COMMUNICATION

Trienamine catalyzed asymmetric synthesis and biological investigation of a cytochalasin B-inspired compound collection†

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Due to their enhanced metabolic needs many cancers need a sufficient supply of glucose, and novel inhibitors of glucose import are in high demand. Cytochalasin B (CB) is a potent natural glucose import inhibitor which also impairs the actin cytoskeleton leading to undesired toxicity. With a view to identifying selective glucose import inhibitors we have developed an enantioselective trienamine catalyzed synthesis of a CB-inspired compound collection. Biological analysis revealed that indeed actin impairment can be distinguished from glucose import inhibition and led to the identification of the first selective glucose import inhibitor based on the basic structural architecture of cytochalasin B.

Fig. 1 Structure of cytochalasin B (CB) with the semi-saturated iso-indolinone motif highlighted and the outline of the synthesis strategy employing enantioselective trienamine organocatalysis.

Introduction

Cancer cells adapt to their often hypoxic environment and satisfy their increased need for fast nutrient utilization and metabolic building block generation by shifting their metabolism from oxidative phosphorylation (OXPHOS) to glycolysis even when sufficient oxygen is available. This reprogramming of energy metabolism, termed the Warburg effect, is considered a hallmark of cancer.[1] To compensate for the lower efficiency of ATP generation in glycolysis as compared to OXPHOS cancer cells upregulate glucose uptake through dysregulated expression of glucose transporters to facilitate import of glucose. In particular, overexpression of glucose transporter GLUT1 has been reported in many types of human cancers.[2] Small molecule inhibition of glucose uptake *via* GLUT1 is a promising strategy for the development of novel anti-cancer drug candidates and inhibitors of GLUT1 and related members of the GLUT family are in high demand.[3] The isoindolinone natural product cytochalasin B (CB; Fig. 1) is a potent GLUT inhibitor[4] widely used as a biological tool compound. However, CB also inhibits actin
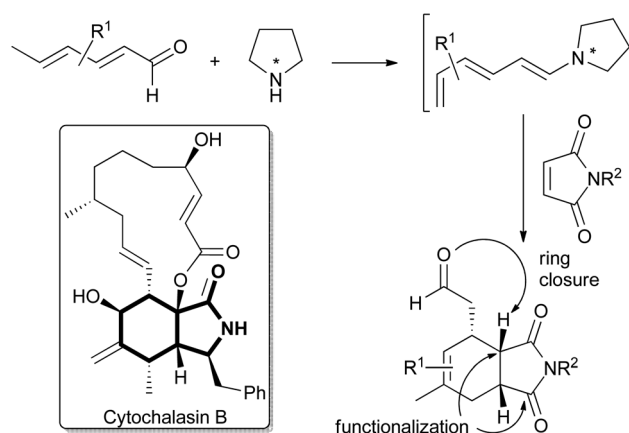
polymerization which prevents its use as a drug[5] and impairs its application as a tool compound in biology. Clearly, the discovery of CB analogs that inhibit GLUT activity but do not impair the actin cytoskeleton would be of major interest. However, to date only sparse data are available that correlate CB activity and structure[4] and a CB derivative that preferentially inhibits glucose uptake has not been identified. We have now synthesized a CB-inspired compound collection employing enantioselective trienamine catalysis as a key transformation. Biological evaluation of the library revealed the first CB-analogue that inhibits glucose uptake in cancer cells but does not impair actin polymerization.

For the synthesis of cytochalasin B and other cytochalasans an inter- or intramolecular Diels–Alder reaction between a diene- or a triene part and an α,β-unsaturated amide has been employed as a key step.[6] Recently, trienamine catalysis has emerged as a powerful method to steer the steric course of asymmetric Diels–Alder reactions,[7] and we decided to employ this method in the preparation of a cytochalasin inspired compound library. Chen and Jorgensen and co-workers have

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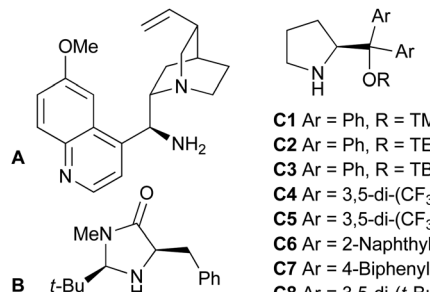†Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ob02272j

described that linear dienals, which in the presence of proline-derived catalysts form asymmetric trienamines, react with highly activated dienophiles such as cyanoacrylates to form Diels–Alder adducts in high enantiomeric excesses.[8]

However, weaker dienophiles, such as maleimides, did not yield the expected products. Subsequently, maleimides have successfully been reacted with branched aryl-[9] and methyl-[10] dienones by using cinchinoa alkaloid derived catalysts. However, the trienamine catalysed reaction between dienals and maleimides which would yield the semi-saturated iso-indolinone core of the cytochalasans (Fig. 1) has to the best of our knowledge not yet been reported.

Results and discussion

We screened various catalysts for the trienamine reaction between dienal **2a**,[11] and maleimide **1a** (Table 1). While we

were unable to isolate any product using the cinchona-based catalyst **A** previously used with aryldienones,[9] the use of the Macmillan catalyst[12] **B** afforded a low yield (21%) of *ent*-**3** after 90 hours in 28% ee. Fortunately, the use of diaryl prolinol silyl ethers **C** proved more successful. The Jorgensen catalysts[13] **C4** and **C5** gave much better ee's of 78% and 81%, respectively. These catalysts, however, significantly decreased the reaction rate compared to the diphenyl analogs **C1** and **C3**. The bulky, more electron rich catalyst **C8** [14] gave the highest ee (82%) with a reaction rate similar to that of the diphenyl substituted catalysts. The choice of solvent had a substantial impact on the ee, and chloroform was the best among the tested solvents. The acid had only small effects on the ee, but the yield increased by using benzoic acid instead of 2-fluorobenzoic acid (85% and 65%, respectively). Decreasing the temperature to −10 °C gave slightly higher ee and also increased the yield. However, as expected, this prolonged the reaction time significantly. The absolute stereochemical outcome of the asymmetric trienamine reaction was confirmed by the preparation of and comparison with a known compound[15] (see the ESI†).

To explore the scope of this reaction, various dienals and maleimides were subjected to trienamine catalysis with catalyst **C8** (Table 2). The alkyl substituted dienals **2b–d** [11] were more unstable than the phenyl substituted dienal **2a**, and a

Table 1 Identification of favorable conditions for the asymmetric trienamine reaction[a]
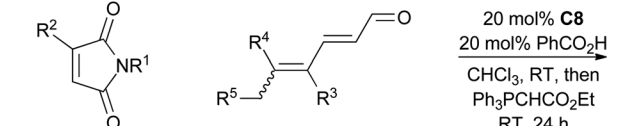
Entry	Cat.	Solvent	Acid	Temp. [°C]	Time [h]	Yield [%]	ee[b] [%]
1	A	CHCl₃	TFA[c]	40	24	n.r.[d]	—
2	B	CHCl₃	OFBA	40	90	21	−28
3	C1	CHCl₃	OFBA	40	18	78	34
4	C2	CHCl₃	OFBA	40	17	85	44
5	C3	CHCl₃	OFBA	40	24	74	46
6	C4	CHCl₃	OFBA	40	40	41	78
7	C5	CHCl₃	OFBA	40	64	68	81
8	C6	CHCl₃	OFBA	40	20	62	24
9	C7	CHCl₃	OFBA	40	21	68	6
10	C8	CHCl₃	OFBA	40	22	65	82
11	C8	PhMe	OFBA	40	19	61	58
12	C8	EtOH	OFBA	40	41	50	76
13	C8	DME	OFBA	40	20	45	45
14	C8	EtOAc	OFBA	40	16	59	49
15	C8	CHCl₃	BA	40	20	85	82
16	C8	CHCl₃	AcOH	40	23	62	80
17	C8	CHCl₃	OFBA	−10	120	79	83

[a] Reaction conditions: **1a** (0.15 mmol), **2a** (0.165 mmol), catalyst (0.03 mmol), acid (0.03 mmol), solvent (1.5 mL). [b] The ee was determined by chiral HPLC using a Chiralpak IA column. [c] 40 mol% acid. [d] n.r. = no reaction. OFBA = 2-fluorobenzoic acid, BA = benzoic acid, TMS = trimethylsilyl, TES = triethylsilyl, TBS = *tert*-butyldimethylsilyl, TFA = trifluoroacetic acid.

Table 2 Scope of the reaction between various maleimides and dienals

Entry	Prod.	R¹, R²	Time 1st step (h)	Yield (%)	ee (%)	Endo : exo	E : Z
1	4a	H, H	22	37	78	18 : 1	9 : 1
2	4b	Bn, H	22	71	72	19 : 1	19 : 1
3	4c	Ph, H	40	75	92	11 : 1	>20 : 1
4	5a	Me, H	24	34	81	9 : 1	>20 : 1
5	5b	Bn, H	45	25	73	>20 : 1	>20 : 1
6	5c	Ph, H	45	46	73	>20 : 1	>20 : 1
7	6a	Bn, H	70	58	85	>20 : 1	14 : 1
8	6b	Ph, H	47	78	85	>20 : 1	15 : 1
9	7a	Me, H	21	71	76	>20 : 1	>20 : 1
10	7b	Bn, H	21	58	81	>20 : 1	>20 : 1
11	7c	Ph, H	21	57	79	>20 : 1	>20 : 1
12	7d	Ph, Me	120	25	82	>20 : 1	>20 : 1

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slightly higher excess (1.5 equiv.) of these dienals was used. The reaction was run at room temperature and the resulting aldehydes were directly treated with a Wittig-reagent to avoid isolation of the somewhat sensitive products. The resulting α,β-unsaturated esters **4–7** were formed with 72–92% ee (Table 2).

The reaction tolerated a variety of maleimides and dienals. However, linear dienals such as hexadienal failed to give any notable conversion under these conditions, and when a high concentration of hexadienal was used, the maleimide instead underwent a Diels–Alder reaction with the dienal rather than the trienamine. This is in agreement with the same observation for aryl dienones.[9] Generally the products were formed in good yields, but compounds **5**, which resulted from the least stable dienal, **2b**, were obtained in lower yields. Also the 2-methyl substituted maleimide gave a slower reaction and lower yield. The *endo*-selectivity was good to excellent in all cases.

The prepared α,β-unsaturated esters are themselves interesting as reactants to construct new, more complex natural

product-like heterocycles, as exemplified by the synthesis of compound **8** by means of a subsequent highly diastereoselective intramolecular Michael addition (Scheme 1). The configuration of the newly formed stereocenter was determined by NOE measurements. Compound **8** has structural similarities to cytotoxic natural products such as quadrone[16] and suberosenol A.[17]

For the synthesis of a CB-inspired compound collection we envisioned reduction of the intermediate aldehyde formed in the cycloaddition reaction to avoid potential reactivity problems. For establishment of a suitable synthesis sequence initially the cycloadducts were synthesized as racemates in a thermal non-enantioselective Diels–Alder reaction as shown in Scheme 2. To this end, the dienes were equipped either with a methyl ether (**9a**) or an allyl ether (**9b**) which would enable subsequent ring closure by means of ring closing metathesis. The obtained imides **10** were then selectively reduced with DIBAL,[18] activated as sulfones, and then coupled with Grignard-reagents using a zinc-mediated reaction[19] to produce **11a–g** with an additional substituent in the pyrrolidine ring by analogy to the structure of CB. Imides with unprotected *N*-H did not provide the expected products, but the corresponding TBS-protected imides conveniently gave the substituted *N*-H amides **11f–g** without a separate deprotection step. Boc-protection of these two amides was necessary for further reactions. To further approximate the CB structure, α-hydroxylation of the amide is necessary. For α-hydroxylation of related compounds the Davis oxaziridine has previously been employed,[20] and **12b–c** were synthesized using similar conditions. To prepare compounds **12a** and **12d**, however, the reaction with
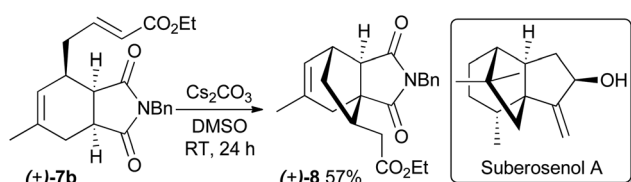
**Scheme 1** Intramolecular Michael addition of **7b**.
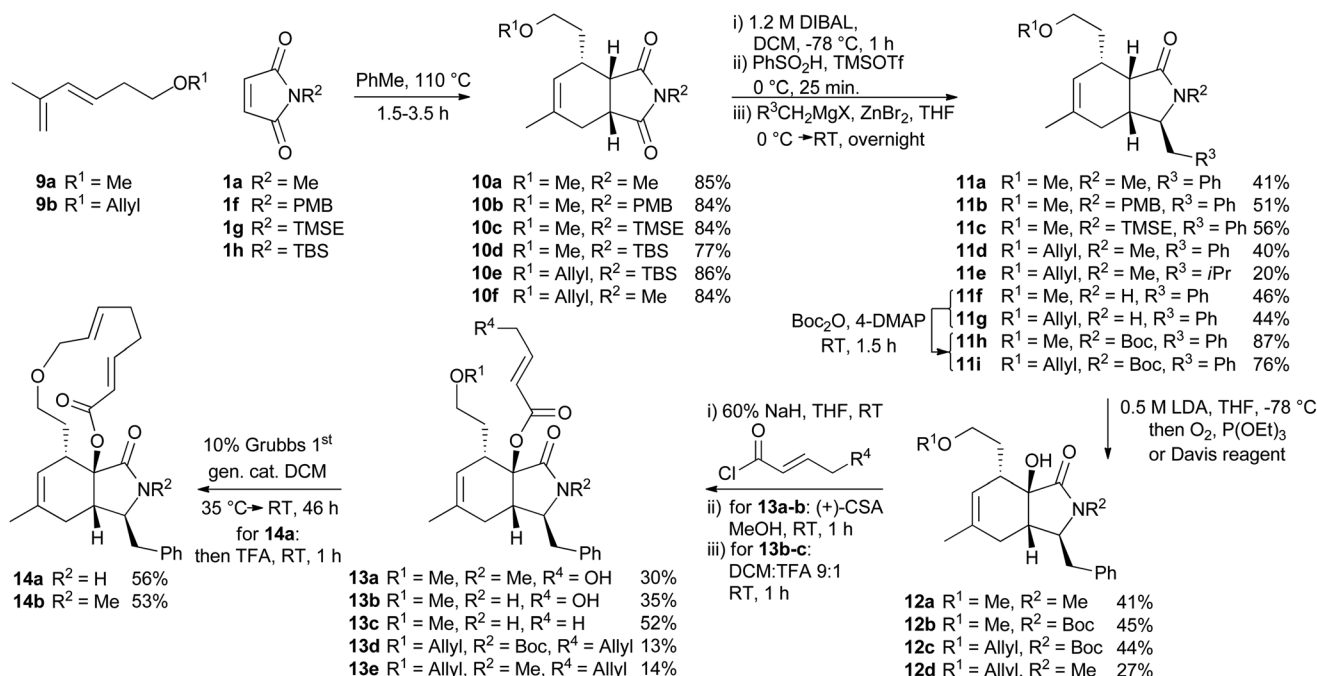
Scheme 2 Synthesis of a CB inspired compound collection. PMB = *para*-methoxybenzyl, TMSE = 2-trimethylsilylethyl, Boc = *tert*-butoxycarbonyl.

dioxygen[18] was more effective. The tertiary alcohols **12** proved unreactive to coupling with acids using coupling reagents such as carbodiimides,[20] PyBOP, or HBTU. The alcohols were also unreactive to acid chlorides and 4-DMAP and required deprotonation with sodium hydride to react with acid chlorides. Finally metathesis of **13d–e** successfully afforded the macrocyclic compounds **14a–b**[21] with the same ring-size as CB. Several of the synthesized compounds were also prepared asymmetrically using trienamine catalysis, followed by

reduction with sodium borohydride, and then either methylation with TMS-diazomethane, or allylation with *tert*-butyl-allyl carbonate[22] (Scheme 3).

The compounds **4–8** and **10–15** were then investigated for inhibition of glucose import in the human colon cancer cell line HCT116 using a 2-deoxyglucose-based assay.[23] Compounds which showed significant inhibition at the initially employed concentration of 180 μM were also analysed at 90 μM and 45 μM. The results revealed that compounds which embody the partially saturated isoindolinone core of CB but lack the medium-sized lactone ring can inhibit glucose import as was in particular observed for **11c** and **12c** (Fig. 2A). The absolute configuration of these cycloadducts is only of minor importance. These compounds carry only a substituent in the cyclohexane ring and a benzyl group in the pyrrolidine. Analogous bicyclic compounds with an additional substituent α to the carbonyl group (**13**) were not active. Notably, compound **14a** which contains the macrocyclic ring was active in the glucose import assay. Bicyclic compound **12c** and tricyclic CB analog **14a** were finally investigated for possible influence on the actin cytoskeleton (Fig. 2B). Very gratifyingly, at 180 μM concentration both compounds did not impair the actin cytoskeleton in cells.
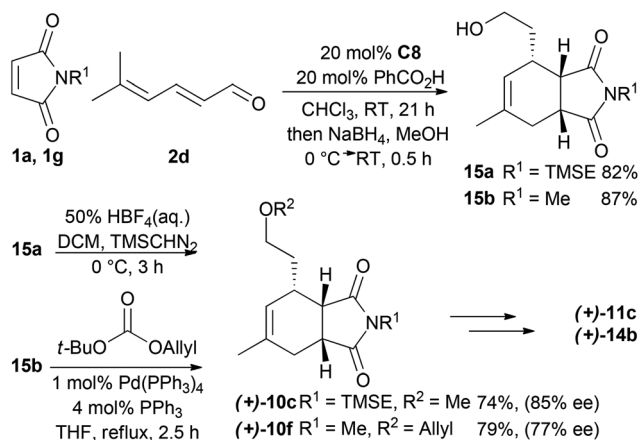
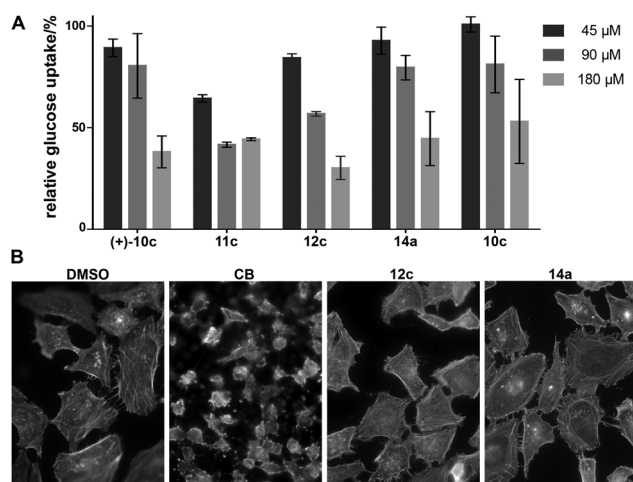**Scheme 3** Asymmetric synthesis of selected compounds.

Fig. 2 Influence of selected compounds on glucose uptake and the actin cytoskeleton. (A) Glucose uptake was determined in HCT116 cells upon treatment with 2-deoxy-D-glucose (2DG) and with the compounds or DMSO as a control for 30 min followed by detection of 2DG uptake by means of an enzyme coupled reaction using glucose-6-phospate dehydrogenase, NADP$^+$ and diaphorase to reduce weakly fluorescent resazurin to highly fluorescent resorufin. Data are shown as mean values (n = 3) ± s.d. and were normalized to DMSO. (B) The influence of **12c** and **14a** on the actin cytoskeleton was investigated in HeLa cells. Cells were treated with the compound or DMSO and CB as a control for 1 h prior to fixation and staining of actin using phalloidin labelled with TRITC. Nuclei were stained with DAPI.

Conclusions

Our results demonstrate that it is indeed possible to differentiate the glucose import inhibiting activity of cytochalasin B from its influence on actin polymerization. Structurally significantly simplified CB analogs **12c** and **14a** are the first compounds that on the one hand resemble the characteristic structural architecture of the natural product at the scaffold level, yet are glucose import inhibitors only. Clearly, these compounds do not reach the potency of the natural product itself, and our data in accord with previous findings[4,5] indicate that structural fine-tuning of the macrocycle and the partially saturated isoindolinone scaffold is necessary for full activity. However, they also suggest that by means of appropriate structure–activity correlation it may be possible to develop potent glucose import inhibitors based on the structure of cytochalasin B which are devoid of undesired impact on the actin cytoskeleton.

Experimental

Detailed experimental procedures and analytical data are described in the ESI.†

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