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New fascaplysin-based CDK4-specific inhibitors: design, synthesis and biological activity

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Received (in Cambridge, UK) 23rd April 2004, Accepted 25th May 2004

First published as an Advance Article on the web 25th June 2004

The first biologically active non-planar analogues of the toxic anti-cancer agent, fascaplysin, have been produced; we present the design, synthesis and biological activity of three tryptamine derivatives.

Traditionally, anti-cancer agents have been discovered by testing natural products for activity *in vivo* against cancer cell lines, this approach has resulted in the discovery of many valuable chemotherapeutic agents such as taxol.[1] Recent progress in understanding the genetics of the biochemical pathways involved in cancer has allowed small molecules to be produced which selectively inhibit specific enzymes involved in tumour formation and proliferation.[2] The advantage of this approach is that the active molecules have a far simpler chemical structure than the complex natural products used traditionally in chemotherapy, and synthesis is therefore more tractable. A notable recent example of this idea are the nutlins which inhibit the protein mdm2 causing the release of the tumour suppressor protein p53.[3]

An area of major current interest in the anti-cancer field is the inhibition of cyclin-dependent kinases, CDKs, by small molecules.[4] CDKs are a vital component of the check-points in the various phases of the cell division cycle[5] – they are required for healthy cell growth and proliferation. CDK4 has a very specific function in the G0/G1 phase of the cell division cycle – the CDK4:cyclin D1 complex phosphorylates the protein retinoblastoma (pRB), an active repressor of the E2F family of transcription factors. CDK4-mediated hyperphosphorylation of pRB facilitates liberation of E2F proteins and allows them to carry out their transcriptional activation roles. This enables the cell to pass through the restriction point, an early G_1 checkpoint, where the cell commits itself to complete one cell division cycle.[6] Inhibition of CDK4 is therefore a vital factor in controlling the rate of cell proliferation. In normal cells this is carried out by CDK4-specific cyclin dependent kinase inhibitors (CKIs) such as p16; in tumour cells, inactivating mutations which result in the underproduction of p16 are common.[7] Moreover, the activating partner cyclin D1 and the catalytic subunit CDK4 are often either overproduced or hyperactivated in many tumour cells. A small molecule inhibitor of CDK4 would result in early G1 arrest of the cell cycle and thus prevent uncontrolled cell growth, the hallmark of all tumour cells. Given that CDK4 is a kinase, the most obvious structures to act as inhibitors of CDK4 are analogues of ATP.[8] It is also known that structures totally unrelated to ATP, such as staurosporin and flavopiridol, are also effective inhibitors of CDK4.[4]

Fascaplysin **1** (Fig. 1), is a pentacyclic quaternary salt originally isolated from the Fijian sponge *Fascaplysinopsis* Bergquist sp,[9] which inhibits the growth of several microbes, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *and Saccharomyces cerevisiae*, and suppresses the proliferation of mouse leukemia cells L-1210 with ED_{50} = 0.2 μm mL^{-1}. Recently, fascaplysin has been reported to specifically inhibit CDK4, causing G1 arrest of tumour (U2-OS, HCT-116) and normal (MRC-5) cells.[10] However, fascaplysin is highly toxic – the potential for its planar structure to intercalate with DNA has been suggested as a possible explanation.[11] The aim of the current study is therefore to devise a potent, non-toxic (non-planar) CDK4 inhibitor based on the structure of fascaplysin.

The proposed strategy (Fig. 1) is to release bonds a, b and c in fascaplysin **1** leading to the tryptamine derivative **7**, this compound contains the structural components of fascaplysin, but it is non-planar and will not intercalate with DNA. Clearly it does not necessarily follow that compound **7** will exhibit CDK4 activity. To investigate this prior to embarking upon synthesis, the CDK4 activity of compound **7** was predicted (Table 1) using *in silico* modelling methods which docked compound **7** into the active site of CDK4. Since there is no experimentally determined 3-dimensional structure of CDK4 currently available, this structure was produced by homology modelling (using Modeller[12]) based on the crystal structures of CDK2[13] and CDK6.[14] Fascaplysin **1**, and the unsubstituted (compound **7a**), Cl (**7b**) and Br (**7c**) derivatives of compound **7**, were docked in turn (using Gold[15]) into the active site of our CDK4 model. Interestingly, compound **7** is predicted to bind to CDK4 with its aromatic rings positioned similarly to those of fascaplysin (Fig. 2), supporting our design strategy for non-planar fascaplysin analogues (Fig. 1). Our docking studies of fascaplysin (Table 1) are consistent with its high experimentally observed affinity for CDK4 (binding with a sub-μM IC_{50}), giving confidence in our *in silico* approach. Our computational studies also predict (Table 1) that compounds **7a–c** will exhibit CDK4 activity.

The synthesis of compound **7** is shown in Scheme 1. Tryptamine **2** was reacted with ethyl chloroformate to give the urethane **3** in

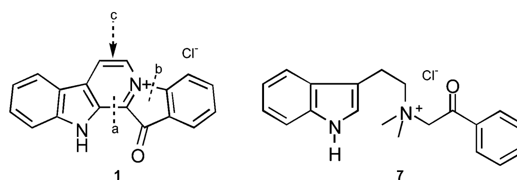

Fig. 1 Strategy: to remove from fascaplysin **1** bonds *a* and *b*, and make *c* a single bond, producing the non-planar tryptamine derivative **7**, in which three of the five rings and the quaternary nitrogen are retained.

Table 1 CDK4 activity

[a]Compound	[b]Predicted: Goldscore	[c]Measured: IC_{50}/μM
7a	41.5	70
7b	41.3	50
7c	43.6	50
Fascaplysin **1**	51.1	0.55

[a] Note that compounds **7a**, **7b** and **7c** are the unsubstituted, Cl and Br derivatives, respectively, of compound **7** (see Scheme 1). [b] Binding "energy" predicted *in silico* using the program Gold;[15] in our experience a Goldscore > 40 is indicative of a significant binding affinity. [c] CDK4:cyclin D1 assay, using RB-152 fusion protein as substrate.[10]

DOI: 10.1039/b406076h

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95% yield.[16] Reduction with lithium aluminium hydride produced *N*-methyltryptamine **4** in 89% yield.[16] This intermediate **4** was then reacted with different 4-substituted bromo acetophenone derivatives to afford the compounds **5a–c**.[17] Clearly, a wide range of diverse compounds can be created at this point in the route, which can be carried out on a large scale without extensive purification. The coupling of *N*-methyl tryptamine **4** with 2-bromoacetophenone, 2-bromo-4′-chloroacetophenone and 2,4′-dibromoacetophenone gave compounds **5a–c** in yields between 49 and 61% after column chromatography. The tertiary amines **5a–c** were unstable after a week when stored at −25 °C, as indicated by NMR. These materials were reacted separately with methyl *p*-toluene sulfonate to furnish the tosylate salts **6a–c**. Ion exchange using Dowex® Cl− 1×8–400 ion exchange resin produced the chloride salts **7a–c** in yields between 46% and 100%.[18]

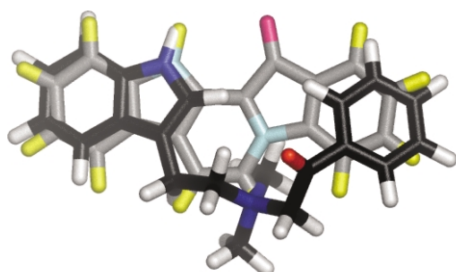
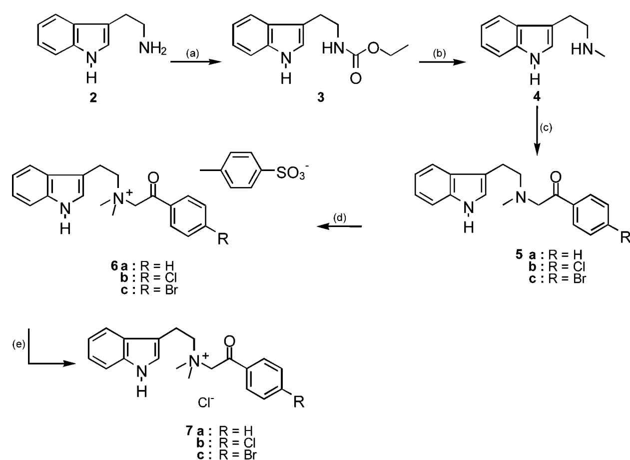


Fig. 2 Relative positions of fascaplysin **1** (C is in grey, N in cyan, O in magenta and H in yellow) and compound **7a** (C in black, N in blue, O in red and H in white) when docked into the active site of CDK4. This prediction concurs with the strategy adopted (Fig. 1).



Scheme 1 *Reagents and conditions.* (a) Ethyl choroformate, NaOH 4M, $CHCl_3$, 3 h, 95%; (b) $LiAlH_4$, THF, N_2, reflux, 1 h, 89%; (c) $BrCH_2COC_6H_4R$, toluene, N_2, $NaHCO_3$, Na_2SO_4, H_2O, 4 h, 49–61%; (d) methyl-*p*-toluenesulfonate, acetonitrile, reflux, 4 h, (e) Dowex® Cl− 1×8–400 ion exchange resin, overnight stirring and column, 46–100%.

The CDK4 activities of fascaplysin **1** and compounds **7a–c** were assayed *in vitro*, IC_{50} values (Table 1) were measured for inhibition of CDK4 using RB-152 fusion protein as a substrate. These results show that compounds **7a–c** are CDK4 active, confirming our predictions. Furthermore, whilst they fall a little (5-fold or so) short of the activities expected of a lead compound, they raise the distinct possibility that potent fascaplysin analogues can be produced in which the toxicity of fascaplysin has been designed out.

In summary, our strategy to design the first biologically active non-planar analogues of fascaplysin has proved successful. Compounds **7a–c** are active against CDK4, and are likely to pave the way for more potent non-planar, and by inference non-toxic, fascaplysin analogues.

This work was supported by Cancer Research UK.

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