Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1463–1466

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel Bicyclic Oxazolone Derivatives as Anti-Angiogenic Agents

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Received 12 November 2001; revised 25 March 2002; accepted 25 March 2002

Abstract—Novel bicyclic tetrahydropyrano[3,2-*d*]oxazolones derivatives, analogues of Fumagillin, were synthesised via a stereo-controlled oxidative-rearrangement of furylcarbinols and subsequent treatment with the appropriate isocyanate. These compounds demonstrated potent antiangiogenic activity.

The development of the anti-angiogenic[1] compound **TNP-470** (**1a**), which is currently undergoing clinical trials,[2] was based on the hemisynthesis[3] of the natural antibiotic fumagillin **1b**.[4] Several total syntheses of the 6-member ring system were described for fumagillin, but none were appropriate for process development (Fig. 1).[5]

Only the importance of the 4-*O* acyl group of **1a** has been previously studied. No other evaluation to determine the importance of the other functionalities around the ring with respect to the pharmacological activity has been assessed. Substitution of the methoxy moiety of **TNP-470** by the oxygen atom of a carbohydrate pyranyl ring was described by our group, with no loss of anti-angiogenic activity.[6] A complementary strategy to investigate the pharmacological contribution of the various substituents around the ring is proposed, via the total synthesis of bicyclic oxazolones **2** (Fig. 2).[7]

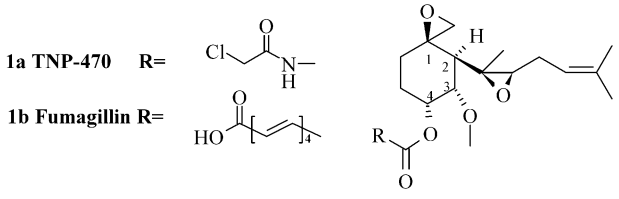

Figure 1. TNP-470 and Fumagillin.

Chemistry

A number of diverse furyl carbinol derivatives **4** was prepared via attack of a lithium acetylide on the appropriate 2-furyl ketone or aldehyde. This synthetic route should permit modification of the epoxide lateral chain, facilitating a study of the steric and electronic effects governing the activity.

Although the oxidation-rearrangement method of the 2-furyl carbinols could be achieved with a variety of oxidizing agents,[8] it was best performed in the presence of *N*-bromosuccinimide in aqueous media.[8a] In these

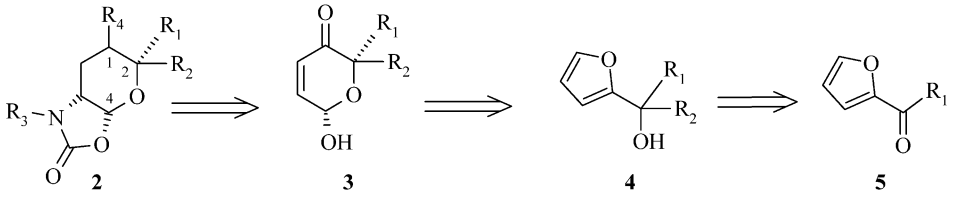

Figure 2. Retrosynthesis of bicyclic oxazolones **2**.

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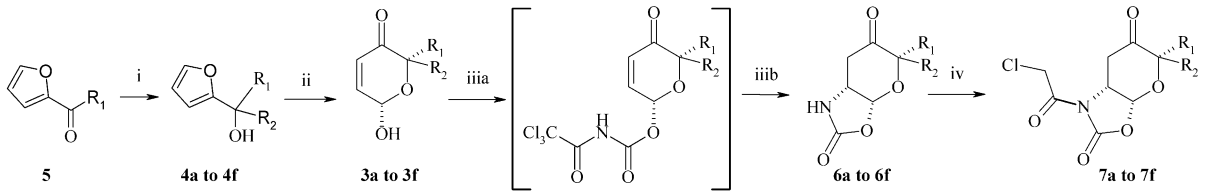
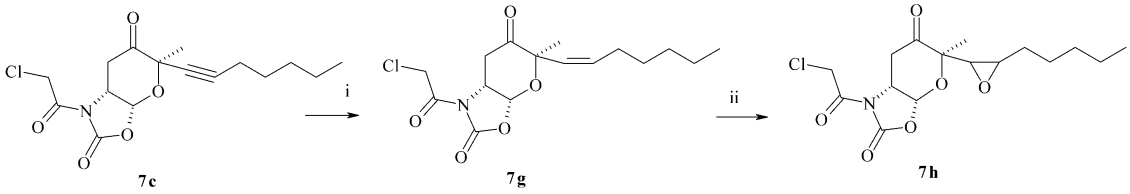


Scheme 1. Structural modification at C_2. (i) R_2Li, THF, −78 °C; (ii) NBS, THF, H_2O, 0 °C; (iii) (a) $CCl_3CON{=}C{=}O$, CH_2Cl_2; (b) $NaHCO_3$, acetone; v: (a) *n*BuLi, THF, −78 °C; (b) $ClCH_2COCl$.



Scheme 2. Structural modification of C_2 side chain. (i) H_2, Pd Lindlar, C_6H_6; (ii) 2,2-dimethyldioxirane, acetone.

equilibrating conditions, the ring closure of the presumed ene-dione intermediate yielded almost exclusively the thermodynamically favored isomer **3**. The C_4-hydroxyl adopted the *anti* configuration with respect to the largest substituent at C_2, probably due to the anomeric effect combined with some steric bias (Scheme 1). A significantly better chemical stability was observed for the C_2 methyl derivative **7b**[9] compared to the corresponding unsubstituted **7a** (Scheme 2, Table 1) presumably due to the absence of an enolizable proton at C_2.

The carbamoylation of pyranone **3** was performed with trichloroacetylisocyanate, producing the corresponding carbamoylated intermediate, which was cyclized upon basic treatment to give the bicyclic ring system **6** as a mixture of isomers (16:1). The preferred isomer contained the largest C_2 chain in a *trans* relative configuration with respect to the oxazolone ring. This bicyclic system was then acetylated on the oxazolone moiety to afford compounds **7a**–**f**.

Side chain modifications at C_2 were achieved by partial hydrogenation to afford the corresponding alkene derivatives **7g**, and subsequent oxidation to epoxides **7h**.

The ketone of synthon **6** could in turn be converted to the corresponding spiroepoxide or hydroxyl (Scheme 3). The spiroepoxide was introduced by either peracid oxidation of the double bond obtained by Wittig olefination to give the *syn* isomer **8a**, or by combination with Corey trimethylsulfonium method to give the *anti* isomer **9g**. The hydroxyl derivative was obtained by reduction of the C_1 ketone of **6**, by sodium borohydride in the presence of cerium chloride. A stereospecific control in favor of the *trans* isomer, could be achieved at low temperature to afford a 4:1 mixture of **10a$_1$**:**10a$_2$**.

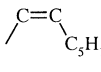
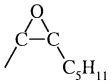
Similar side chain modulations as above were achieved by partial hydrogenation to afford the corresponding alkene derivatives **9g**, and epoxides **11h** and **12h**.

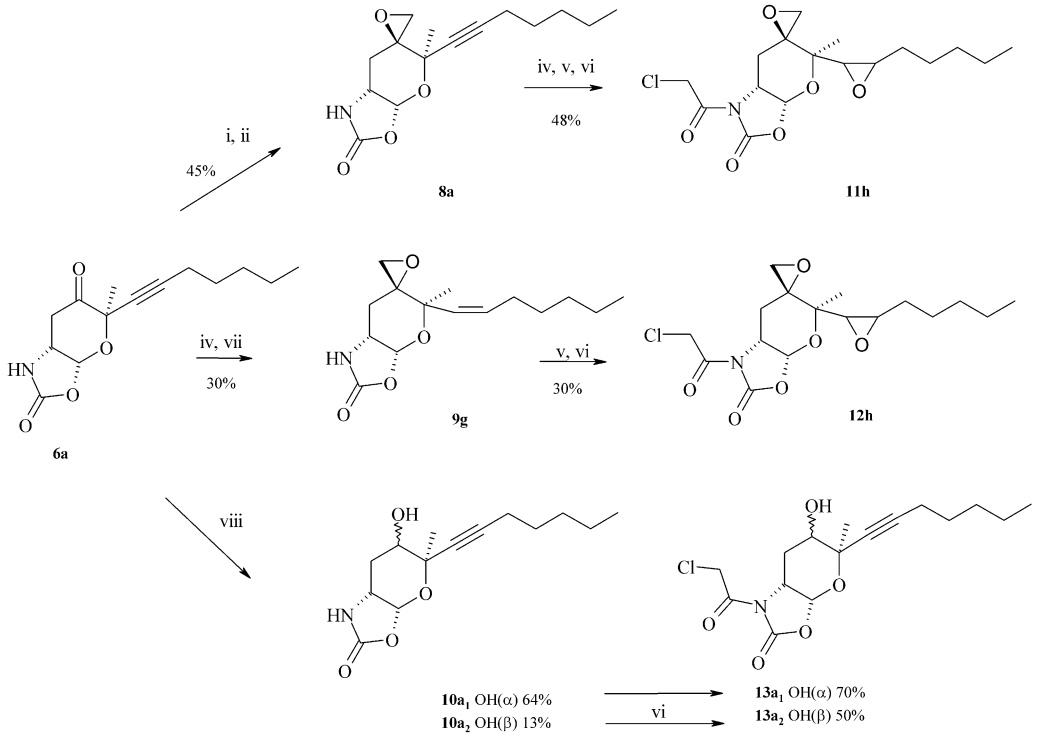
Biological Activity

The anti-angiogenic activities of the compounds were assessed by inhibition of the vascular growth in the chick embryo chorioallantoic membrane (CAM assay)[10,11] at 125 nmol.

It is noteworthy that the mono- and the disubstituted pyranyl analogues **7a** ($R_1 = H$) and **7b** ($R_1 = Me$), retained significant CAM activity (87% and 60%, respectively). As mentioned above, structural modification at the C_2 lipophilic chain was performed on

Table 1.

Compd	a	b	c	d	e	f	g	h
R$_1$	—H	—CH_3	—CH_3	—C≡C—C_5H_{11}	—CH_3	—CH_3	—CH_3	—CH_3
R$_2$	—C_7H_{15}	—C_7H_{15}	—C≡C—C_5H_{11}	—CH_3	—C≡C—OMe	—C≡C—C_4H_8Ph		
4	85%	94%	93%	93%	33%	91%	—	—
3	94%	85%	90%	90%	90%	92%	—	—
6	50%	80%	84%	5%	83%	80%	—	—
7	30%	70%	70%	50%	53%	70%	98%	100%



Scheme 3. Structural modifications at C_1. (i) CH_3PPh_3Br, KOtBu, THF, 60 °C; (ii) MCPBA, CH_2Cl_2, rt; (iii) (a) nBuLi, THF, −78 °C; (b) $ClCH_2COCl$; (iv) H_2, Pd lindar, C_6H_6; (v) 2,2-dimethyldioxirane, acetone; (vi) (a) *n*BuLi, THF, −78 °C; (b) $ClCH_2COCl$; (vii) $(CH_3)_3SI$, nBuLi, rt; (viii) $NaBH_4$, $CeCl_3$, THF, −78 °C.

Table 2. Biological activity

N		1b	7a	7b	7c	7d	7e	7f	7g	7h	11h	12h	$13a_1$	$13a_2$
CAM assay[11,12] (%)		85	87	60	70	50	60	60	50	58	19	nt[a]	40	25
IC_{50} (μM)[13]	A431	5	79	78	47	5	64	56	81	116	33	93	52	75
	L1210	8	96	28	31	32	74	27	81	193	22	89	>100	>100

[a]nt, not tested due to poor solubility.

disubstituted compound **7b**, due to its better chemical stability compared to **7a**, although there was a slight loss of antiangiogenic effect (Table 2).

First, the separation of the *cis*/*trans* mixture at the level of the synthon **6** (**6c**:**6d** as a 16:1 mixture) gave us access to the two isomers **7c** and **7d**, respectively. In agreement with the C_2/C_4 *trans* relative configuration of **1**, the antiangiogenic activity of the *trans* isomer **7c** was slightly more pronounced than the *cis* isomer **7d** (70% vs 50%). Since the *tran*s stereochemistry was more readily obtainable via the synthetic route, it was conserved throughout the series.

The replacement of the ethynyl moiety of the side chain of **7c** by either a *cis* double bond (**7g**) or by an epoxide (**7h**) decreased only moderately the CAM activity (50% and 58%, respectively vs 70%).

The modification of the C_2 side chain terminus, by introduction of either a methoxy (**7e**) or a phenyl (**7f**), did not significantly alter the CAM activity (60%). Thus the heptynyl side chain of **7c** was conserved to investigate the C_1 functionality.

Unexpectedly, unlike for **1b**, the presence of a spiro-epoxide at C_1 (**11h**) was detrimental for antiangiogenic activity compared to the corresponding ketone (**7c**).

The reduction of the ketone of synthon **6c** led to a separable mixture of α- and β-hydroxy stereoisomers at C_1, which were then converted to the chloroacetyls $\mathbf{13a_1}$ and $\mathbf{13a_2}$, respectively. The activities in the CAM assay of these compounds were also significantly weaker compared to the corresponding ketone **7c**, especially in the β-configuration (40% and 25%, respectively vs 70%).

The antitumoral activity was assessed in the inhibition of proliferation of various tumor cells including A431 (human epidermoid carcinoma) and L1210 (murine leukemia).[12] All the compounds showed at least a 10-fold decrease of their antiproliferative activities compared to **1b**, with an exception for the activity of **7d** which showed similar activity on A431. Two of the most potent compounds in the CAM (with less cytotoxicity than **1b**), **7a** and **7f**, were evaluated in vivo in mice, in the melanoma M5076 (sc) model, with a daily administration (J1–12 ip). At 240 mg/kg, these compounds

showed a T/C^{13} determined day 13 of 40% and 57%, respectively, while **1b** at 30 mg/kg showed a T/C of 22%. These results point out that the in vitro CAM antiangiogenic test is not sufficient to predict antitumoral activity in vivo (Table 2). However, the relative low stability of the derivatives described here, $t_{1/2} < 30$ min, could explain the discrepancy between their in vitro and in vivo pharmacological activity.

A versatile synthetic route leading to pyran analogues of **1b** has been developed, where a stereocontrolled oxidation-rearrangement of 2-furyl-carbinols led to the formation of a series of original tetrahydropyrano[3,2-*d*]oxazolones.[7]

Key structural features for antiangiogenic activity of the pyran analogues of **1b** have been identified. Epoxide functionalities at C_1 and C_2 are not required for antiangiogenic activity and a large variety of lipophilic chains can be introduced at C_2 with the relative stereochemistry of **1b** (*trans* stereochemistry between C_2 and C_4).

Although the mechanism of action of **1b** and its analogues remains uncertain, it appears that for the most active compounds, the spatial arrangements occupied by the lipophilic chain with respect to the alkylating functionality can be superimposed, suggesting a highly stereospecific mechanism of action.

Acknowledgements

The authors wish to thank Dr. Patrick Casara for corrections and relevant comments in the preparation of this manuscript.

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9. All synthetic intermediates and final compounds gave satisfactory ^1H NMR, ^{13}C NMR, mass spectral, and elemental analysis data, in full agreement with their assigned structures and stereochemistries. Experimental procedures and analytical data have been described in ref 7.

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13. T/C: Test/Control tumor volume.