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Synthesis of chromenoindole derivatives from *Robinia pseudoacacia*†

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An unusual tetrahydrochromeno[4,3-*b*]indole type analogue of medicarpin has been isolated from the roots of the Black Locust and subsequently has been synthesized together with an aromatic derivative which shows considerable biological activity against a range of significant targets.

Introduction

The phytoalexin medicarpin (**3**) is found in a wide range of legumes, such as barrel medic (*Medicago truncatula*, a Mediterranean form of clover) and alfalfa, where it is formed upon fungal infection of the plant.[1] During the last couple of decades, several studies have been conducted to investigate the various biological activities with this plant protective/defensive pterocarpan (for a review see ref. 1). Not surprisingly, medicarpin exhibits a good fungicidal activity against a range of pathogenic fungi,[2] including *Cladosporium cladosporioides*.[3] The compound is also effective against other microbes, including certain bacteria,[4] and has shown the ability to induce apoptosis in human lung fibroblasts and peripheral lymphocytes.[5] In the context of human health, medicarpin and its analogue coumestrol form a group of tetracyclic isoflavone-derivatives with phyto-estrogenic properties and have a potential impact on bone formation.[6] Interestingly, medicarpin is also present in the dried root of *Taverniera abyssinica*, and forms part of an analgesic and antipyretic Ethiopian drug called "Dingetegna" which, like medicarpin itself, shows strong nematicidal activity, for instance against *Caenorhabditis elegans*.[7]

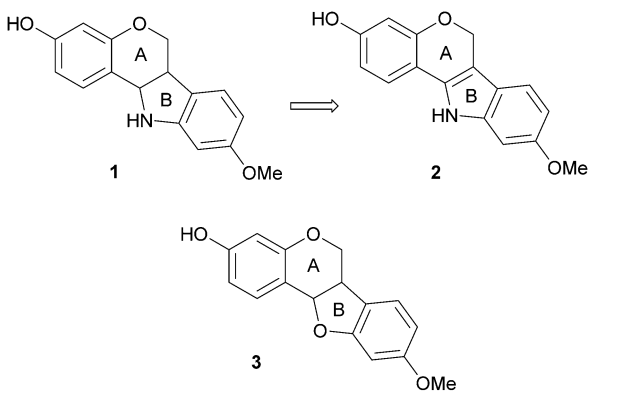
Results and discussion

(1) Isolation of a natural chromeno[4,3-*b*]indole derivative from the roots of *Robinia pseudoacacia*

As part of our ongoing research into natural products associated with a distinct biological activity, we have isolated a rather unusual compound **1** (Scheme 1) of the tetrahydro-chromeno-[4,3-*b*]indole type, a nitrogen-analogue of medicarpin (**3**), from the roots of the Black Locust (*Robinia pseudoacacia*), a tree known to be rich in (biologically active) flavonoids (for details see ESI†). This compound is rather unusual for a range of reasons. First of all, medicarpin (**3**) is synthesized from its (open) isoflavone derivative formononetin by formal ring closure involving B and the keto-function in ring A. In sharp contrast, the biosynthesis of **1**, which is hitherto unknown, would have to proceed *via* an imine, which would be fairly unusual. Secondly, whilst the overall three-dimensional structure of this compound may be similar to the one of medicarpin, the presence of the nitrogen (instead of the oxygen) atom may result in a distinctly different biological "chemistry" and hence activity, for instance regarding electrostatic interactions and metal binding.

(2) Initial screening for possible biological activity

In the next step, we have therefore studied some of the most obvious potential activities of **1**, such as (a) antioxidant activity (which is often associated with flavones), (b) aromatase inhibition (as found for coumestrol, see ref. 6) and cytotoxicity against (c) nematodes and (d) against a specific leukemia cell



Scheme 1 Chemical structures of the novel tetrahydro chromeno-[4,3-*b*]indole **1**, its chemical dihydro precursor **2** and its known oxygen analogue medicarpin (**3**).

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† Electronic supplementary information (ESI) available: Isolation of compound **1** from a natural source, details of chemical syntheses, NMR spectra and details of *in vitro* activity assays. See DOI: 10.1039/c3md00213f

line (human promyelocytic leukemia cells, HL-60). The results obtained for compound **1** in these assays have been mostly disappointing, with low or no activities observed in the DPPH antioxidant assay (IC_{50} > 300 μM), the aromatase inhibition assay (IC_{50} > 300 μM) and the *Steinernema feltiae* nematode assay (no significant toxicity observed at concentrations up to 200 μM and 48 h of incubation). At closer consideration, these results may not be too surprising, however, as compound **1** does not possess the typical kind of hydroquinone ring responsible for the antioxidant activity of many flavonoids and also lacks the two free hydroxyl-groups of coumestrol required for interactions within the estrogen pathway.

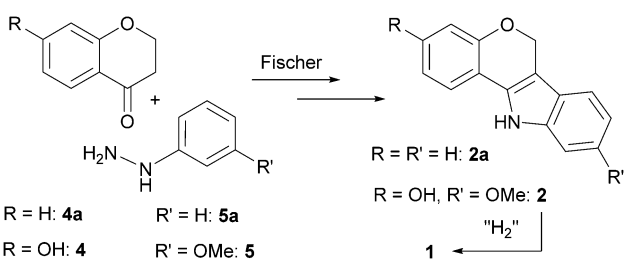
Compound **1**, however, did exhibit an interesting cytotoxicity against HL-60 cells, with cell survival of 72% at 40 μM and just 27% at 200 μM of compound **1**. Whilst this activity is rather modest when compared to classical cytotoxic molecules, it is still rather impressive for a natural product which has not yet been optimized for a specific activity.

(3) Strategy to synthesize dihydrochromeno[4,3-*b*]indole derivatives (compare 2) as precursors for 1

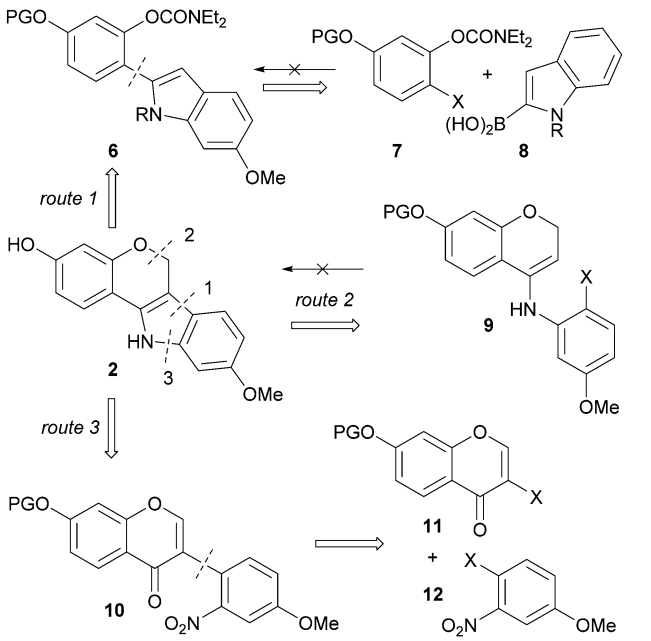
Due to the fact that the heterocyclic backbone of **1** is rather unknown and that only small amounts of the natural compound were available, a total synthesis of **1** seemed to be worthwhile. Ultimately, this should allow us to better understand the cytotoxic effects associated with compound **1**, to access the various derivatives of this novel class of natural chromeno[4,3-*b*]indoles and, possibly, also derive at more active compounds which ultimately may be useful for pharmacological application. Hence a strategy has been developed to synthesize these compounds **1**/**2** and to extend the initial pharmacological studies of **1** and of similar compounds.

The synthesis of the non-substituted dihydrochromeno-[4,3-*b*]indole skeleton **2a** was described by Buu-Hoï[8] using the classical Fischer indole approach (Scheme 2). Besides the low yield (15%), this route is impracticable for the synthesis of substituted derivatives such as **2** because of the need for specific chromanones (*e.g.* **4**) as starting material and because of a notable lack of regioselectivity in the indole ring formation with substituted phenylhydrazines like **5**.

Numerous additional methods have been described for the synthesis of indole derivatives.[9] For the highly condensed heterocyclic system **2** we first explored two routes following the retrosynthesis depicted in Scheme 3. The Suzuki coupling[10] between carbamoyl aryl halides **7** and an indole boronic ester **8**



Scheme 2 Fischer approach to the dihydrochromeno[4,3-*b*]-indole skeleton **4**.



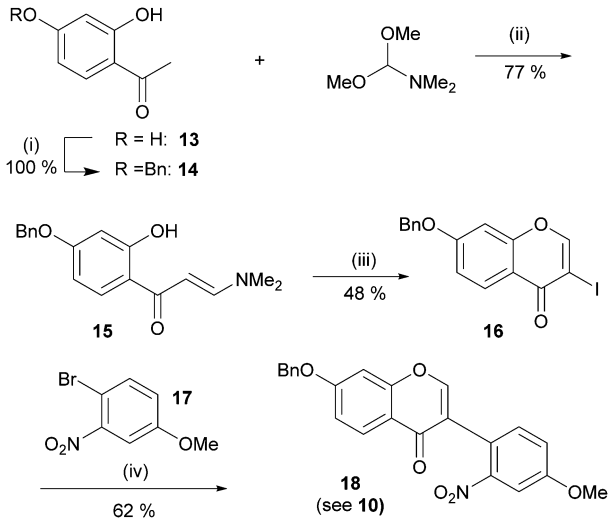
Scheme 3 Retrosynthesis for compound **2** and previous attempts for synthesis (PG: protective group). Schemes 3/4 (old) combined, the text was adapted.

(to **6**) resulted in low yields and efficiency (route 1). Attempts to cyclize haloenamins **9** (available through enamine formation by Buchwald–Hartwig coupling[11] as an intermediate step) failed (route 2).

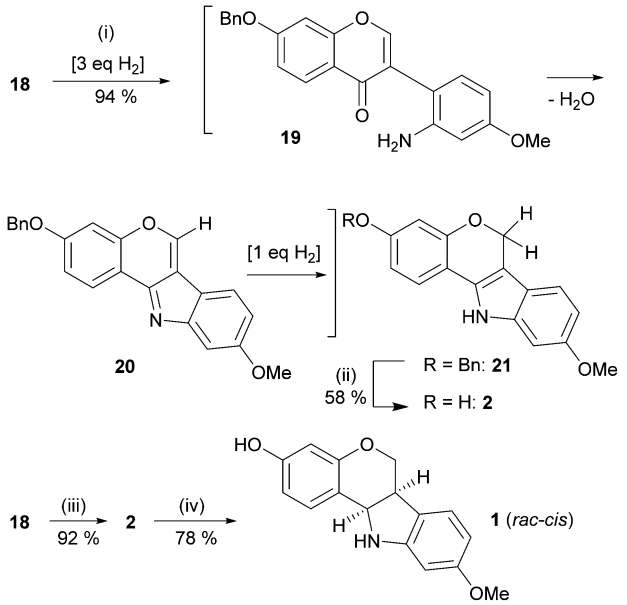
Hence, an alternative strategy had to be employed. Several methods used to prepare condensed indole compounds are based on C–C-cross-coupling followed by cyclization. For instance, Ullmann type coupling between an α-halo enone and an α-halo nitroarene followed by hydrogenation to the corresponding anilines and (spontaneous) ring closure leads to indoles (similar to the "Reissert synthesis").[12] Based on this strategy (route 3), the indole structure of **2** would be accessible by such a sequence starting with a halochromenone **11** and an α-halo nitroarene **12** and formation of **10** in the first step.

For reasons of accessibility and stability we synthesized the 3-iodochromenone **16** from a mono-protected dihydroxy acetophenone **14** by aldol condensation with dimethylformamide dimethylacetal and subsequent iodo-induced cyclization[13] in 37% overall yield. Ullmann type cross coupling with the bromo nitroanisole **16** yielded the 3-(*o*-nitroaryl)chromenone **18** (Scheme 4).

Several reaction conditions for a hydrogenation cascade from **18** to the dihydrochromenoindol **2** were set up also considering the solubility of **18** and some intermediates (Scheme 5). With H_2–Pd–C in MeOH or EtOAc[12] mainly a mixture of **20** and **21** was obtained whereas H_2–Pt–C in THF[14] gave **21** in high yield. But **21** could be debenzylated to **2** only under harsh reaction conditions *via* catalytic transfer hydrogenation using 1,4-cyclohexadiene as a hydrogen donor.[15] Due to this additional cleavage step, we tried to proceed with the synthesis without the phenol protecting group, but this approach led to difficult work-up conditions in the first step already.



Scheme 4 Synthesis of the indole precursor **18**. *Reaction conditions*: (i) K_2CO_3, BnCl, MeCN, reflux; (ii) 90 °C; (iii) I_2, $CHCl_3$, r.t.; (iv) Cu, $Pd_2(dba)_3$, DMSO, 70 °C.



Scheme 5 Hydrogenation steps for the synthesis of **1** from **18**. *Reaction conditions*: (i) 10% Pt/C, THF, 15 bar H_2; (ii) cyclohexadiene, EtOAc, Pd/C, 70 °C; (iii) 10% Pt/C, $Pd(OH)_2$/C, THF, 5 bar H_2; (iv) $NaBH_3CN$, HOAc, r.t. Stereo formula for **1** was added.

Finally, by adding Pearlman's catalyst [$Pd(OH)_2$/C][16] to the platinum catalyst, the cyclization as well as the deprotection occurred in one step to give the dihydrochromenoindole **2** in high yield.

Since the reaction conditions for the catalytic hydrogenation of indoles are drastic,[14] we decided to reduce the double bond using $NaBH_3CN$ in HOAc[17] and obtained the desired indoline **1**, now synthesized for the first time in a six-step synthesis and 16% overall yield. The spectroscopic data of the synthetic sample are identical to those of the isolated compound (see ESI†). As expected[18] and clearly verified for the medicarpin analogue **3**,[19] the chroman and indoline subunits of this tetrahydrochromeno-indole **1** are *cis* annulated for reasons of strong thermodynamical preference.[20] The natural compound was isolated as a racemate.

(4) Biological activity associated with compounds 1 and 2

Based on the literature describing various biological activities associated with medicarpin and coumestrol, and taking into account the notable activities initially seen for the isolated compound, it was decided to take a closer look at possible – and possibly useful – biological activities associated with synthetic, chemically pure compound **1** and its unsaturated analogue **2**. Here, it was expected that the pure compound **1** – as well as its "medicarpin-like" analogue **2** – may be more active compared to the isolated material. Both compounds were considered together, as they clearly differ in their three-dimensional shape and also chemical reactivity (see Fig. 1). They were tested against relevant targets, such as the human colon cancer cell line HCT-116 (this cell line is more representative of a typical cancer cell than HL-60).

Fig. 2 shows the biological activity of **1** and **2** in the HCT-116 cell culture assay. Whilst the natural substance **1** shows no significant toxicity up to a concentration of 100 μM (higher concentrations are not considered as pharmaceutically relevant), the unsaturated analogue **2** is fairly cytotoxic against these cancer cells, with an IC_{50} of 66.1 μM. These results are rather intriguing. Whilst they confirm that compound **1** is not particularly toxic against human cancer cells (as already expected when using the extracted compound), they also highlight the quite significant change of activity when moving from the 2-pyrroline to the pyrrole ring which in **2** forms part of an indole moiety.

While these initial results highlight a notable activity associated with the indole-containing compound **2**, they cannot yet explain why this compound is active whilst compound **1** is not. One may speculate that **2**, as a planar, tetracyclic molecule may interact with DNA (*e.g. via* intercalation) or enzymes involved in the synthesis and/or replication of DNA. Here, the indole ring present in **2** may enhance binding to DNA, possibly by coordinating to a shared Mg^{2+} cation. Indeed, indoles, such as the amino acid tryptophan, are known to interact quite strongly with metal ions, such as Mg^{2+}, and, besides the tetracyclic

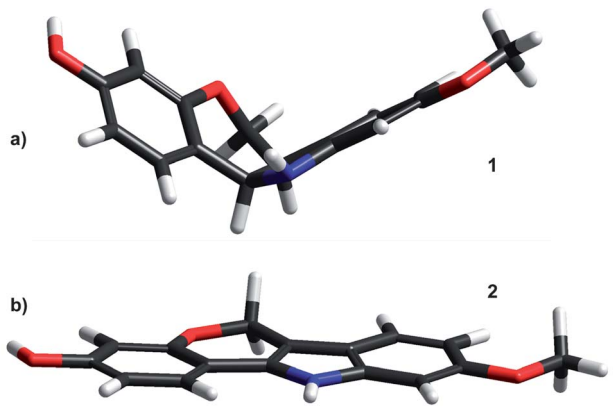


Fig. 1 3D shapes of **1** and **2** calculated by AM1.[21]

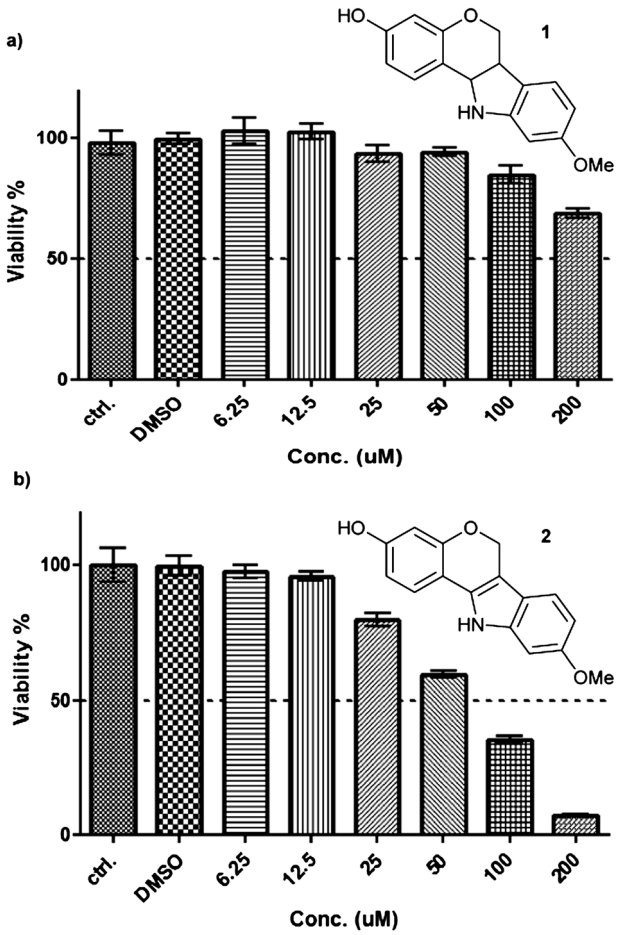


Fig. 2 Biological activity of **1** and **2** in the HCT-116 cell culture assay.

scaffold, one of the most apparent (bio-)chemical aspects found in the structure of compound **2** is the apparent cation-π metal binding site at the indole moiety. Interestingly, the latter is notably absent in medicarpin **3** and is also clearly different in pyrroline **1**, which despite featuring a nitrogen atom lacks this particular π-system.

Conclusions

In summary, the biological activities associated with compound **2** are rather interesting and may form the basis for more extensive research on structural analogues of this "not quite natural" analogue of natural compound **1**. Here, isomers with the OH group in the vicinity of the nitrogen may be of particular interest as far as metal binding is concerned. At the same time, the furan analogue of medicarpin may be of interest, considering that the presence of an aromatic ring in this oxygen-analogue may result in a similar activity as found for compound **2**. From a more pharmacological perspective, issues related to the spectrum of activity (against different cell types), selectivity (*e.g.* for specific cancer cells) and bioavailability also need to be considered. And finally, the biochemical mechanism of action needs to be studied in more detail, as it is still unclear why compound **2** is rather cytotoxic whilst compound **1** is not. As compounds **1**, **2** and various analogues are now easily accessible *via* the synthetic route described as part of this study, more detailed investigations are now feasible and bode well for future drug development.

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