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Synthesis and biological evaluation of novel fumagillin and ovalicin analogues†

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A promising approach among the numerous efforts to cure cancer is the interruption of the tumour-induced formation of new blood vessels (angiogenesis). By suppressing angiogenesis with drugs, the tumour can neither grow to a life threatening size, nor metastasize. The natural product fumagillin **1** and the structurally related ovalicin **2** are two of the most potent anti-angiogenic compounds. Here, we report the design and synthesis of novel fumagillin and ovalicin analogues lacking reactive epoxy functionalities, which were thought to be responsible for the severe toxic side-effects observed. We also report a new synthetic approach and the determination of the anti-angiogenic properties of these compounds in endothelial cells.

Introduction

Claiming more than 2.5 million new victims every year, cancer is one of the leading causes of death among the Western population.[1] Consequently, there are numerous efforts to develop effective therapies for treating this complex disease. One promising approach is the interruption of the tumour induced angiogenesis, the formation of new blood vessels. This strategy, which was initially proposed by J. Folkman, targets the nutrient and oxygen supply of the tumour.[2] Without initiating and maintaining a connection to the vascular system, a tumour can not grow beyond the size of a few cubic millimetres. By suppressing angiogenesis with drugs, the tumour can neither grow to a life threatening size, nor metastasize. In addition to the application in oncology, anti-angiogenic drugs are in demand for various diseases that are associated with pathological angiogenesis. These include rheumatoid arthritis and diabetic retinopathy, the major cause of blindness in Europe.[3–5]

The natural product fumagillin **1** and the structurally related ovalicin **2** are two of the most potent anti-angiogenic compounds (Scheme 1).[6–8] Both fumagillin and ovalicin bind covalently to the active site of the enzyme methionine-aminopeptidase type 2 (MetAP2) and irreversibly block its proteolytic activity.[8–10] The exact relation between the inactivation of MetAP2 and the anti-angiogenic effect of fumagillin is not understood, though there seems to exist vast evidence for causal coherence.[8,11–14] However, Phillips and coworkers could recently demonstrate that depletion of MetAP2 by siRNA does not inhibit endothelial cell growth.[15] Moreover, MetAP2-depleted endothelial cells remain responsive to inhibition by fumagillin. These findings suggest that MetAP2 function is not required for endothelial cell proliferation. As we have shown recently, fumagillin inhibits the expression of the transcription factor Ets-1, which is an essential positive regulator of angiogenesis.[16]

TNP-470 **3** (Scheme 1), a semisynthetic derivative of fumagillin, reduces the proliferation of endothelial cells with an IC_{50} value of 2.5×10^{-11} M, and has already entered several clinical studies for the treatment of solid tumours including Kaposi sarcoma, prostate, kidney and cervix carcinomas.[7,17–21] Apart from a very short physiological half-life, TNP-470 causes severe side effects such as ataxia, vertigo, drowsiness and agitation.[18,22–24] The side effects can be attributed to the reactive functional groups. Therefore, extensive efforts are being made to develop new derivatives, especially those that lack the reactive epoxides as, *e.g.*, in the recently synthesised Fumagalone.[25]

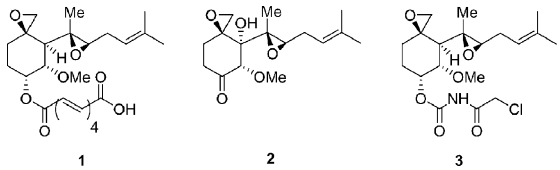
The present investigation and synthesis of fumagillin and ovalicin analogues is mostly based on semisynthetic strategies, starting from the natural products. This approach limited us mainly to modifications of the C6–O position which can be liberated by saponification of the ester moiety and derivatives that are accessible by ring opening of the spiroepoxide with suitable nucleophiles.[14,26–28] The relevance of the other functional groups, especially the isoprenoid side chain, has been barely studied. Recently, Eustache *et al.* presented a strategy that allows modifications of the side chain and the access to ring-substituted analogues.[29] To date, eight and four total syntheses have been reported for fumagillin and ovalicin, respectively.[30–42] However, none seemed to be applicable or practicable for our intentions.

Here we report the design of novel fumagillin and ovalicin analogues lacking one or both reactive epoxides. We also report a new synthetic route for the synthesis of these analogues, and the determination of their anti-angiogenic properties in endothelial cells.

Previous findings show that the conversion of the spiroepoxide to a methylene group results in a considerable reduction of the biological activity (IC_{50} 10^{-7} M).[13,43] Thus, the spiroepoxide is essential for the activity of fumagillin.

On the other hand the epoxide of the side chain has no major impact on the biological activity. As previous studies show the transformation to a carbon–carbon double bond, however, reduces the potency (IC_{50} 5×10^{-9} M for endothelial cell proliferation).[13] Therefore, we planned to retain the polar

† Electronic supplementary information (ESI) available: experimental data. See http://www.rsc.org/suppdata/ob/b5/b503163j/



Scheme 1 Fumagillin **1**, ovalicin **2** and TNP470 **3**.

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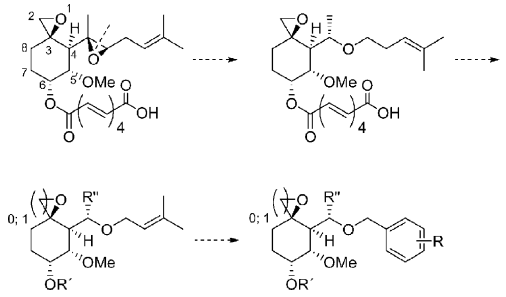
character of this position. This can be achieved by the formal scission of the carbon–carbon bond of the oxirane, which is equivalent to the transformation to an ether (Scheme 2). Furthermore, the oxygen can be replaced by other heteroatoms or appropriate functional groups. In all cases, the incision increases the total chain length by one methylene unit. Therefore, the distal portion of the side chain needs to be shortened accordingly. The simplest case yields an allyl ether. Apart from the dimethylallyl moiety, other functional groups such as aromatic substituents seem to be rational.[44]



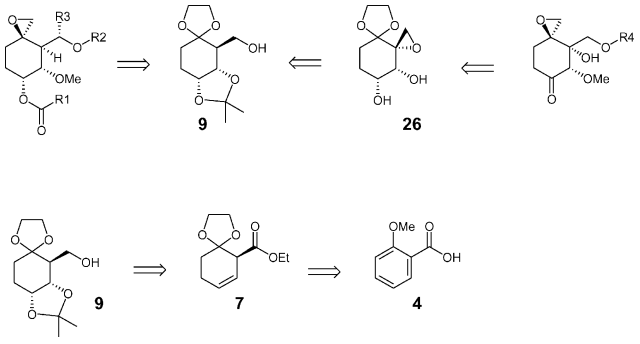
Scheme 2 Novel fumagillin analogues.

Although recent studies had shown that the spiroepoxide is crucial for the exceptional potency, the substitution of this oxirane with an inert functionality seemed to be very attractive. We reasoned that the replacement of the epoxide by a ketone was interesting, since it would not extensively affect the geometry of the central scaffold or the spatial requirements.

Due to the abundance of biological data on TNP-470 and the ease of its synthesis, we considered the substitution of the polyunsaturated ester of fumagillin with the chloroacetyl carbamate of TNP-470. Furthermore, we were interested in utilizing the recently described 3,4,5-trimethoxy cinnamic acid ester.[14]

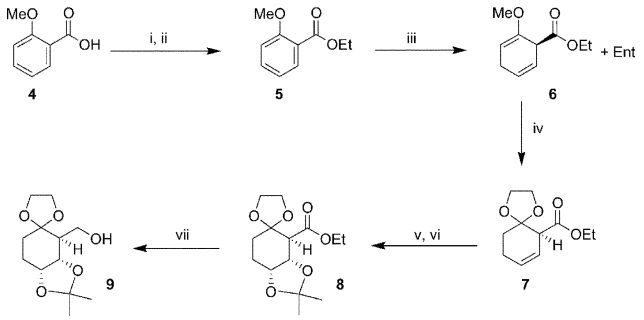
Results and discussion

The retrosynthetic analysis is shown in Scheme 3. Both the fumagillin and the ovalicin analogues, which feature an additional tertiary hydroxy group, can formally be dissected into four basic elements that are linked by a central cyclohexane scaffold. The individual residues comprise two vicinal, syn-oriented oxy substituents, which can be ascribed to a bishydroxylation, an adjacent anti-oriented alkyl substituent that can be utilized to direct the stereospecific outcome of the bishydroxylation and an adjoining spiroepoxide that can retrosynthetically be considered as a ketone. The synthesis strategy should be designed in a way that allows a wide variation of the alkyl side chain, a broad choice of the acyl substituent and the modification and replacement of the spiroepoxide, respectively. Both, the fumagillin and the ovalicin series can be derived from one common precursor **9**.



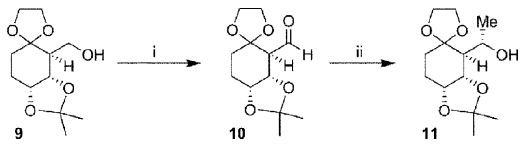
Scheme 3 Retrosynthetic analysis.

The racemic primary alcohol **9** is accessible starting from inexpensive and commercially available 2-methoxy benzoic acid **4** that was cleanly converted with quantitative yields to the corresponding ethyl ester **5** (Scheme 4). The aromatic ester was then reduced with sodium in liquid ammonia under modified Birch conditions to yield the 2,5-cyclohexadiene **6**.[45] The enol ether resembles an activated ketone that reacted under acidic conditions in ethylene glycol–DMF to form the spiroketal **7**. Osmium-catalyzed bishydroxylation of the remaining double bond yielded the desired diol as a single diastereomer in excellent yields. Both newly generated hydroxyl functionalities were then protected as acetonide **7** followed by the lithium aluminium hydride reduction of the ethyl ester to afford the central intermediate **9** in 7 steps and 46% overall yield.



Scheme 4 Synthesis of alcohol **9**; (i) oxalyl chloride, DCM, 0 °C to 25 °C; (ii) EtOH, pyridine, DCM, 0 to 25 °C (100%, two steps); (iii) Na, NH_3–THF, −78 to −33 °C (84%); (iv) ethylene glycol, *p*-tosOH, DMF, 65 °C (70%); (v) *N*-methyl morpholine *N*-oxide, OsO_4, acetone–water, 25 °C; (vi) 2,2-dimethoxy propane, *p*-tosOH, DMF, 65 °C (83%, two steps); (vii) LAH, THF, 0 to 25 °C (95%).

For the synthesis of the fumagillin series, alcohol **9** was directly alkylated with alkyl halides or oxidized with IBX in acetone to yield the α-chiral aldehyde **10** in excellent yields, which was then reacted with methyl magnesium bromide in THF at −78 °C to afford the secondary alcohol **11** as a single diastereomer (Scheme 5).[46]

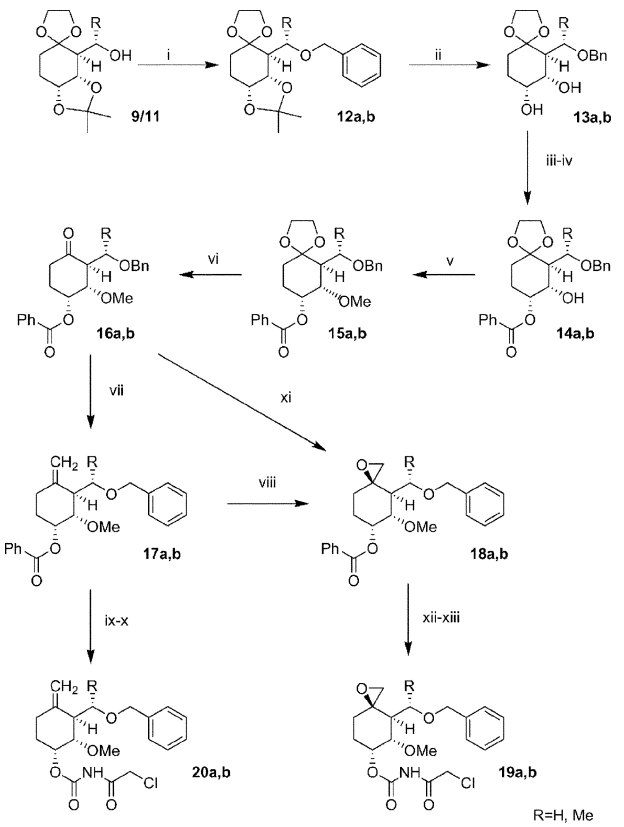


Scheme 5 Introduction of methyl branching; (i) IBX, acetone, reflux (98%); (ii) methyl magnesium bromide, THF, −78°C (96%).

The secondary alcohol **11** was analogously alkylated to alcohol **9** by treatment with NaH in DMF followed by addition of alkylhalides to yield the desired ether **12**. The vicinal diol could selectively be deprotected by acid catalyzed transketalization with ethylene glycol in DMF to afford compound **13** (Scheme 6). Conversion of the diol **13** to the dibutyl tin ketal proved to be a useful way to discriminate between the axial and equatorial hydroxyl groups, since the tin ketal reacted with benzoyl chloride in THF at low temperatures with a regioselectivity of 24 : 1 to yield benzoate **14**.[47] The undesired regioisomer could easily be separated by column chromatography and reisolated as starting material **13** after saponification.

Diazomethane with catalytic amounts of boron trifluoride etherate was the reagent of choice for the methylation of the remaining alcohol functionality of **14**. The spiroketal of compound **15** that protected the carbonyl group and needed to be removed at this step, proved to be exceptionally stable. All standard methods for deprotection resulted in reisolation or decomposition of starting material.[48] However, the use of catalytic amounts of cerium ammonium nitrate in aqueous acetonitrile, that was recently described by Marko *et al.* cleanly

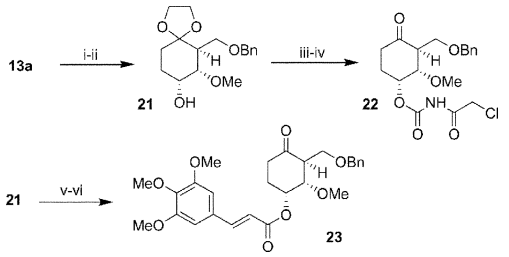
yielded the desired ketone **16a** and **16b**, respectively.[49] An additional advantage of this procedure with regard to the adjacent stereogenic center is the fact that the deprotection can be carried out under neutral conditions (Scheme 6).



Scheme 6 Synthesis of fumagillin analogues **19a,b** and **20a,b**; (i) BnBr, NaH, Bu_4NI, 0 to 25°C (95–98%); (ii) ethylene glycol, *p*-tosOH, DMF, 65 °C (98–100%); (iii) Bu_2SnO, MeOH, reflux; (iv) BzCl, THF, −40 to 25 °C (91–92%, two steps); (v) diazomethane, $BF_3{\cdot}Et_2O$, DCM, −78°C; (vi) cerium ammonium nitrate, MeCN–water, 65 °C (94–95% two steps); (vii) Nysted reagent; $TiCl_4$, −78 to 25 °C (65%); (viii) mCPBA, DCM, 0 °C (96% R = H, 6%); (ix) NaOH, MeOH, 25 °C (quant.); (x) chloroacetyl isocyanate, DCM, 0 °C (86–92%); (xi) trimethyl sulfoxonium iodide, NaH, 25 °C (22%); (xii) NaOH, MeOH, 25 °C (quant.); (xiii) chloroacetyl isocyanate, DCM, 0 °C (81–94%).

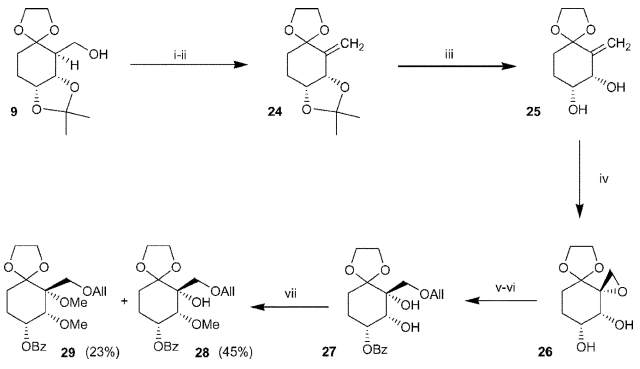
The direct epoxidation of ketone **16** with trimethylsulfoxonium ylide was only moderately successful in case of the unbranched side chain (R = H). The derivatives with a bulkier side chain yielded, depending on the reaction time and equivalents of sulfur ylide, only the α,β-unsaturated ketone and the cyclopropanated consecutive product, respectively.[35,38,50,51] However, a two step detour *via* olefin **17** with subsequent epoxidation of the double bond with *m*-chloroperbenzoic acid, turned out to be a valuable alternative. The derivative with the unbranched side chain was converted to the desired epoxide **18a** as the only product, whereas compound **17b** (R = Me) yielded a mixture of both diastereomeric epoxides. However, both diastereomers could be easily separated by column chromatography.[52]

After saponification of the benzoate, the free hydroxy group readily reacted in the last step with chloroacetyl isocyanate to form carbamate **19**.[27,28] In a similar approach, the olefin **17** was converted to the epoxide-lacking derivative **20**. Minor modifications of the synthetic route gave access to the cyclohexanone derivatives **22** and **23**. For this purpose, diol **13a** was converted to the dibutyl tin ketal as described above. Interestingly, the equatorial hydroxyl group exhibits the higher reactivity with alkyl halides. This feature was utilized in the reaction of the tin ketal with methyl iodide to give the desired methyl ether with a regioselectivity of 9 : 1. The remaining hydroxyl group was then acylated with either chloroacetyl isocyanate or trimethoxy cinnamic acid chloride, followed by the CAN catalyzed deprotection of the spiroketal to afford ketone **22** and ketone **23**, respectively (Scheme 7).



Scheme 7 Synthesis of fumagillin analogues **22** and **23**; (i) Bu_2SnO, MeOH, reflux; (ii) MeI, DMF, 25 °C (53%, two steps); (iii) chloroacetyl isocyanate, DCM, 0 °C; (iv) cerium ammonium nitrate, MeCN–water, 65 °C (41%, two steps); (v) 3,4,5-trimethoxy cinnamic acid chloride, DMAP, DCM, 25 °C; (vi) cerium ammonium nitrate, MeCN–water, 65 °C (46%, two steps).

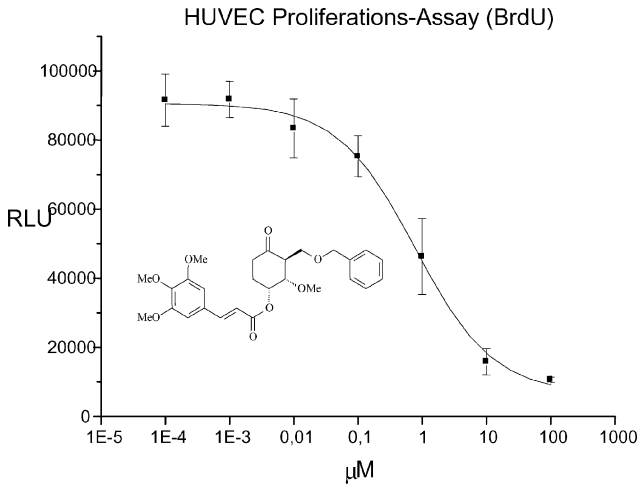
The tertiary hydroxyl group in 4-position, which is characteristic of the ovalicin series, was introduced starting from the central intermediate alcohol **9** (Scheme 8). Mesitylation followed by potassium *tert*-butoxide mediated elimination generated the exocyclic olefin **24** in 85% yield. After deprotection of the acetonide, we took advantage of the free allylic hydroxyl group of **25** that served as a directing neighbor group for the vanadium catalyzed epoxidation of the double bond to give compound **26** in good yields.[53] Of special interest is the dibutyltinoxide mediated epoxide opening by the alcohol that is used as solvent for the tin ketal formation in order to selectively benzoylate the axial hydroxyl group of diol **26**. Selective methylation of the secondary alcohol of compound **27** over the tertiary alcohol was accomplished with diazomethane with moderate selectivity to yield **28** in 45% yield with the bismethylated byproduct **29** in 23% yield. The subsequent transformations were carried out with minor modifications according to the fumagillin series.



Scheme 8 Synthesis of ovalicin analogues; (i) mesitoyl chloride, NEt_3, DCM; (ii) KO*t*Bu, DMF 0 °C (85% two steps); (iii) ethylene glycol, *p*-tosOH, DMF, 65°C (90%); (iv) *t*BuOOH, $VO(acac)_2$, DCM (67%); (v) Bu_2SnO, allyl alcohol, reflux; (vi) BzCl, THF, −40 to 25 °C (53%); (vii) diazomethane, $BF_3{\cdot}Et_2O$, DCM, −78°C.

All individual fumagillin and ovalicin derivatives were tested for their anti-angiogenic properties in an endothelial cell proliferation assay (Scheme 9, Table 1). For active compounds, the IC_{50} values were determined (as shown for compound **23** in Scheme 9). We were pleased to find that compound **23**, which lacks both reactive epoxides, inhibits the proliferation of endothelial cells in a dose dependent manner down to high nanomolar concentrations.

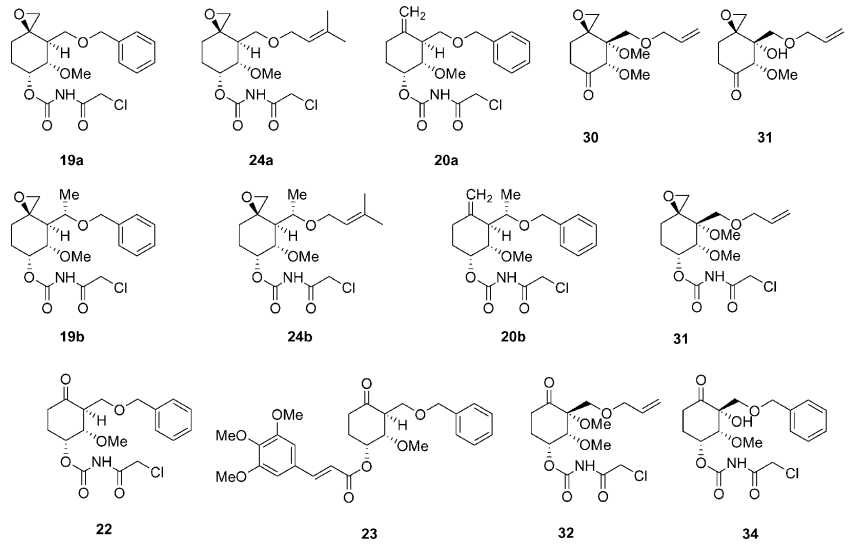
The fact that the presence of the spiroepoxide has no major impact on the activity in comparison to the corresponding ketones or methylene derivatives, suggests that these compounds might bind with a modified geometry their target, resulting in an incapacitated orientation of the epoxide.



Scheme 9 Dose dependent inhibition of cell proliferation (HUVEC) by compound **23**.

Table 1 Biological activity: inhibition of cell proliferation (HUVEC)

Compound	IC_{50}/µM
19a	1.5
19b	20
20a	11
20b	12
22	3.6
23	0.8
24a	3
24b	20
30	9
31	35
32	27
33	15
34	53



Scheme 10 Synthesised fumagillin and ovalicin analogues.

The comparison of compounds **19** and **24** shows that the benzyl group is a suitable substitute for the isoprenoid side chain. However, the introduction of the methyl branch results in a reduction of activity.

Conclusion

Our approach to novel analogues of fumagillin and ovalicin has proved to be very efficient and versatile. It offers also access to structure–activity relationship studies. Apart from modifications of the C6–*O*-acyl moiety, the synthetic approach enables an extensive variation of the alkyl side chain as well as the substitution of the spiroepoxide by alternative functionalities (see Scheme 10). Starting from 2-methoxybenzoic acid the desired target compounds were synthesised in 13 to 19 steps. All reactions afforded high yields and can be easily carried out on a multi-gram scale.

Compound **23** is of particular interest. Exhibiting an IC_{50} value of 0.5–1 µM, it was the most potent inhibitor of endothelial cell proliferation among the tested fumagillin analogues. This derivative has no reactive functionality and represents a promising lead structure for the development of potent anti-angiogenic drugs lacking the reactivity of fumagillin.

For experimental data refer to the electronic supplementary information.

Acknowledgements

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