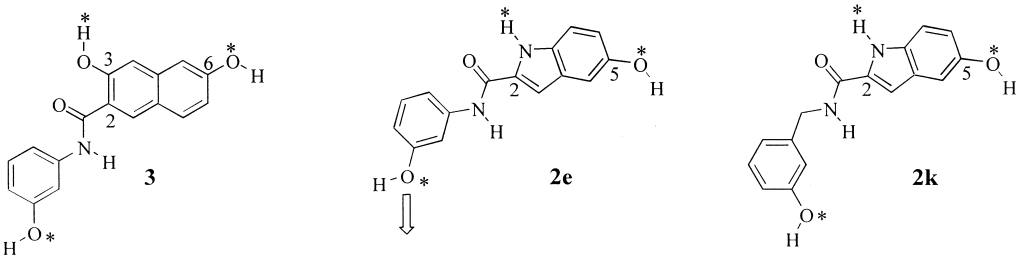
As in the preceding paper,[5] the rank order activity of this series of $pp60^{c\text{-}src}$ inhibitors was first determined at a constant inhibitor concentration (Table 1). The same inhibitor concentration (100 μM) was used for the current indole series of inhibitors, the previous naphthalene series of inhibitors, and five non-ATP competitive literature PTK inhibitors (see Part 1 of this paper). This allowed an efficient rank order comparison of 59 compounds in total under identical assay conditions.

The modeling studies predicted that a hydroxy group at carbon 5 of the indole scaffold would be optimal. Comparison of the 5-hydroxy indole inhibitor **2k** (74%) with the analogous 6-hydroxy indole inhibitor **2u** (56%) and 4-hydroxy indole inhibitor **2x** (60%) confirms this prediction, although the preference is not strong. The prediction that a hydroxy group at carbon 5 will improve the activity (relative to no hydroxy group) is confirmed by comparing the 5-hydroxy indole inhibitor **2l** (54%) with the corresponding des-hydroxy inhibitor **2w** (36%).

Extending the indole inhibitors as aryl amides at carbon 2 improved potency, as expected based upon the previous naphthalene inhibitors. For example, the *meta*-hydroxybenzyl amide indole **2k** gives 74% inhibition at 100 μM whereas the analogous methyl ester **1a** gives only 40% inhibition at 500 μM. Interestingly, comparing the 5,6-dihydroxy ethyl ester **1b** (28%) to the corresponding aryl amide **2t** (26%) shows that the *simultaneous* presence of the second hydroxy at carbon 6 prevents the potency enhancement normally provided by the otherwise preferred *meta*-hydroxybenzyl amide side chain. This amide side chain was the best of the current series when the 5-hydroxyl group is present alone (**2k**, 74%) and still gave good inhibition when a 6-hydroxy group was present alone (**2u**, 56%). Also, the simultaneous presence of two hydroxy groups at carbons 5 and 6 seems well tolerated in the absence of an amide side chain (**1b** versus **1a** and **2e**). This data suggests that a change in the binding orientation of the indole scaffold may have occurred due to the presence of the second hydroxy group and that a different amide side chain may now be preferred. The optimal combination of side chains at carbons 4–7 (including functional group replacements for hydroxy groups[8]) and amide side chains is currently under investigation.

In general, the indole scaffold structure–activity relationships (SARs) revealed by the data in Table 1 parallels that reported in the preceding paper for the naphthalene scaffold. In both cases positioning a hydroxy group on the scaffold analogous to the Tyr 1162 OH, as identified by modeling studies, provided the highest potency. Moving this hydroxy group to one of the adjacent carbons reduced the potency, but not dramatically, in both cases. Extending both scaffolds with aryl amides at the position identified by the modeling studies to mimic the Tyr 1162 peptide bond improved the potency. With both scaffolds, substitution of a methoxy group for the hydroxy groups on the amide side chain usually reduced potency, and did so to a greater extent with the longer benzylamide side chain (e.g. **2k**, 74% versus **2h**, 7% compared to **2e**, 30% versus **2b**, 21%). The major difference in the SARs for these two scaffolds is that the 5-hydroxyindole scaffold prefers the longer *m*-hydroxybenzyl amide side chain (**2k**, 74% versus **2e**, 30%) whereas the analogous 3,6-dihydroxynaphthalene scaffold prefers the shorter amide side chain derived from *m*-hydroxyaniline. The 5-hydroxyindole scaffold showed essentially no preference for the position of the hydroxyl group on the shorter amide side chain (**2d–f**) whereas with the longer hydroxybenzyl amide side chain a significant preference for the *meta* position was observed (**2j–l**). In the case of the 3,6-dihydroxynaphthalene scaffold the opposite was observed.

Additional molecular modeling studies were carried out to further probe the preference for a longer amide side



Scheme 3.

chain with the indole scaffold. The most active naphthalene inhibitor **3** from the previous report was used as a template upon which the analogous indole inhibitor **2e** and the homologated indole inhibitor **2k** were superimposed. The three most important side-chain functionalities in naphthalene inhibitor **3** are considered to be the 6-hydroxy group (H-bond donor and acceptor), the hydrogen from the 3-hydroxy group (H-bond donor), and the side-chain hydroxy group (H-bond acceptor) based upon the rational design and SAR for both series of inhibitors. This three point pharmacophore model is identified in both series by asterisks in Scheme 3.

The 'multifit', energy minimization and 'fit atoms' facilities within SYBYL™ (6.4, Tripos, St Louis) were used in sequence to superimpose **2e** and **2k** onto **3**. This overall fitting process was carried out with spring constants (multifit) and weights (fit atoms) chosen such that the highest emphasis was on optimally superimposing the scaffold pharmacophore Os and Hs (100), followed by the side chain Os (10) and then the intervening amide bond (1). The 'multifit' process adjusted the conformations for maximum pharmacophore fit, the subsequent minimization produced the nearest local minimum energy conformations and finally the 'fit atoms' process produced the best pharmacophore superimposition of these minimized conformations. As expected, the scaffold pharmacophore Os and Hs of both **2e** and **2k** superimposed closely and similarly upon the corresponding atoms in **3** (all within ca. 0.50 Å). However, the side chain pharmacophore Os of **2e** and **2k** differed significantly in their superimposition on the corresponding O of **3**, with displacements of 1.8 Å versus only 0.08 Å, respectively. This close fit of the three key pharmacophore sites between **2k** and **3** provides a rationalization for their potency differing by only a factor of 2.4 (IC_{50}s 38 μM versus 16 μM, respectively).

Extending the amide side chain by another carbon atom reduced the activity (**2m**, 21% versus **2l**, 54%). Adding a methyl group to the benzylic carbon of **2k**, in either stereochemistry, greatly reduced the activity (**2y**, 15% and **2z**, 13% versus **2k**, 74%). Replacing the side-chain hydroxy group (in the *para* position) with a carboxylate anion (**2n**, 0% versus **2l**, 54% and **2p**, 7% versus **2f**, 45%) reduced the activity whereas the corresponding methyl esters (**2o**, 11% and **2q**, 32%, respectively) showed a smaller loss of potency. Importantly, replacing the side chain hydroxy group with a fluorine maintained much of the potency (**2s**, 57% versus **2k**, 74% and **2r**, 21% versus **2e**, 30%). Consequently, the fluoro analogue **2s** has only one hydroxy group left for potential Phase II metabolism (e.g. glucuronide formation), and this remaining hydroxy group is a current target for replacement.[8]

Using the same method as in the preceding paper,[5] the most potent inhibitor from the current indole series (**2k**) was analyzed for ATP competition by monitoring the % inhibition at increasing [ATP] while holding the inhibitor concentration constant. Since the [ATP] had little effect on the % inhibition,[9] **2k** is non-competitive with respect to ATP under these assay conditions.

In summary, an indole scaffold has been designed, and an initial SAR carried out, for the development of non-ATP competitive $pp60^{c\text{-}src}$ inhibitors. The potency of the best indole-based inhibitor from the current series was found to be close to that of the best naphthalene-based inhibitor from the preceding paper.

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