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# Aryl–indolyl maleimides as inhibitors of CaMKIIδ. Part 3: Importance of the indole orientation

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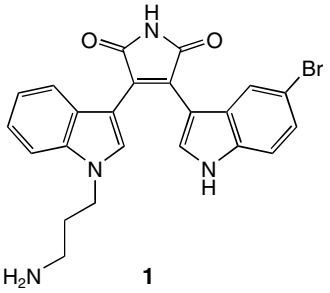
**Abstract**—A family of aryl-substituted maleimides was prepared and studied for their activity against calmodulin dependant kinase. Inhibitory activities against the enzyme ranged from 10 nM to >20 μM and were dependant upon both the nature of the aryl group and the tether joining the basic amine to the indolyl maleimide core of the inhibitors. 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 activate the family of $Ca^{2+}$/calmodulin-dependant protein kinases (CaMK)s.[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[9,10] identified compound **1** (Fig. 1) as a 34 nM inhibitor of CaMKIIδ.

Docking compound **1** into a homology model of CaMKIIδ verified previously identified interactions within the ATP binding site and hinge region.[9,10] Of particular interest are hydrogen bonding interactions involving Glu100 and Glu106 in conjunction with potentially optimal utilization of the hydrophobic binding pocket by the bromoindole moiety.




**1**

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

While exploring the SAR of compound **1**, we noted that repeat assays of previously described compounds[9] frequently resulted in increases of potency by as much as 10-fold. Following up on the composition of the original stock solutions, LCMS analysis revealed the presence a new peak (<20% as measured by HPLC) with identical mass to the original structure. These observations were made in comparison with freshly prepared solutions and stock solutions that were approximately three weeks old. Finally, this chemical transformation was noted in all samples where increased activities were observed. Representative results are shown in Table 1.[11]

We speculated that these observations were based on a photochemically induced Cope rearrangement (observed

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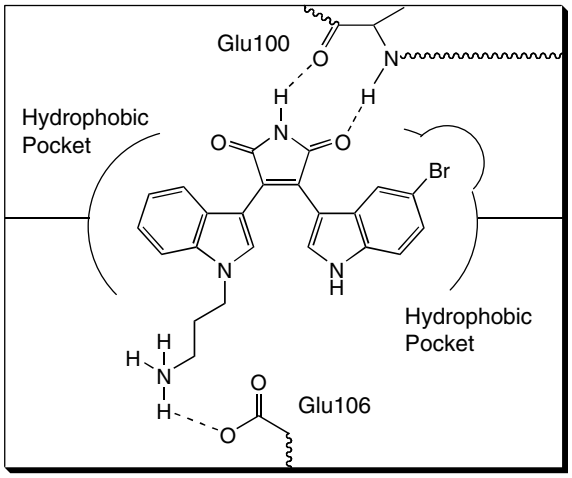
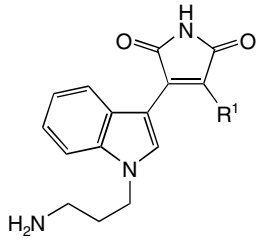
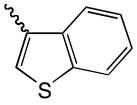



**Figure 2.** Interactions between compound **1** and the CaMKIIδ catalytic site as indicated by homology modeling.

**Table 1.** Potency variations between fresh and older samples



| Compound | $R^1$ | $IC_{50}$ (μM) fresh | $IC_{50}$ (μM) old |
|---|---|---|---|
| **2** |  | 0.36 ± 0.01 ($n$ = 2) | <0.039 |
| **3** |  | 1.35 ± 0.64 ($n$ = 2) | 0.23 |

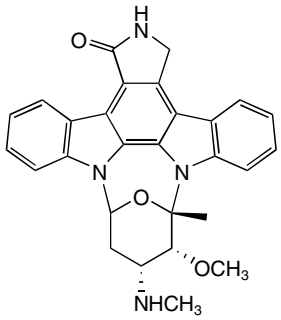
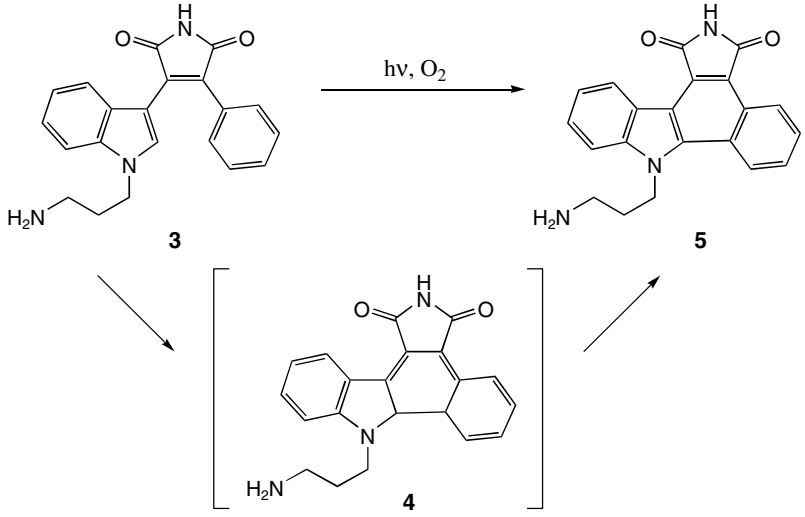


**Figure 3.** Structure of staurosporine.

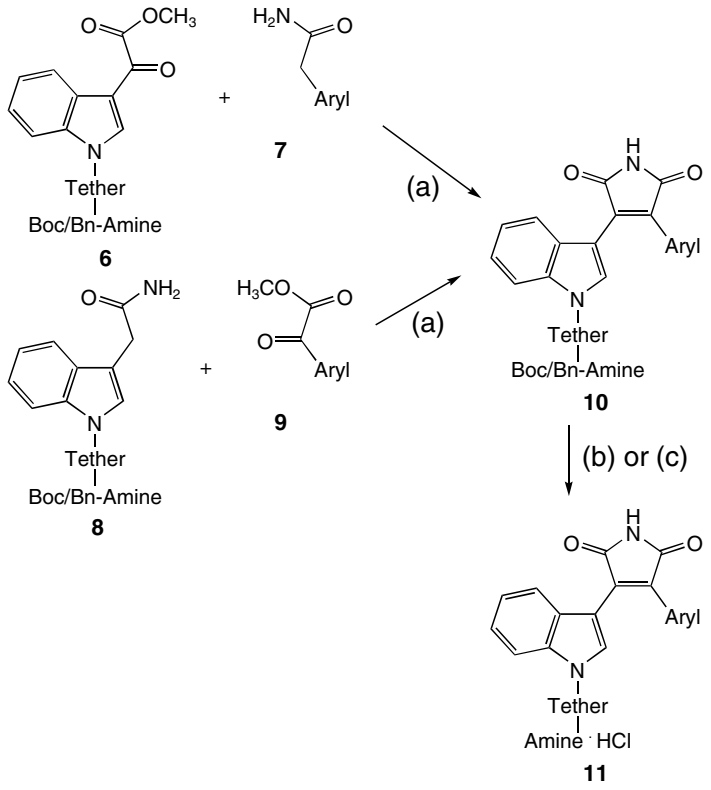
by LCMS analysis) followed by spontaneous oxidation as illustrated in Scheme 1. A survey of the literature revealed that such transformations are known.[12] Furthermore, according to our homology model, these planar structures are not postulated to bind in the same manner as the parent structures. A binding region exists adjacent to the ATP binding site which accommodates known potent and non-specific kinase inhibitors such as staurosporine (Fig. 3).[13]

In order to address the problem of spontaneous cyclization, we designed structures with impaired abilities to undergo the initial transformation to intermediates similar to structure **4**. Consequently, all compounds in this phase of our studies either incorporated substituents to sterically block cyclization or possessed interrupted conjugated systems incapable of participating in Cope-type rearrangements. Preparation of these compounds is generally illustrated in Scheme 2. Specifically, acetamides and methylglyoxalates were combined under basic conditions to generate maleimides.[9,10] Deprotection of the amino group yielded the final compounds.

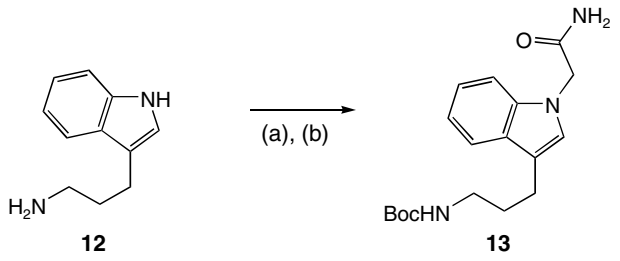
In order to generate the specific compounds of this study, indole *N*-acetamides were required. As shown in Scheme 3, commercially available aminopropylindole **12** was protected as its Boc analog and treated with




**Scheme 1.** Cyclization and oxidation of bis-aryl maleimides.




**Scheme 2.** Reagents: (a) $^{t}$BuOK, THF, 40–80%; (b) HCl, Dioxane, MeOH, 100%; (c) $H_2$, 10% Pd/C, MeOH, 50–80%.




**Scheme 3.** Reagents: (a) $(Boc)_2O$, $CH_2Cl_2$, 100%; (b) iodoacetamide, NaH, DMF, 70%.

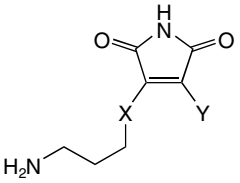
iodoacetamide in the presence of sodium hydride giving the required structure **13**. The conditions for the alkylation of indoles proved general and were used in the preparation of all indole *N*-acetamides required for this study. These compounds were then substituted for indole-3-acetamides **8** illustrated in Scheme 2. Finally, the preparation of the indazole based maleimide **19** was accomplished using published procedures for the synthesis of related structures.[14]

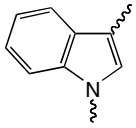
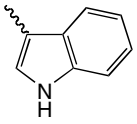
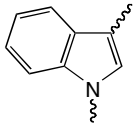
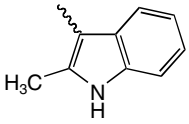
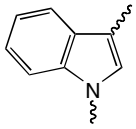
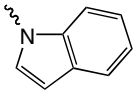
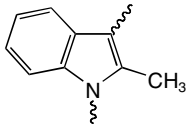
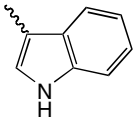
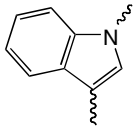
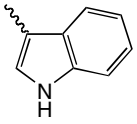
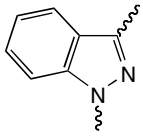
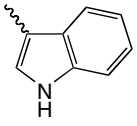
As shown in Table 2, an interesting SAR evolved out of our attempts to generate cyclization impaired structures. Compared to compound **14**,[9] inserting a methyl group on either the right hand or left hand indole ring was generally tolerated. This modification, intended to introduce a steric deterrent to cyclization, resulted in a 2-fold loss in potency for both compounds. Furthermore, replacing the left hand indole with an indazole was fully tolerated with no detrimental effects on potency. This modification was designed to block the oxidation step of the proposed cyclization. Finally, by flipping the indole rings, the conjugated system required for Cope-type cyclizations was blocked. These modifications provided the most striking observations, with potency highly dependant upon which indole ring was flipped. As shown, while compound **16** exhibited greater than a 10-fold reduction in activity, compound **18** was shown to be 2-fold more potent than the parent. Thus, this compound became our next generation lead.

Continuing with our improved lead, SAR studies were designed to assess the importance of the aminopropyl side chain. Previous reports, as well as our homology model, have alluded to the need for a basic group. Consequently, our SAR focused on tether rigidity and substitutions on the amine. All compounds in this study were prepared using chemistry described in this and previous reports.[9,10] Furthermore, all substituted indoles were obtained from commercial sources.

As shown in Table 3, the conversion of the amine to an alcohol lent further support to the importance of the presence of a basic group by causing a 30-fold drop in potency. Furthermore, converting the primary amine to a tertiary amine resulted in a 4-fold drop in potency. Cyclization of the aminopropyl group into a rigid piperidine was also not helpful and resulted in a significant loss in potency. An apparent breakthrough came when studying the importance of the tether length between the indole and the amine. Specifically, reducing the tether length from 3 to 2 carbons resulted in a 2-fold in-

**Table 2.** Activity of cyclization impaired bis-aryl maleimides compared to previously reported parent inhibitor[9,10]



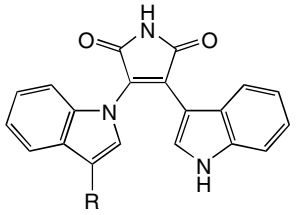
| Compound | X | Y | $IC_{50}$ (μM) |
|---|---|---|---|
| **14** |  |  | 0.38 ($n = 1$) |
| **15** |  |  | 0.75 ± 0.31 ($n = 2$) |
| **16** |  |  | 4.56 ($n = 1$) |
| **17** |  |  | 0.74 ($n = 1$) |
| **18** |  |  | 0.19 ± 0.01 ($n = 2$) |
| **19** |  |  | 0.38 ± 0.06 ($n = 2$) |

crease in activity. As no significant benefit was noted for the mono-methylation of the aminoethyl side chain, both tryptamine and *N*-methyltryptamine became our next platforms for SAR studies.

Having identified the utility of tryptamine in our analoging efforts, our attention turned to the importance of substitutions on the 5-position of the right hand indole ring. These studies were based on our early success in the identification of compound **1** coupled with our proposed binding model (Fig. 2). All compounds for this study were prepared using chemistry already described in this and previous reports.[9,10]

As shown in Table 4, analogs based on tryptamine and *N*-methyltryptamine were prepared and studied. For indole modifications that were made on both tryptamine and *N*-methyltryptamine, no significant differences were noted in enzyme inhibitory activity. With respect to specific indole substituents, Cl provided the best results. Incorporating more electron withdrawing substituents

**Table 3.** SAR related to the aminopropyl side chain



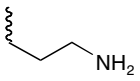
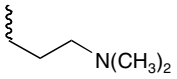
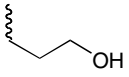
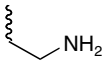
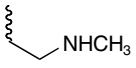
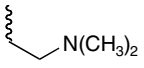
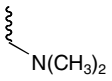
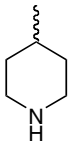
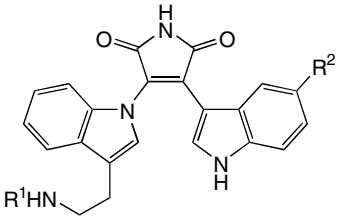
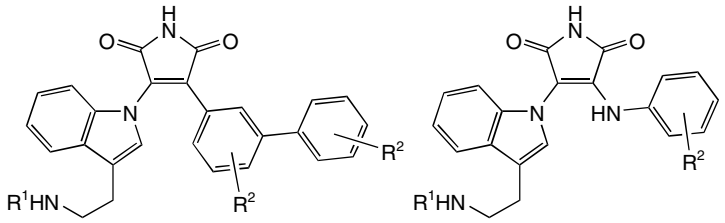
| Compound | R | $IC_{50}$ (μM) |
|---|---|---|
| **18** |  | 0.19 ± 0.01 ($n = 2$) |
| **20** |  | 0.74 ($n = 1$) |
| **21** |  | 6.00 ($n = 1$) |
| **22** |  | 0.11 ± 0.05 ($n = 2$) |
| **23** |  | 0.15 ($n = 1$) |
| **24** |  | 0.56 ± 0.10 ($n = 2$) |
| **25** |  | 2.41 ± 0.20 ($n = 2$) |
| **26** |  | 0.68 ± 0.003 ($n = 2$) |

**Table 4.** SAR of 5-substituted indoles



| Compound | $R^1$ | $R^2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **22** | H | H | 0.11 ± 0.05 ($n = 2$) |
| **23** | $CH_3$ | H | 0.15 ($n = 1$) |
| **27** | H | Cl | 0.010 ($n = 1$) |
| **28** | $CH_3$ | Cl | 0.013 ($n = 1$) |
| **29** | H | F | 0.038 ± 0.008 ($n = 2$) |
| **30** | H | $CF_3$ | 0.032 ($n = 1$) |
| **31** | H | CN | 0.046 ± 0.004 ($n = 2$) |
| **32** | $CH_3$ | $CH_2CH_3$ | 0.11 ± 0.03 ($n = 3$) |
| **33** | H | $OCH_3$ | 0.17 ± 0.06 ($n = 2$) |
| **34** | $CH_3$ | $CO_2CH_3$ | 0.021 ± 0.003 ($n = 2$) |
| **35** | $CH_3$ | $CO_2H$ | 0.38 ± 0.10 ($n = 2$) |
| **36** | $CH_3$ | $CONH_2$ | 0.19 ($n = 1$) |
| **37** | $CH_3$ | $CONHCH_3$ | 0.28 ($n = 1$) |
| **38** | $CH_3$ | $CON(CH_3)_2$ | 11.85 ($n = 1$) |




$R^1$ = H, $CH_3$
$R^2$ = H, Cl, Br, $OCH_3$, $CO_2H$, $CO_2CH_3$, $CONH_2$

**Figure 4.** Biaryl- and aniline-based maleimides studied as CaMKIIδ inhibitors.

such as F, $CF_3$ and CN resulted in 3- to 4-fold reductions in potency. This trend carried forward into carboxyl-based substituents. One dramatic observation is the activity change noted when moving from a primary to a secondary to a tertiary amide. While the primary and secondary amides are comparable in potency against CaMKIIδ, the tertiary amide is 60-fold weaker than the primary. Finally, the incorporation of electron donating substituents such as ethyl and methoxy results in 10- to 20-fold reductions in potency. While these modifications did not yield analogs with activity comparable to the corresponding Cl analog, these results may be due to steric interactions with the binding site associated with the C–O–C or C–C–C bond angles.

In addition to the compounds presented herein, additional SAR studies were carried out incorporating biaryl substituents and aniline-based groups. These structures, generically illustrated in Figure 4, were further substituted with various electron donating and withdrawing groups ranging from halogens to alkoxy groups to carboxyl-based functionalities. While the data are not included in this report, it is important to note that no benefit was realized when these groups were used as replacements for the right hand indole system. Further details will be presented in a future report.

In summary, novel bis-aryl maleimides were prepared and tested for activity against CaMKIIδ. Problems associated with spontaneous cyclization of the parent structures to staurosporine-type scaffolds were addressed and solved through the inversion of the orientation of one of the indole rings. Optimization of the basic side chain resulted in the identification of tryptamine and *N*-methyltryptamine as core building blocks. Finally, the incorporation of 5-chloroindole resulted in an almost 50-fold increase in enzyme activity compared to the parent compound **14**.

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11. 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 $CaCl_2$, 8.3μg/ml calmodulin, 25 μM [His 5] Autocamtide-2, 120 nM [g-33P]ATP) and the reaction mixture incubated at rt for 3 minutes. 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 3 times with 1% phosphoric acid and 1 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).
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