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# Aryl-indolyl maleimides as inhibitors of CaMKIIδ. Part 2: SAR of the amine tether

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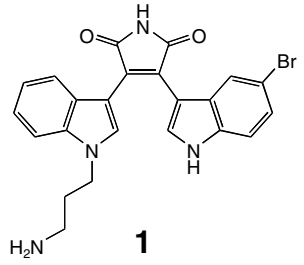
**Abstract**—A family of aryl-substituted maleimides was prepared and studied for their activity against calmodulin-dependant kinase. Inhibitory activities against the enzyme ranged from 34 nM to >20 μM and were dependant upon both the nature of the aryl group and the tether joining the basic amine to the indolyl maleimide core. Key interactions with the kinase ATP site and hinge region, predicted by homology modeling, were confirmed.


Calcium is critical to cardiac[1–3] and neuropathic[2,4,5] signaling pathways. Additionally, calcium is an important second messenger controlling apoptosis, cell cycle regulation, gene expression, and hormone signaling processes.[6] To induce these responses, calcium forms a complex with calmodulin in order to and activate the family of $Ca^{2+}$/calmodulin-dependant protein kinases (CaMKs).[6]

The family of CaMKs consists of three types, among which multiple tissue specific isoforms are known. For example, CaMKIIα and β are found primarily in neural tissue[7] while CaMKIIδ is found in cardiac tissue.[8] Previous work in our group[9] identified compound **1** (Fig. 1) as a 34 nM inhibitor of CaMKIIδ.

As shown in Figure 2, compound **1** was docked into a homology model of CaMKIIδ and key interactions within the ATP binding site and hinge region, previously identified, were verified.[9,10] Specific interactions include dual hydrogen bonds between the Glu100 hinge backbone and the maleimide as well as a salt bridge between Glu106 and the terminal amine. Additionally, optimal utilization of the hydrophobic binding pocket by the bromoindole was noted.




**1**

**Figure 1.** Lead aryl-indolyl maleimide.

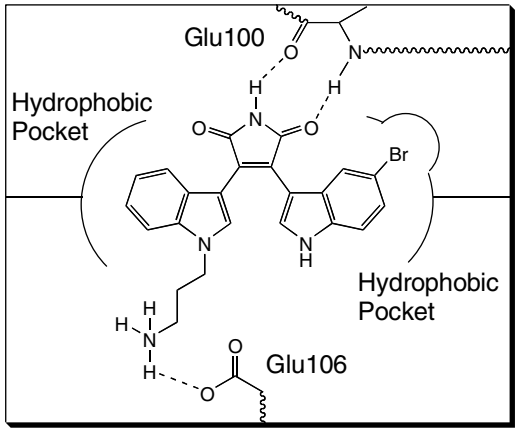



**Figure 2.** Interactions between compound **1** and the CaMKIIδ catalytic site as indicated by homology modeling.

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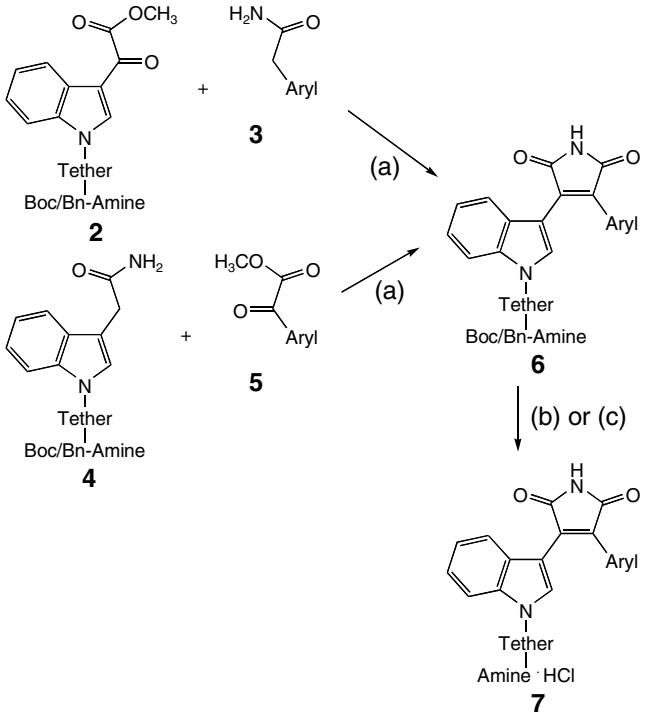
Based on the observation that the amine is tethered to the indolyl maleimide core via a flexible linker, our group became interested in the possibility of improving inhibitory activity by constraining the tether or utilizing additional substituents. In order to realize this goal, previously described chemistry[9] was employed where aryl glyoxalates were reacted with aryl acetamides to yield the desired maleimides under basic conditions (Scheme 1).[11] This chemistry reduced the synthetic problems to the preparation of suitably functionalized indole-3-acetamides or methyl indole-3-glyoxalates.

Referring to the structures listed in Tables 1 and 2, compounds **6a–c**, **7b–f**, and compounds **7h–i** were prepared according to Scheme 1 using acetamides prepared from commercially available arylacetic acids as shown in Scheme 2. Of the required glyoxylates, commercially available methyl 3-indoleglyoxylate and methyl (1-methylindole)-3-glyoxylate were utilized. The 1-(3-aminopropylindole) glyoxylate required for compounds **7a–d** was prepared as previously described.[9]

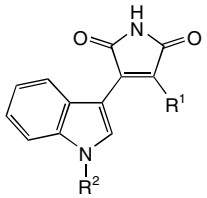
Regarding the structures in Table 4, the required indoleglyoxylates were prepared from commercially available 3-(1-indole)- and 4-(1-indole)piperidine. As shown in Scheme 3, protection of the piperidine nitrogen was followed by treatment with oxalyl chloride and methanol giving the desired compounds **2**.

Having addressed various patterns of rigidity and the structural requirements of the amine-tether combination, interest turned to actual substitutions on the tether itself. In accessing this SAR, compound **7q** (Table 5) was prepared using the chemistry described in Scheme 4. As illustrated, compound **7h** (prepared using previously described chemistry)[9] was first reacted with epibromohydrin and then with ammonia thus providing the desired 1,2-amino alcohol.




**Scheme 1.** Reagents: (a) $^{t}$BuOK, THF, 40–80%; (b) HCl, dioxane, MeOH, 100%; (c) $H_2$, 10% Pd/C, MeOH, 50–80%.

**Table 1.** SAR indicating the relationship between the amino group and potency



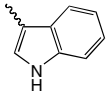
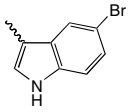
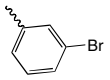
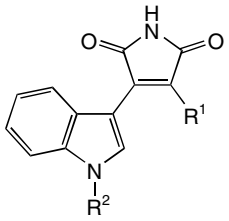
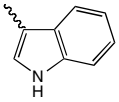
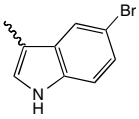
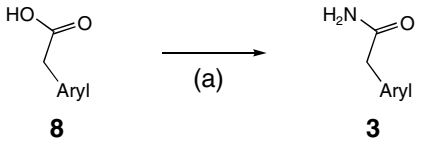

| Compound | $R^1$ | $R^2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **7a**[13] |  | $CH_2CH_2CH_2NH_2$ | 0.38 ($n$ = 1) |
| **7g**[13] | | H | 1.19 ($n$ = 1) |
| **7b** |  | $CH_2CH_2CH_2NH_2$ | 0.034 ± 0.009 ($n$ = 3) |
| **7e** | | $CH_3$ | 0.87 ($n$ = 1) |
| **7c** |  | $CH_2CH_2CH_2NH_2$ | 1.35 ± 0.64 ($n$ = 2) |
| **7f** | | $CH_3$ | >20 ($n$ = 1) |
| **7h** | | H | >20 ($n$ = 1) |
| **7d** |  | $CH_2CH_2CH_2NH_2$ | 0.18 ± 0.06 ($n$ = 3) |
| **7i** | | H | 3.53 ($n$ = 1) |

**Table 2.** SAR indicating the dependence of amine basicity on potency



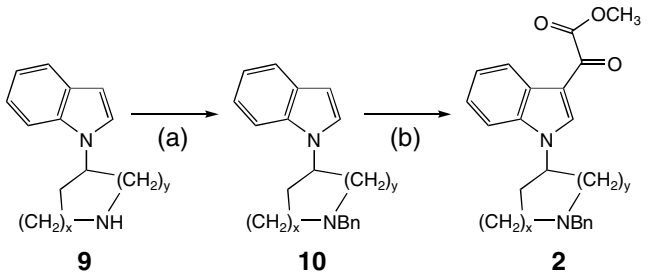

| Compound | $R^1$ | $R^2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **7a**[13] |  | $CH_2CH_2CH_2NH_2$ | 0.38 ($n$ = 1) |
| **6a** | | $CH_2CH_2CH_2NHBoc$ | 3.81 ± 0.25 ($n$ = 2) |
| **7b** |  | $CH_2CH_2CH_2NH_2$ | 0.034 ± 0.009 ($n$ = 3) |
| **6b** | | $CH_2CH_2CH_2NHBoc$ | 7.54 ($n$ = 1) |
| **7c** |  | $CH_2CH_2CH_2NH_2$ | 1.35 ± 0.64 ($n$ = 2) |
| **6c** | | $CH_2CH_2CH_2NHBoc$ | >20 ($n$ = 2) |

$IC_{50}$ values[12] for all tested compounds are listed in the following tables. As shown in Table 1, the importance



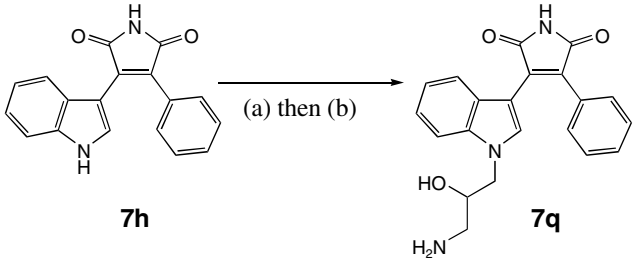

8 3

**Scheme 2.** Reagents: (a) CDI, THF then $NH_3$ (0.5 M in MeOH), 94%.




9 10 2

**Scheme 3.** $x = 1$, $y = 2$ or $x = 2$, $y = 1$. Reagents: (a) BnCl, $K_2CO_3$, $H_2O$, $CH_2Cl_2$, 60–70%; (b) oxalyl chloride, $CH_2Cl_2$ then MeOH, 70–80%.




7h 7q

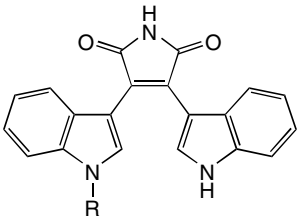
**Scheme 4.** Reagents and condition: (a) NaH, DMF, epibromohydrin; (b) $NH_3/H_2O$, 100 °C, sealed tube, 24% (2 steps).

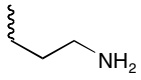
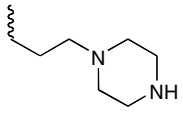
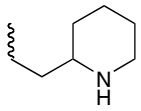
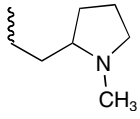
of an amine tethered to the indole moiety was confirmed via the study of a series of amines as compared to indoles with N–$CH_3$ or N–H substitutions. The data illustrate a drop in potency from 3- to 20-fold over the corresponding amine-based compounds.

As an extension to the importance of an amino group tethered to the indole, the importance of basicity was addressed via a comparison of the potency of amine-based analogs to their corresponding NH-Boc derivatives. The data, shown in Table 2 and summarized from a previous report,[9] demonstrate that elimination of the amine basicity results in potency losses ranging from 10- to 220-fold. While one can argue that the steric bulk of the Boc group could be interfering with the ability of the inhibitors to bind in the active site, homology modeling indicates that the salt bridge is near the solvent front and that there is sufficient space available to accommodate large groups. Furthermore, if binding was truly impaired, one might expect substantially larger reductions in potency for parent compounds that do not possess optimized hydrophobic groups such as 5-bromoindole.

In dealing with entropic factors, a study was conducted in order to elucidate the effect of tying the amine group into a more rigid cyclic structure. The data, summarized in Table 3, indicate that such modifications result in potency losses ranging from 5- to 8-fold based on the parent tethered primary amine. These results may simply result from the inhibitors' inabilities to present the amine groups to Glu106 while maintaining low-energy conformations.

**Table 3.** SAR related to the incorporation of cyclic amines connected to the indole via flexible tethers



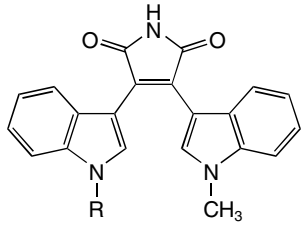

| Compound | R | $IC_{50}$ (μM) |
|---|---|---|
| **7a**[13] |  | 0.38 ($n = 1$) |
| **7j**[14] |  | 2.02 ($n = 1$) |
| **7k**[14] |  | 1.45 ($n = 1$) |
| **7l**[13] |  | 1.89 ($n = 1$) |

Continuing with the exploration into the entropic effects of flexible tethers, rigidified tethers were incorporated into our studies. As illustrated in Table 4, such modifications were generally well tolerated with equipotent activities noted between the parent structure and the 3-piperidyl analog. Conversion of a cyclic secondary amine to a bulky tertiary amine did result in further loss in potency presumably due to an interruption in the ability of the inhibitor to present the rigid amine to Glu106 in a low-energy conformation.

Finally, with respect to direct substitution of the tether with additional functional groups, addition of a hydroxyl group demonstrated the potential for increased activity. As shown in Table 5, incorporation of this group onto the parent phenyl maleimide resulted in a 3-fold increase in potency. While this is only a representative example, this result supports further exploration in this area. Specifically, the stereochemistry and the nature of the functional group substitution are of interest.

In summary, synthetic and commercially available arylindolyl maleimides were studied for their activity against CaMKIIδ. A specific emphasis was placed on

**Table 4.** SAR related to the incorporation of rigid cyclic amines directly connected to the indole



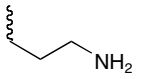
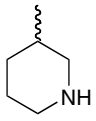
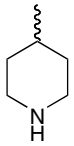
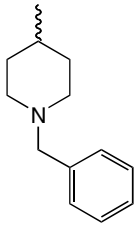
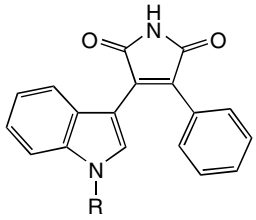
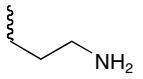
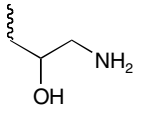
| Compound | R | $IC_{50}$ (μM) |
|---|---|---|
| **7m**[13] |  | 0.31 ($n$ = 1) |
| **7n** |  | 0.30 ($n$ = 1) |
| **7o** |  | 0.64 ($n$ = 1) |
| **7p** |  | 1.87 ± 0.18 ($n$ = 2) |

**Table 5.** Effect of hydroxyl substitution on tether



| Compound | R | $IC_{50}$ (μM) |
|---|---|---|
| **7c** |  | 1.35 ± 0.64 ($n$ = 2) |
| **7q** |  | 0.45 ($n$ = 1) |

observing the influence of the amine tether on potency. While primary acyclic amines generally demonstrated better activity than cyclic secondary or cyclic tertiary amines, rigid amine-tether combinations exhibited equipotent activity to the previously identified leads. In one specific example, hydroxylation of the tether indicated that enhanced activity over the parent may be achieved.

## References and notes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9. Levy, D. E.; Wang, D.-X.; Lu, Q.; Chen, Z.; Perumattam, J.; Xu, Y.-J.; Liclican, A.; Higaki, J.; Dong, H.; Laney, M.; Mavunkel, B.; Dugar, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2390.
10. An homology model of autoinhibited CaMKIIδ was built based on crystal structure 1A06 of autoinhibited rat CaMKI. Because rat CaMKI shows high sequence homology with CaMKIIδ, this model was used to study inhibitors that were not ATP competitive. Due to the lack of availability of a crystal structure of activated CaMKIIδ, homology models were built based on crystal structures 1CDK, 1PHK, and 1KOB. From these homology models, we selected the one that best explained the SAR. The accuracy of this model was validated using point mutation studies.
11. Faul, M. M.; Winneroski, L. L.; Krumrich, C. A. *J. Org. Chem.* **1998**, *63*, 6053.
12. Assays were performed with inhibitor or suitable control solvent added 10 μl per well in a 96-well microtiter plate (Corning, NY). CaMKIIδ was diluted in enzyme buffer (50 mM PIPES pH 7, 0.2 mg/ml BSA, 1 mM DTT) and added 10 μl per well. Reactions were initiated with 30 μl reaction buffer (62.5 mM PIPES pH 7, 0.25 mg/ml BSA, 33.3 mM $MgCl_2$, 83 μM ATP, 0.4 mM $CaCl_2$, 8.3 μg/ml calmodulin, 25 μM [His 5] autocamtide-2, 120 nM [g-33P]ATP) and incubated at rt for 3 min. Reactions were terminated by transferring 25 μl to a UNIFILTER 96-well P81microplate (Whatman, UK), pre-wet with 15 μl 1% phosphoric acid. After 10 min, the plate was washed three times with 1% phosphoric acid and one time with 95% ethanol on a BiomekFX (Beckman Coulter, CA) equipped with a vacuum manifold. Plates were dried for approximately 60 min, scintillant was added to the wells, and the plates were read on a TopCount NXT Microplate Scintillation and Luminescence Counter (Perkin-Elmer, MA).
13. The following compounds were obtained from Calbiochem–Novabiochem: **7a** (cat # 203294), **7g** (cat # 203297), **7l** (cat # 203292) and **7m** (cat # 557508).
14. The following compounds were obtained from Sigma: **7j** (cat # B3681) and **7k** (cat # B3556).