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BISINDOLYLMALEIMIDE INHIBITORS OF PROTEIN KINASE C. FURTHER CONFORMATIONAL RESTRICTION OF A TERTIARY AMINE SIDE CHAIN.

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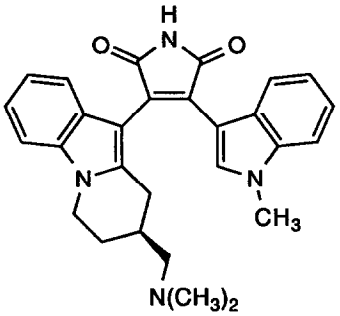
Abstract: A pharmacophore model for the localisation of the cationic binding site of bisindolylmaleimide PKC inhibitors is described. This model has been used to guide selection of further conformationally restricted tertiary amine analogues, culminating in the identification and synthesis of the potent and highly selective PKC inhibitor, Ro 32-0557.

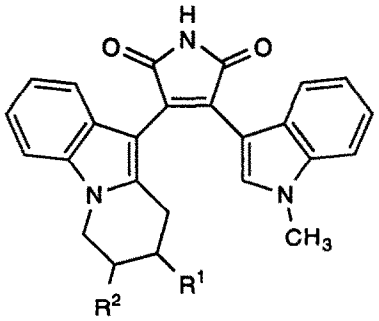
The regulation of the phosphorylation state of intracellular proteins by protein kinases and phosphatases is believed to be critical for the transmission of an extracellular message from the cell surface through the cytosol to the nucleus. Indeed, it has been estimated that one third of the proteins expressed in a typical mammalian cell are covalently bonded to phosphate.[1]

Protein kinase C (PKC) is a serine/threonine specific protein kinase family[2] which is implicated in the pathogenesis of a variety of diseases.[3] We are interested in the therapeutic potential of PKC inhibitors for, *inter alia*, the treatment of autoimmune diseases such as rheumatoid arthritis. Recently, we have described a series of potent and highly selective bisindolylmaleimide PKC inhibitors[4] which culminated in the synthesis of an orally active compound, Ro 32-0432 (**1**).[5] This compound is able to prevent T cell activation *in vitro* and initial studies suggest that the tertiary amine is required for *in vivo* efficacy. The potency of tertiary amines against isolated PKC, however, was slightly reduced compared to the corresponding primary and secondary amines. We now report our efforts to further conformationally restrict the tertiary amine side chain of Ro 32-0432 in an attempt to obtain more potent analogues *in vitro* which should lead to more efficacious compounds *in vivo*.



1 Ro 32-0432

In our earlier work we postulated that bisindolylmaleimides and the non-selective protein kinase inhibitor, staurosporine, adopt a common mode of binding.[6] Amine containing side chains attached to an indole nitrogen were modelled to place the amine nitrogen proximal to the amine of staurosporine. On conformational restriction of this side chain it became clear that the best fits were obtained when the boat conformer of staurosporine was used as a template. The best fit, however, was obtained for amine **2** whereas regioisomer **4** was found to be a more potent PKC inhibitor. This strongly suggests that the spatial position of the nitrogen in staurosporine boat is not optimal for our inhibitors. We therefore required a more sophisticated model to facilitate the design of more potent tertiary amine inhibitors.



2 $R^1 = H$, $R^2 = CH_2NH_2$
3 $R^1 = (CH_2)_2NH_2$, $R^2 = H$
4 $R^1 = CH_2NH_2$, $R^2 = H$

Conceptually, there are four possibilities for further conformational constraint of the amine side chain by formation of another ring. Either a fused or spiro ring could be envisaged in which the nitrogen can be appended to or form part of the new ring. It has already been demonstrated that 5-, 6- and 7-membered rings can be accomodated for fused [1,2-a]indole inhibitors.[4] In addition the size of the newly formed ring could vary considerably, as can the point of attachment. A large number of potential targets therefore exist, all of which would be synthetically demanding. In order to concentrate on those targets most likely to show improved potency we decided to generate an amine pharmacophore model for these inhibitors based upon amines **2**, **3** and **4**, three of the most potent compounds described to date.[4] We reasoned that such a model would permit the rational selection of likely successful candidates from all possible compounds and should enable targetted synthetic endeavours.

Both enantiomers of compounds **2**, **3**, and **4** were modelled on to the amine of staurosporine boat and the spatial position of the amine nitrogen of the enantiomer which gave the best fit was recorded. This information was then used to define an amine pharmacophore for further conformationally restricted inhibitors. Candidate compounds were then modelled effectively to bring their respective amine nitrogens proximal to the centroid of the triangle representing the amine binding site. A number of analogues were thus selected for synthesis.[7] One of

the most promising targets identified by this approach was the trans-fused pyrrolidine **6**. The best fit for pyrrolidine **6** brought the nitrogen to 0.64 Å from the pharmacophore centroid with a 0.1kcal mol^{-1} enthalpy penalty (figure 1). The process was repeated for the enantiomer **5** which did not fit quite as well (0.65 Å, 0.37kcal mol^{-1}).

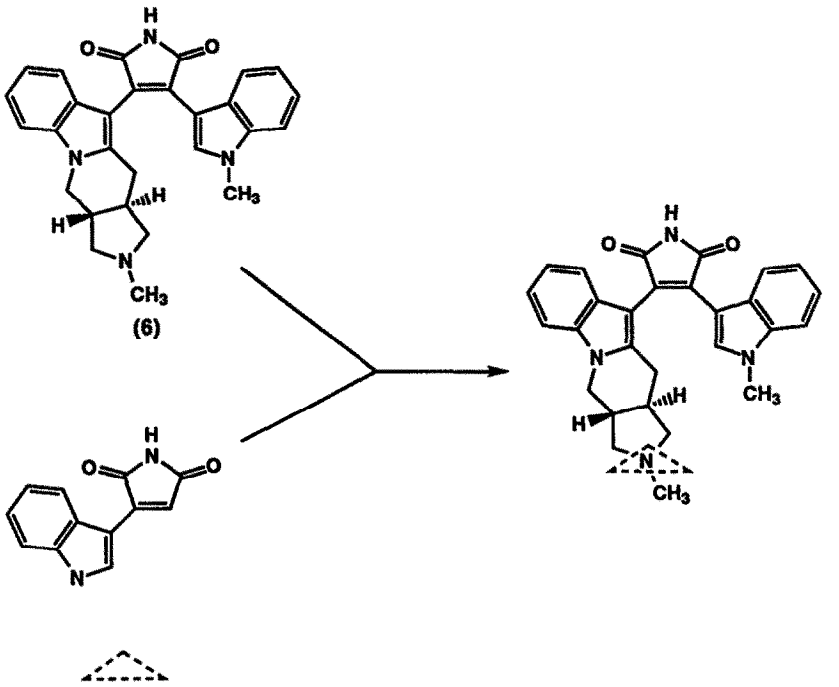
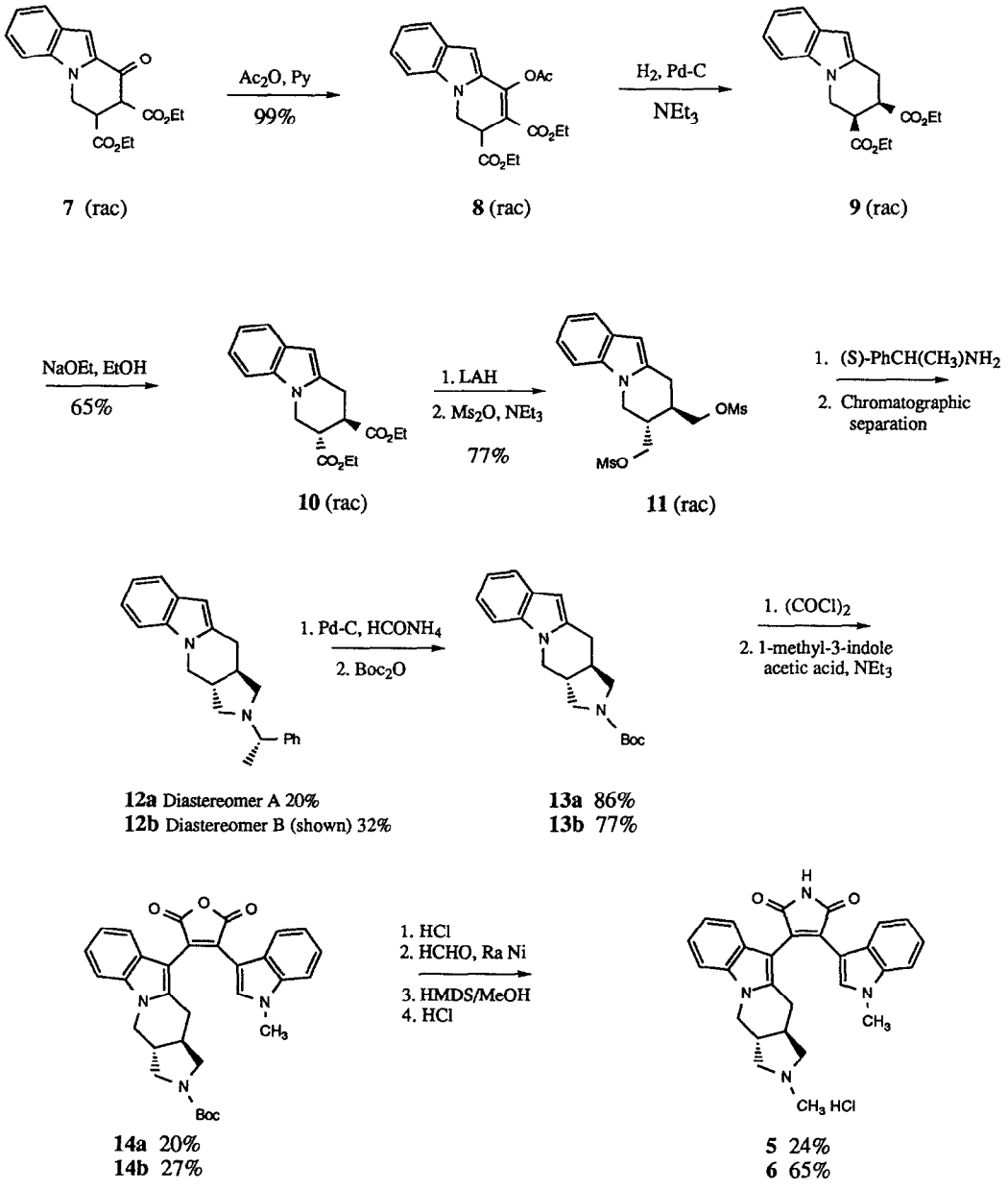


Figure 1 Schematic representation of Ro 32-0557 (6) matched onto the amine pharmacophore model.

The pyrrolidines **5** and **6** were synthesised from the readily available ketodiester **7** (see scheme).[8,9] The enolacetate **8** was hydrogenated to give directly the cis-diester **9**, which was readily epimerised to the corresponding trans-diester **10** with sodium ethoxide in ethanol. This procedure relied upon the differential solubilities of the two compounds; the trans-diester being considerably less soluble in ethanol and this diastereomer crystallised preferentially. Reduction to the trans-diol with lithium aluminium hydride followed by activation of the hydroxyl groups gave the dimesylate **11**, which was readily reacted with (S)-α-methylbenzylamine. The resulting diastereomers **12** were separable by chromatography. The absolute configuration of **12b** was unambiguously established by X-ray crystallography.[10] Each diastereomer was deprotected by transfer hydrogenation, protected as the t-butoxycarbamate and coupled to give the required anhydrides **14**.[11] N-Deprotection followed by reductive alkylation with formaldehyde and sodium cyanoborohydride gave the N-methylated amines. The anhydrides were converted into the corresponding imides

with HMDS/methanol[12] to afford the desired materials **5** and **6** in good yield.



Scheme

	IC_{50} (nM)		
	PKC	**cAMP-dependent protein kinase (PKA)**	**Phosphorylase kinase (PhK)**
Ro 32-0432(**1**)	17.2±5.4 (6)	22375±3090 (5)	15625±3400 (4)
Ro 32-0556(**5**)	8 (1)	5500 (1)	3800±700 (2)
Ro 32-0557(**6**)	5±1 (3)	2800±130 (4)	1688±238 (4)

Table 1

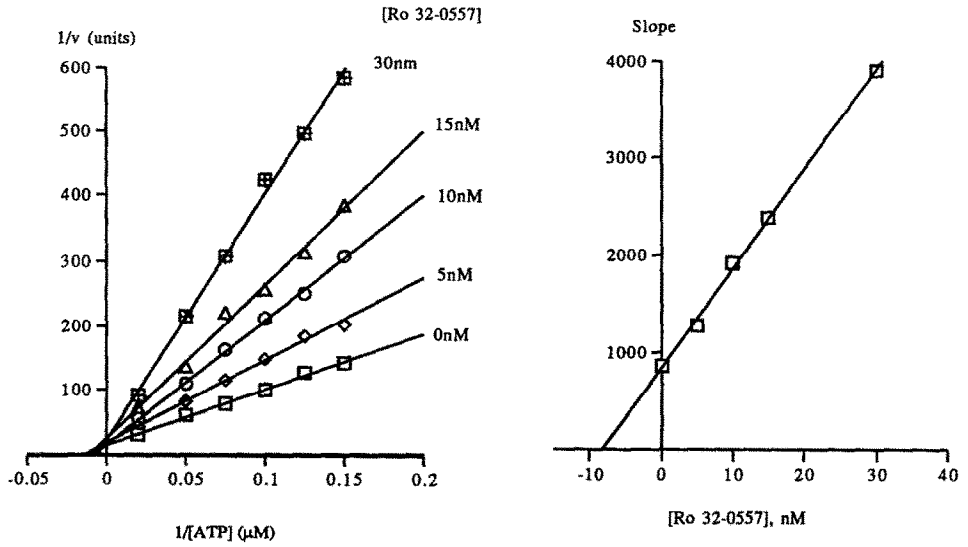
The pyrrolidines **5** and **6** are more potent than Ro 32-0432 as protein kinase C inhibitors and both retain a greater than 300 fold selectivity for PKC over related kinases (table 1). The most potent inhibitor, Ro 32-0557, was also evaluated against a number of PKC isoenzymes, showing a similar profile to Ro 32-0432 but with greater selectivity for the conventional isoenzymes over the calcium independent ε isoenzyme (table 2).[13]

	IC_{50}(nM)					
		PKC Isoenzyme				
	Rat Brain	α	βI	βII	γ	ε
Ro 32-0432 (**1**)	21±4.3 (2)	8.8±3.2 (2)	28±7.1 (2)	30.5±13.4 (2)	36.5±6.4 (2)	105±21.2 (2)
Ro 32-0557 (**6**)	6.8±1.7	2.85±0.64	5.3±0.42	6.8±1.9	5.4±0.57	48±19.8

Table 2

The inhibition of the ßI isoenzyme of PKC by Ro 32-0557 is fully competitive with respect to ATP with a Ki of 9nM (figure 2). Despite the improved *in vitro* potency Ro 32-0557 was not significantly more active than Ro 32-0432 in the developing adjuvant arthritis model. An investigation of the pharmacokinetics of this compound should identify the reasons for this and direct further optimisation in an attempt to improve the *in vivo* efficacy of these inhibitors.

In conclusion, the pharmacophore model described here has been successfully applied to guide the selection of further conformationally constrained tertiary amine side chains of bisindolylmaleimides culminating in the identification of highly potent and selective inhibitors of PKC.



(a) (b)

Figure 2 Lineweaver-Burk (a) and secondary re-plot (b) for the inhibition of the βI isoenzyme of PKC by Ro 32-0557 (6)

References and Notes

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