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# Aryl–indolyl maleimides as inhibitors of CaMKIIδ. Part 1: SAR of the aryl region

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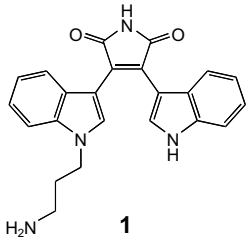
**Abstract**—A family of aryl-substituted maleimides was prepared and studied for their activity against calmodulin dependant kinase. Inhibitory activities against the enzyme ranged from 34 nM to >20 μM and were dependant upon both the nature of the aryl group and the hydrogen bond donating potential of the maleimide ring. Key interactions with the kinase ATP site and hinge region were found to be consistent with homology modeling predictions.


Calcium signaling is a critical component of biological pathways leading to cardiac[1–3] and neuropathic[2,4,5] responses. Additionally, calcium serves as an important second messenger in processes including apoptosis, cell cycle regulation, gene expression, and hormone signaling.[6] In order to induce these responses, calcium utilizes calmodulin as a ubiquitous intracellular receptor. The resulting calcium-calmodulin complex binds to and activates the family of $Ca^{2+}$/calmodulin-dependant protein kinases (CaMK)s.[6]

The family of CaMKs consists of three types (CaMKI, CaMKII and CaMKIV). Furthermore, CaMKII is known to be a family of four isoforms (α, β, γ, and δ) distributed in various tissue types. For example, CaMKIIα and β are found primarily in the brain,[7] while CaMKIIδ is found in the heart.[8] Utilizing recombinant CaMKIIδ, commercially available compound **1** (Fig. 1)[9] was shown to inhibit the enzymatic activity with an $IC_{50}$ of 380 nM.

As shown in Figrue 2, compound **1** was docked into a homology model of CaMKIIδ and key interactions within the ATP binding site and hinge region were identified. Specific interactions include dual hydrogen bonds




**1**

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

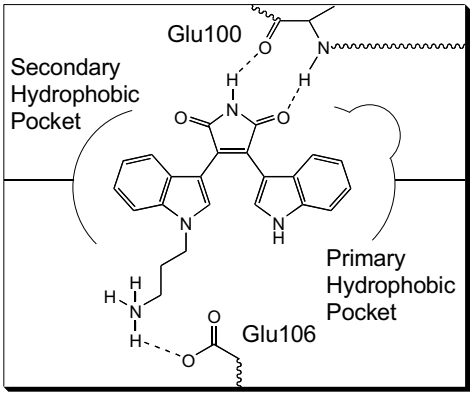



**Figure 2.** Interactions between compound 1 and the CaMKIIδ catalytic site as indicated by homology modeling.

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between the Glu100 hinge backbone and the maleimide as well as a salt bridge between Glu106 and the terminal amine. Additional hydrophobic pockets with varying geometry were noted.

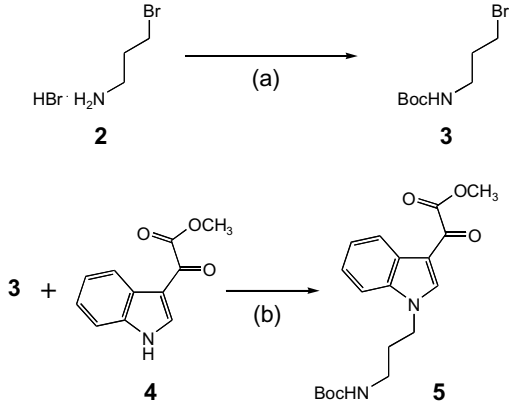
In order to develop our understanding of the structural requirements necessary to inhibit CaMKIIδ, chemistry was employed where an aryl glyoxalate was reacted with an aryl acetamide to yield the desired maleimides under basic conditions.[10] Because the glyoxalate and acetamide components were interchangeable, we were able to utilize a diverse menu of commercially available starting materials.

With the aryl component serving as the structural variable in this study, enablement of the SAR depended upon the availability of the required 3-acetamido and 3-methylglyoxalyl indoles. Preparation of the glyoxalate, as shown in Scheme 1, began by treating 3-bromopropylamine hydrobromide **2** with di-*tert*-butyl dicarbonate and diisopropylethylamine giving the desired 3-bromopropyl-*tert*-butylcarbamate **3**. Compound **3** was then coupled with commercially available methyl 3-indoleglyoxalate **4** giving the required substituted indoleglyoxalate **5**.

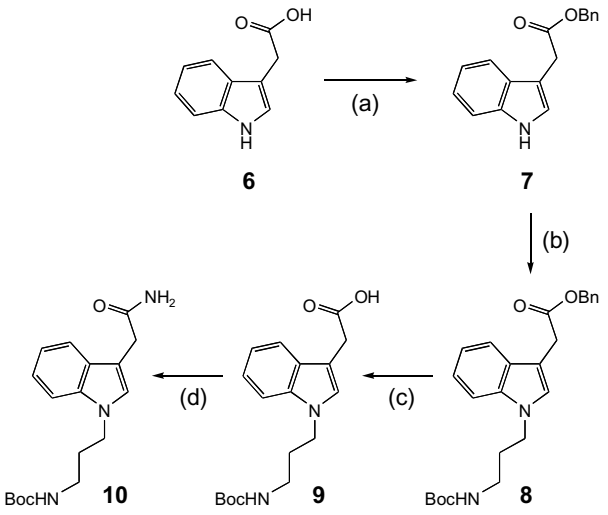
Preparation of the acetamide, as illustrated in Scheme 2, began with the conversion of commercially available 3-indole acetic acid **6** to its corresponding benzyl ester **7** utilizing benzyl bromide and cesium carbonate. Compound **7** was then coupled with compound **3** utilizing conditions already described, and yielding compound **8**. The benzyl group was removed via catalytic hydrogenation giving carboxylic acid **9**. Final conversion to acetamide **10** was achieved utilizing carbonyldiimidazole and ammonia.

Continuing with compound **10**, commercially available methyl arylglyoxalates **11** were utilized in the preparation of target aryl-indole maleimides **13**. Alternatively, commercially available arylacetic acids were converted to their corresponding arylacetamides **12** utilizing chemistry identical to conditions (d) shown in Scheme 2. These aryl acetamides were combined with compound **5** yielding additional target aryl-indole maleimides, **13**. All compounds **13** were converted to their corresponding primary amine hydrochlorides **14** under acidic conditions. The chemistry utilized in these transformations is illustrated in Scheme 3. In order to study the importance of the basic amine, as shown in the homology model, examples of compounds **13** were assayed in addition to all of the deprotected analogs **14**.

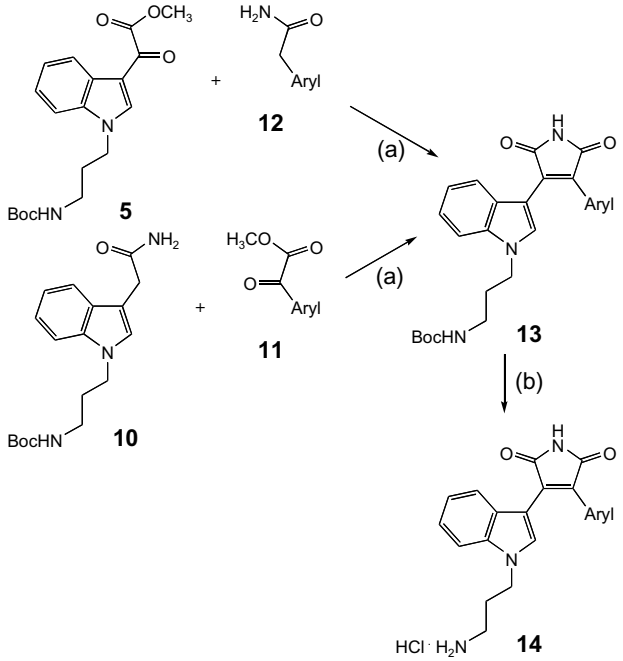
Finally, in order to verify the importance of the hydrogen bond associated with the maleimide NH, *N*-methyl maleimides were prepared. As shown in Scheme 4, examples of compounds **13** were treated with methyl



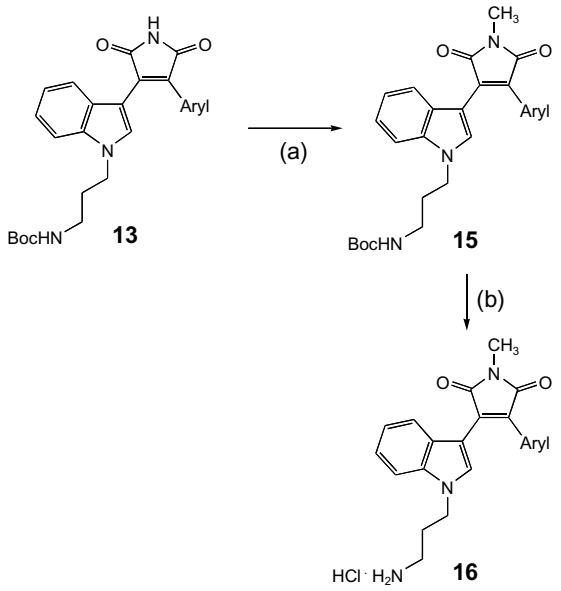

**Scheme 1.** Reagents: (a) $(Boc)_2O$, DIEA, $CH_2Cl_2$, 100%; (b) NaH, DMF, 78%.




**Scheme 2.** Reagents: (a) BnBr, $Cs_2CO_3$, $CH_3CN$, 96%; (b) **3**, NaH, DMF, 60%; (c) $H_2$, 10%Pd/C, MeOH, 87%; (d) CDI, THF then $NH_3$ (0.5 M in MeOH), 94%.



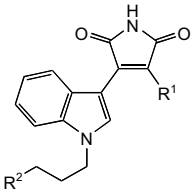

**Scheme 3.** Reagents: (a) $^tBuOK$, THF, 40–80%; (b) HCl, dioxane, MeOH, 100%.




**Scheme 4.** Reagents: (a) MeI, $Cs_2CO_3$, $CH_3CN$, 90–100%; (b) HCl, Dioxane, MeOH, 100%.

iodide and cesium carbonate to form the methylated analogs **15**. Final cleavage of the carbamate under acidic conditions yielded the desired primary amines as their hydrochloride salts **16**.

**Table 1.** SAR based on fused bicycloaryl groups ($R^1$)



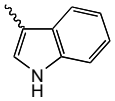
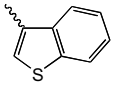
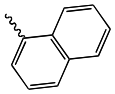
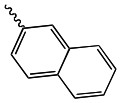
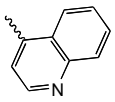
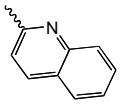
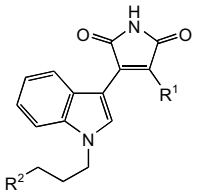
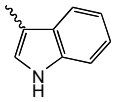
| Compound | $R^1$ | $R^2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **1**[9] |  | $NH_2$ | 0.38 ($n$ = 1) |
| **13a** | | NHBoc | 3.81 ± 0.25 ($n$ = 2) |
| **14b** |  | $NH_2$ | 0.36 ± 0.01 ($n$ = 2) |
| **13b** | | NHBoc | >20 ($n$ = 2) |
| **14c** |  | $NH_2$ | 0.29 ($n$ = 1) |
| **14d** |  | $NH_2$ | 0.50 ± 0.05 ($n$ = 2) |
| **14e** |  | $NH_2$ | 0.63 ± 0.04 ($n$ = 2) |
| **14f** |  | $NH_2$ | >20 ($n$ = 2) |

**Table 2.** SAR based on monocyclic aryl and heteroaryl groups ($R^1$)

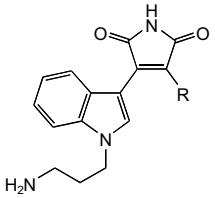


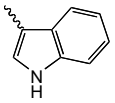
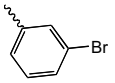
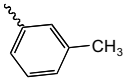
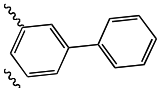
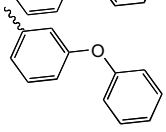
| Compound | $R^1$ | $R^2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **1**[9] |  | $NH_2$ | 0.38 ($n$ = 1) |
| **13a** | | NHBoc | 3.81 ± 0.25 ($n$ = 2) |
| **14g** |  | $NH_2$ | 1.35 ± 0.64 ($n$ = 2) |
| **13g** | | NHBoc | >20 ($n$ = 2) |
| **14h** |  | $NH_2$ | 1.58 ($n$ = 1) |
| **14i** |  | $NH_2$ | 0.54 ($n$ = 1) |
| **14j** |  | $NH_2$ | 1.60 ($n$ = 1) |
| **14k** |  | $NH_2$ | 1.49 ± 0.47 ($n$ = 2) |
| **14l** |  | $NH_2$ | 1.19 ($n$ = 1) |
| **13l** | | NHBoc | >20 ($n$ = 1) |

$IC_{50}$ values[11] for all tested compounds are listed in the following tables. The data contained in Tables 1–4 refer to the general structure shown in Figure 1. As shown in Table 1, multiple fused bicycloaryl groups were studied in an effort to understand the importance of the indole moiety. As the data suggest, fused bicycloaryl groups were tolerated with $IC_{50}$ values ranging from 0.38 to 0.63 μM. Of particular interest is the lower potency noted for compound **14f**. This supports the possibility of significant lone-pair/lone-pair repulsion between the quinoline nitrogen and the adjacent maleimide carbonyl thus raising the energy of the bound conformation.

In an effort to expand the SAR beyond fused bicycloaryl groups, monocyclic aryl and heteroaryl modifications were studied. As shown in Table 2, monocyclic substituents were less tolerated than the bicyclic groups shown in Table 1. In general, these modifications resulted in a 3- to 4-fold loss in potency compared to compound 1. A notable exception is the 3-pyridyl analog **14i** suggesting the possibility of a new interaction within the active site. However, evidence did not exist that these relatively small groups were sufficiently capable of taking full advantage of the active site geometry. Therefore, a series of substituted monocyclic aryl groups was studied.

**Table 3.** SAR based on substituted monocyclic aryl and substituted monocyclic heteroaryl groups



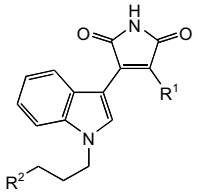
| Compound | R | $IC_{50}$ (μM) |
|---|---|---|
| **1**[9] |  | 0.38 ($n$ = 1) |
| **14i** |  | 0.54 ($n$ = 1) |
| **14m** |  | 0.18 ± 0.06 ($n$ = 3) |
| **14n** |  | 0.28 ± 0.07 ($n$ = 2) |
| **14o** |  | 1.32 ± 0.26 ($n$ = 2) |
| **14p** |  | 1.68 ($n$ = 1) |

Based on the relatively higher potency noted for the 3-pyridyl analog **14i** compared to all other monocyclic derivatives, a series of meta-substituted phenyl analogs were prepared. The data, summarized in Table 3, indicate that small groups such as bromo **14m** or methyl **14n** can further enhance potency through more optimal hydrophobic interactions. However, larger groups such as phenyl **14o** or phenoxy **14p** caused reductions in potency. This indicated that the steric capacity of the hydrophobic pocket is too small to accommodate such groups.

With the promising results noted resulting from placement of small groups on the phenyl ring, similar studies were applied to the parent indole compound **1**. As shown in Table 4, with the exception of inverting the orientation of the indole ring, substitutions on this group were generally well tolerated. Of particular note was the 5-bromoindole analog **14t** which, according to the homology model, makes highly efficient use of the hydrophobic site geometry shown in Figure 2.

Having probed the indole-region hydrophobic site geometry, attention was directed to the predicted hydrogen bonding interactions between the maleimide and the hinge region. As shown in Table 5, the *N*-methyl maleimide variants of several analogs were prepared and assayed. In all the cases, a significant reduction in potency was noted. The apparent imbalance between the 10-fold reduction noted for compounds **16a**, **16b**, and **16g** as compared to **16t** can be rationalized based on the methyl group forcing a shift in the binding geometry. The larger substituted indole fits very well when the

**Table 4.** SAR based on modifications to the indole ring system ($R^1$)



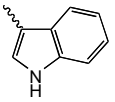
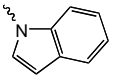
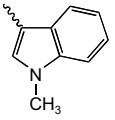
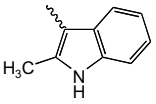
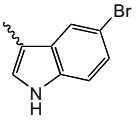
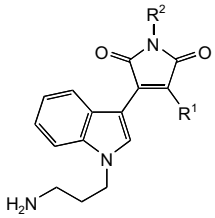
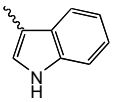
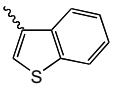
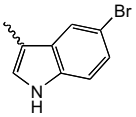
| Compound | $R^1$ | $R^2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **1**[9] |  | $NH_2$ | 0.38 ($n$ = 1) |
| **13a** | | NHBoc | 3.81 ± 0.25 ($n$ = 2) |
| **14q** |  | $NH_2$ | 4.56 ($n$ = 1) |
| **14r**[12] |  | $NH_2$ | 0.31 ($n$ = 1) |
| **14s** |  | $NH_2$ | 0.75 ± 0.31 (n = 2) |
| **13s** | | NHBoc | 7.61 ($n$ = 1) |
| **14t** |  | $NH_2$ | 0.034 ± 0.009 (n = 3) |
| **13t** | | NHBoc | 7.54 ($n$ = 1) |

**Table 5.** SAR based on methylation of the maleimide nitrogen



| Compound | $R^1$ | $R^2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **1**[9] |  | H | 0.38 ($n$ = 1) |
| **13a** | | $CH_3$ | 3.81 ± 0.25 ($n$ = 2) |
| **14b** |  | H | 0.36 ± 0.01 ($n$ = 2) |
| **16b** | | $CH_3$ | 2.32 ($n$ = 1) |
| **14g** |  | H | 1.35 ± 0.64 ($n$ = 2) |
| **16g** | | $CH_3$ | 15.02 ($n$ = 1) |
| **14t** |  | H | 0.034 ± 0.01 ($n$ = 3) |
| **16t** | | $CH_3$ | >20 ($n$ = 1) |

hinge interaction is intact but fits very poorly if the orientation of the inhibitor in the active site is shifted.

In summary, novel inhibitors of CaMKIIδ were prepared. SAR efforts supported homology model predictions pointing to critical hydrogen bonds between the primary amine and Glu106 as well as between the maleimide and Glu100 of the hinge region. These data are supported by the decreased potency associated with NHBoc and *N*-methyl maleimide groups. Inhibitory activity was optimized through incorporation of a 5-bromoindole generating compound **14t** possessing an $IC_{50}$ of 34 nM.

## References and notes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9. Compound **1** was obtained from Calbiochem–Novabiochem Corporation, catalog # 203294.
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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 pH7, 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 pH7, 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 3 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).
12. Compound **14r** was obtained from Calbiochem–Novabiochem Corporation, catalog # 557508.