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The design and synthesis of novel 3-[2-indol-1-yl-ethyl]-1*H*-indole derivatives as selective inhibitors of CDK4

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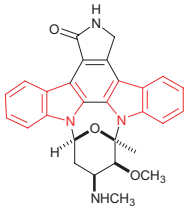
Abstract—We present the design, synthesis and biological activity of novel 3-[2-indol-1-yl-ethyl]-1*H*-indole selective inhibitors of CDK4.

The cyclin dependant kinases (CDKs)[1] perform a vital role in the different checkpoints of the cell division cycle. In particular, CDK4/cyclin D1 controls the restriction point, a threshold that all quiescent cells must cross in order to divide. At this checkpoint, CDK4/cyclin D1 phosphorylates the retinoblastoma protein (pRB), a repressor of cell division. Phosphorylation leads to the release of E2F, which stimulates the transition across the restriction point where the cell is committed to division. The inhibition of CDK4 by natural inhibitor proteins, CKIs, acts as a brake for cell division and prevents unrestricted cell proliferation of normal cells. Underexpression or inactivation of CKI's is common in tumour cells. Consequently small molecules that can act as inhibitors of CDK's provide a potential means of controlling the uninhibited cell growth of a tumour.



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Kinases in general act by catalysing the reaction between the γ-phosphate of ATP and the hydroxyl group of serine, threonine or tyrosine residues in a substrate protein. Small molecule mimics of ATP are therefore effective inhibitors of a range of kinases. The diindolyl natural product staurosporine **1**[2] is one of the best ATP competitive kinase inhibitors, having IC_{50} values in the nanometer range for the serine/threonine kinases, protein kinase C (PKC), CDK2, CDK4 and CDK6. In the structure of **1** the two indole rings connected via the 2 and 3 positions are highlighted in red for clarity. Despite its high level of activity, poor selectivity limits the usefulness of staurosporine. As a result of the potency of staurosporine **1**, diindolyl containing compounds have become a major focus in the discovery of new inhibitors of the CDKs.[3] The cancer preventive properties of indole-3-carbinol and 3,3′-diindolymethane, which are present in cruciferous vegetables such as broccoli, have been reported.[4]

In contrast to the non-specific inhibition exhibited by staurosporin, the natural product fascaplysin[5,6] **2** (Fig. 1) specifically inhibits CDK4/cyclin D_1 and, importantly, is also active on cancer cell lines.[6] Moreover, fascaplysin shows a covalent link between the 2 and 3 positions of one indole ring and the 1 and 2 positions of the second modified indole ring, respectively. The use of fascaplysin as an anti-cancer drug is, however, precluded by its toxicity, which is thought to arise from intercalation of its planar structure in DNA.[7] We seek to synthesise new biologically active inhibitors of CDK4/

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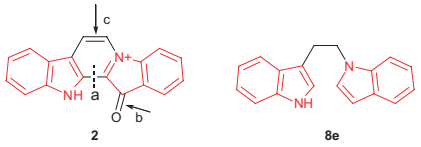
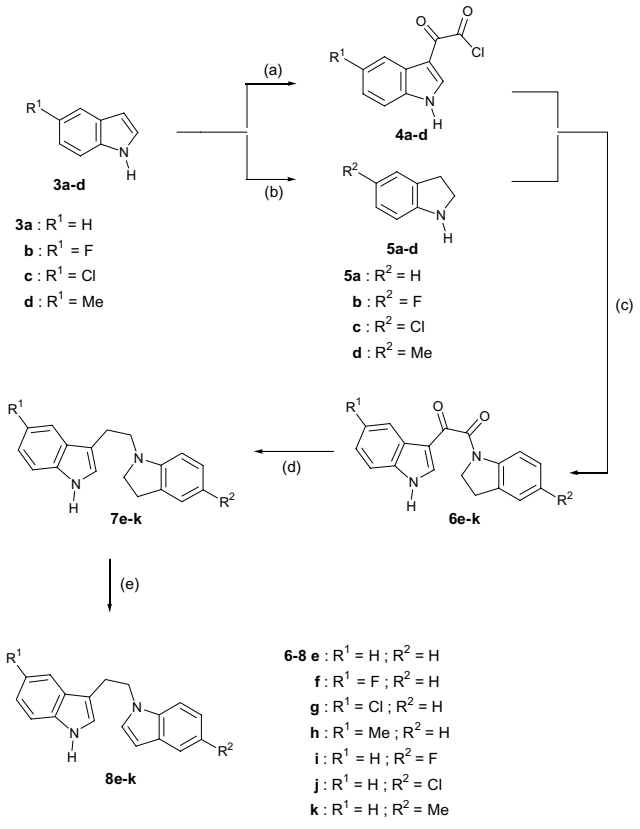


Figure 1. Strategy adopted to convert fascaplysin **2** into the 3-[2-indol-1-yl-ethyl]-1*H*-indole **8e**. Bond 'a' and carbonyl group 'b' of fascaplysin are removed and 'c' is made into a single bond.

cyclin D_1 based on the structure of fascaplysin. Recently we prepared the first biologically active non-planar analogues of fascaplysin,[8] which contained a single indole ring. The potency of diindolyl containing compounds raises the possibility that a di-indolyl derivative of fascaplysin may be more biologically active than our earlier derivatives.[8] Our design strategy is to start with the structure of fascaplysin **2**, cleave bond 'a', remove the carbonyl group 'b' and convert double bond 'c' into a single bond, leading to the indolyl-ethyl indole **8e** (Fig. 1). Here we report the synthesis and biological activity of non-planar indolyl-ethyl indole derivatives based on fascaplysin **8e**–**k** (Scheme 1).

Prior to embarking on the syntheses, we used in silico modelling methods to predict the affinity of the compounds **8e**–**k** (Scheme 1) towards the kinase CDK4 (Table 1). As in our previous study,[8] we docked structures **8e**–**k** into the active site of our model of CDK4.[9] Compounds **8e**–**k** were predicted to bind in a mode overlapping with fascaplysin (illustrated in Fig. 2) and were all predicted to exhibit CDK4 activity (Table 1). Encouraged by these positive in silico results, we proceeded to synthesise **8e**–**k**.



Scheme 1. Reagents and conditions. (a) $(COCl)_2$, dry ether, N_2, 0–5 °C, 1.5 h, 80–91%; (b) $NaBH_3CN$ 95%, glacial acetic acid, N_2, 15–17 °C, 2 h, 85–90%; (c) K_2CO_3, dry THF, N_2, **5** was added at 0 °C then 2 h stirring at rt, 78–90%; (d) $LiAlH_4$, dry THF, dry ether, N_2, reflux, 6 h, 57–99%; (e) MnO_2 activated, $CHCl_3$, reflux, 60 h, 41–95%.

Table 1. CDK4/CDK2 activity

Compound[a]	Predicted:Goldscore[b]	Measured:IC_{50}/μM[c]
8e	47.3	>500 (>500)[d]
8f	44.1	50 (200)[d]
8g	45.2	>500 (>500)[d]
8h	43.7	150 (400)[d]
8i	43.7	95 (>500)[d]
8j	43.1	140 (430)[d]
8k	43.5	130 (>500)[d]
Fascaplysin **2**	51.1[8]	0.55[8] (>500)[d]

[a] Note that compounds **8f**–**k** are the substituted derivatives of compound **8e**.

[b] Binding 'energy' predicted in silico using the program Gold.[16] In our experience a Goldscore predicted >40 is indicative of a significant binding affinity.

[c] CDK4-cyclin D1 assay, using RB-152 fusion protein as substrate.[6]

[d] Brackets denote the IC_{50} values measured for CDK2 activity.[6]

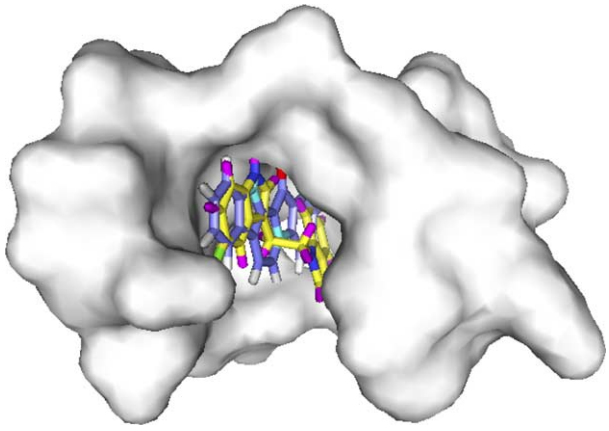


Figure 2. Predicted positions of fascaplysin **2** (C is in slate, N in cyan, O in red, H in white) and indolyl-ethyl indole derivative **8f** (C is in yellow, N in blue, F in green, H in magenta) in the active site of our CDK4 model.[9] Complexes modelled using molecular docking[9] (Gold[17]).

The indole derivatives **3a**–**d** were reacted with oxalyl chloride to afford the indolyl-oxo-acetyl chlorides **4a**–**d** in 80–91% yields.[12] The intermediate dihydroindoles **5a**–**d** were obtained in 85–90% yield by reacting each compound **3a**–**d** with sodium cyanoborohydride 95%.[13] Coupling of **4a**–**d** with **5a**–**d** produced the indolyl-dihydro-indolyl-ethane diones **6e**–**k** in 78–90% yield.[14] Reduction of each compound **6** with lithium aluminium hydride gave the respective dihydro-indolyl-ethyl indoles **7e**–**k** in 57–99% yield.[15] Final oxidation was performed using activated manganese(IV) oxide. Under these conditions, indolyl-ethyl indoles **8e**–**k** were obtained in 41–95% yield.[14]

The CDK4 activities of compounds **8e–k** were assayed in vitro; IC_{50} values (Table 1) were measured for inhibition of CDK4 using RB-152 fusion protein as a substrate.[16] These results confirm our predictions that the indolyl-ethyl indole derivatives **8f**,**h–k** are CDK4 active; **8e** and **8g** are, contrary to our predictions, inactive. Interestingly, the two most active compounds, **8f** and **8i**, contain a fluorine atom in the R^1 and R^2 position, respectively. Our modelling suggests that a fluorine at R^1 (**8f**) is close enough to interact with the guanidinium of Arg 101,[17] and that in contrast Cl or Me are too bulky to occupy this position, resulting in an alternative binding mode for **8g** and **8h**. The in silico results indicate that the R^2 substituents (**8i**,**j**,**k**) lie in a region lined by Val 72, Phe 93, Ala 157 and the Lys 35/Asp 158 salt bridge. The importance of the electronic and steric effects of substituents is demonstrated by the inactivity of the parent system **8e**.

All the CDK4-active indolyl-ethyl indole derivatives synthesised are also CDK4 selective compared to CDK2 (Table 1). This suggests that the indolyl-ethyl indole scaffold is, as we postulated, a good starting point for the development of CDK4 selective compounds.

In conclusion we have designed, modelled, synthesised and tested a series of 3-[2-indol-1-yl-ethyl]-1*H*-indole structures as inhibitors of CDK4. The best compound, **8f**, has an IC_{50} of 50 μM. The compounds prepared are conceived as non-planar analogues of fascaplysin, and all the CDK4-active compounds are also selective for CDK4 compared to CDK2. Compounds with substituents in both indole rings of the 3-[2-indol-1-yl-ethyl]-1*H*-indole including the difluoro derivative are under investigation.

Typical experimental for the final step in the synthesis of **8e**: To a solution of **7e** (0.90 g, 3.41 mmol) in 19.5 mL of chloroform, under nitrogen atmosphere, was added activated MnO_2 (2.08 g, 24 mmol). The mixture was heated under reflux for 60 h. After being cooled to room temperature, the resulting mixture was filtered on Celite and the filtrate was evaporated under reduced pressure. Purification of the crude product by column chromatography on silica gel (EtOAc/petroleum ether = 10/90 then 30/70 as gradient of eluant) gave the 3-[2-indol-1-yl-ethyl]-1*H*-indole **8e** (0.49 g, 1.88 mmol, 55% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 3.31 (2H, td, *J* 7.2 and 0.6 Hz), 4.46 (2H, t, *J* 7.2 Hz), 6.48 (1H, dd, *J* 3.3 and 0.6 Hz), 6.77 (1H, d, *J* 2.1 Hz), 6.99 (1H, d, *J* 3.3 Hz), 7.12–7.29 (4H, m), 7.38–7.42 (2H, m), 7.63–7.69 (2H, m), 7.92 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 26.33 (CH_2), 46.99 (CH_2), 100.83 (CH), 109.39 (CH), 111.30 (CH), 112.63 (Cq), 118.48 (CH), 119.26 (CH), 119.58 (CH), 120.99 (CH), 121.37 (CH), 122.20 (CH), 122.30 (CH), 127.17 (Cq), 128.02 (CH), 128.68 (Cq), 135.85 (Cq), 136.24 (Cq). R_f (EtOAc/petroleum ether = 20/80) 0.33. Melting point 154–155 °C. Mass spectroscopy FAB$^+$: M^+ 260, MH^+ 261. Accurate mass found M^+, 260.13122; $C_{18}H_{16}N_2$ requires 260.13135.

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