# Indirubin inhibitors of *Leishmania mexicana* CRK3 Cyclin Dependent Kinase


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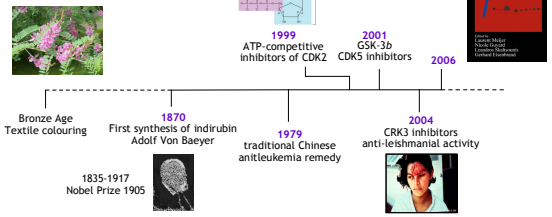
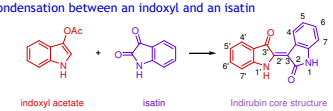
The indirubins are known inhibitors of cyclin dependent kinases (CDKs), including CRK3 from the protozoan parasite *Leishmania mexicana*, which is essential for proliferation of the disease Leishmaniasis.[1] Subsequent investigations into the pharmacological properties of the indirubins show them to be competitive ATP-binding site inhibitors of the CDKs.[2] The structural basis for selectivity and potency has been clarified with the crystallisation of a number of the target protein kinases in complex with indirubin inhibitors. In our hands, indirubins substituted at both the 3´ and 5-positions exhibit significant selectivity as inhibitors of CRK3 compared with CDK1/cyclin B *in vitro*. Immobilisation of our indirubin scaffold onto Affi-Gel beads *via* a polyethylene glycol linker has been carried out for use in affinity matrix chromatography experiments designed to access and confirm both known and potentially new protein targets.[3] The overall aim of this study is to identify an indirubin lead compound that is selective against CRK3 for future anti-Leishmanial drug development.




## Chemistry of the indirubin family

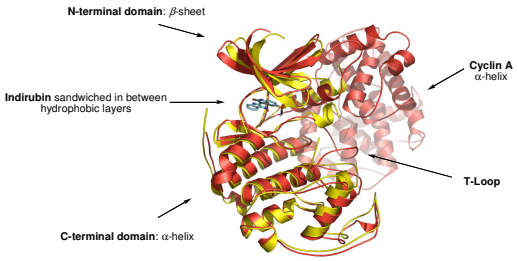
Classical Synthesis: condensation between an indoxyl and an isatin




Conditions: *either* **basic** (i) $Na_2CO_3$ (2.5 eq.), MeOH, rt, 6 h
*or* **acidic** (ii) AcOH (conc.), HCl (cat.), rt, 24 h

- Fully conjugated aromatic structure, completely flat molecule, ability to π-stack
- Highly aqueous insoluble, poor absorption and hence, low cellular activity
- Very high melting point > 300 ˚C and intense colour
- >50 commercially available isatins BUT only a few commercially available indoxyl acetate precursors

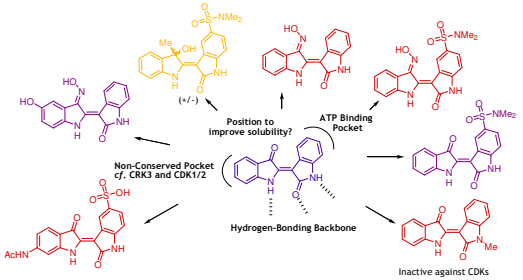
## Binding mode in CDK2




- CRK3 has a relatively high amino acid homology (57%) compared to the mammalian kinases CDK1 and CDK2
- There are non-conserved residues in the active site which can be targeted for drug development

• Protein Crystallography carried out within the laboratory of Dr Malcolm Walkinshaw by Dr Iain McNae

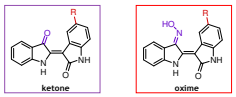
## Indirubin to more drug-like molecules




- Substituents in the 5-position sit in the region of binding site normally occupied by the α-phosphate of ATP and interactions with specific residues in the pocket are thought to account for the increase in potency
- 3´-ketone to 3´-oxime increases potency against CDKs *in vitro* and retains activity in cell-based assays due to its improved membrane permeability

• Analogues shown are active against the parasite, awaiting $IC_{50}$ data.

## Optimisation of 5-substituted analogues




| R | CRK3 Ketone $IC_{50}$ (μM) | CRK3 Oxime $IC_{50}$ (μM) | CDK1 Oxime $IC_{50}$ (μM) | R | CRK3 Ketone $IC_{50}$ (μM) | CRK3 Oxime $IC_{50}$ (μM) | CDK1 Oxime $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|
| H | >10 | >10 | nd | $NO_2$ | >100 | 0.027 | 0.169 nM |
| $OCF_3$ | >100 | 1.154 | 7.033 | $SO_3H$ | 0.049 | 0.029 | nd |
| F | nd | >25 | 1.431 | $SO_2NH_2$ | >15 | 0.060 | nd |
| Cl | nd | >70 | 0.271 | $SO_2NHMe$ | nd | 0.443 | 13.00 |
| Br | nd | >10 | 0.010 | $SO_2NMe_2$ | nd | 0.006 | 14.00 |
| I | 3.056 | 0.899 | 0.034 | $SO_2NH(CH_2)_2NMe_2$ | 2.527 | 0.449 | 9.200 |

• Preliminary indirubins were synthesised in the laboratory of Dr Nicholas J. Westwood by Dr David Taddei
• $IC_{50}$ values determined by a radiation assay carried out in the laboratory of Dr Karen M. Grant by Dr Christopher M. Wells

- **Sulfonamide substituents are the most active AND the most selective between CDK1 and CRK3**
- Decision to prepare a range of sulfonamides using parallel synthesis techniques

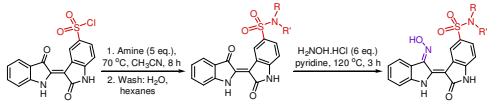
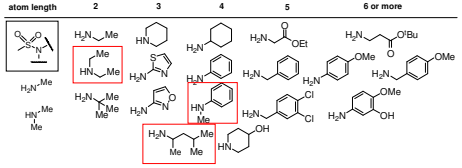



Table of successfully synthesised sulfonamides:




• Amines highlighted show the best activity against the parasite, awaiting $IC_{50}$ data.

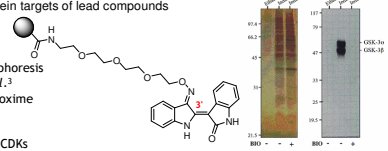

References:

1. Grant, K. M.; Dunion, M. H.; Yardley, V.; Skaltsounis, A. L.; Marko, D.; Eisenbrand, G.; Croft, S. L.; Meijer, L.; Mottram, J. C. *Antimicrobial Agents and Chemotherapy*, **2004**, *48*, 3033-3042.
2. Jautelat, R.; Brumby, T.; Schafer, M.; Briem, H.; Eisenbrand, G.; Schwahn, S.; Kruger, M.; Lucking, U.; Prien, O.; Siemeister, G. *ChemBioChem*, **2005**, *6*, 531-540.
3. Meijer, L.; Skaltsounis, A. L.; Magiatis, P.; Polychronopoulos, P.; Knockaert, M.; Leost, M.; Ryan, X. Z. P.; Vonica, C. A.; Brivanlou, A.; Dajani, R.; Crovace, C.; Tarricone, C.; Musacchio, A.; Roe, S. M.; Pearl, L.; Greengard, P. *Chemistry & Biology*, **2003**, *10*, 1255-1266.

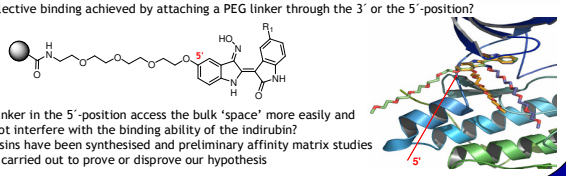

## Affinity matrix chromatography

Method of identifying known and novel protein targets of lead compounds

- Immobilisation of lead compound onto Affi-Gel beads *via* a PEG linker
- Bound proteins identified by Gel Electrophoresis
- **3´-position** has been used by Meijer *et al*.[3]
- Demonstrated 6-bromo-indirubin-3´-monoxime selectivity towards GSK-3*β*
- Literature shown that groups located on the **3´-oxime** decrease potency towards CDKs

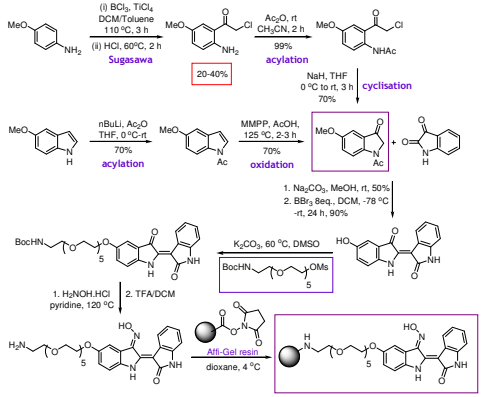



Optimal selective binding achieved by attaching a PEG linker through the 3´ or the 5´-position?




- Can a linker in the 5´-position access the bulk 'space' more easily and thus, not interfere with the binding ability of the indirubin?
- Both resins have been synthesised and preliminary affinity matrix studies will be carried out to prove or disprove our hypothesis

## Synthesis of resin-5´-PEG linker indirubin




## Synthesis of PEG linkers