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Synthesis and Biological Evaluation of Novel Bisindolylmaleimides that Inhibit Vascular Endothelial Cell Proliferation

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Abstract—A novel class of bisindolylmaleimides were synthesized and antiproliferative activities (HUVECs and three tumor cell lines) of these compounds were investigated. Two water-soluble derivatives, **10** and **12**, possessing a dimethylaminoalkoxy side chain in their structure, showed interesting activity and selectivity on HUVECs proliferation.

Angiogenesis plays an important role in cancer during tumor growth and also in the processes of invasion and metastasis.[1,2] As vascular endothelial cell proliferation is a key step in new vessel formation, it can be considered as a potential target in anticancer therapy.

Recently, antiangiogenic activity of staurosporine, a potent non-selective PKC inhibitor, has been described.[3] Staurosporine was active in the CAM assay and inhibits vascular endothelial cell proliferation in a picomolar range, but the main problem is its lack of selectivity, due to the inhibition of several protein kinases. Many series of analogues, with bisindolylmaleimide structure, have been synthesized, searching for selective angiogenesis or PKC inhibitors.[4,5] Some examples of staurosporine analogues with antiangiogenic properties are indicated in Figure 1. A potent β-isozyme selective PKC inhibitor (**1**) has been reported as a VEGF stimulated endothelial cell growth inhibitor.[4a,5] In addition, a staurosporine-related indolocarbazole (**2**), in which the sugar moiety was modified, has shown in vitro antiangiogenic activity, evaluated by the observation of the morphological change in calf pulmonary artery endothelial cells (sandwich method).[6] These two compounds showed more specific activity than staurosporine because they had no or limited toxicity on endothelial cell culture.

An inhibitor of vascular endothelial cell growth that doesn't show tumor cell antiproliferative activity, that is

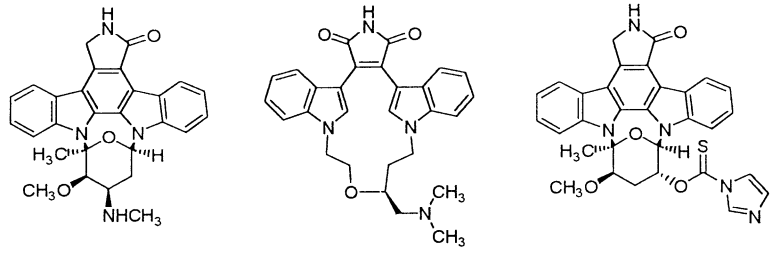

Figure 1.

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with a selective toxicity, would be a desirable anti-angiogenic drug.[7] Thus, an inhibitor with these characteristics could be useful because it would only affect capillary growth and undesirable side effects could be avoided. Thereby, our aim was to explore the anti-angiogenic activity of staurosporine analogues in which a substituted alkoxy chain was attached to the indolic nitrogen of the bisindolylmaleimide, leading to less complex structures (**I**). Furthermore, in a previous work, we have synthesized a series of antiproliferative *N*-substituted 3,4-diphenyl-1*H*-pyrrole-2,5-diones **(II)**,[8] and it was observed that aminoalkyl side chains play an important role in the activity. Therefore, a dialkylaminoalkyl group has been introduced in the imidic nitrogen.

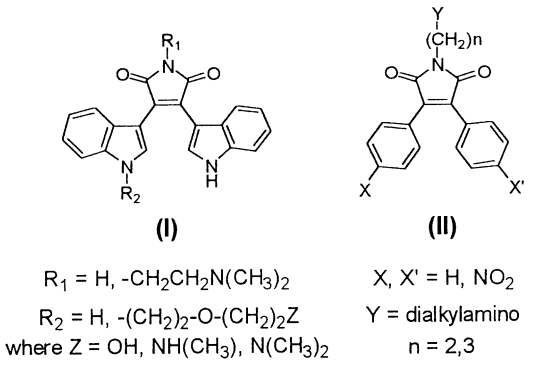


Figure 2.

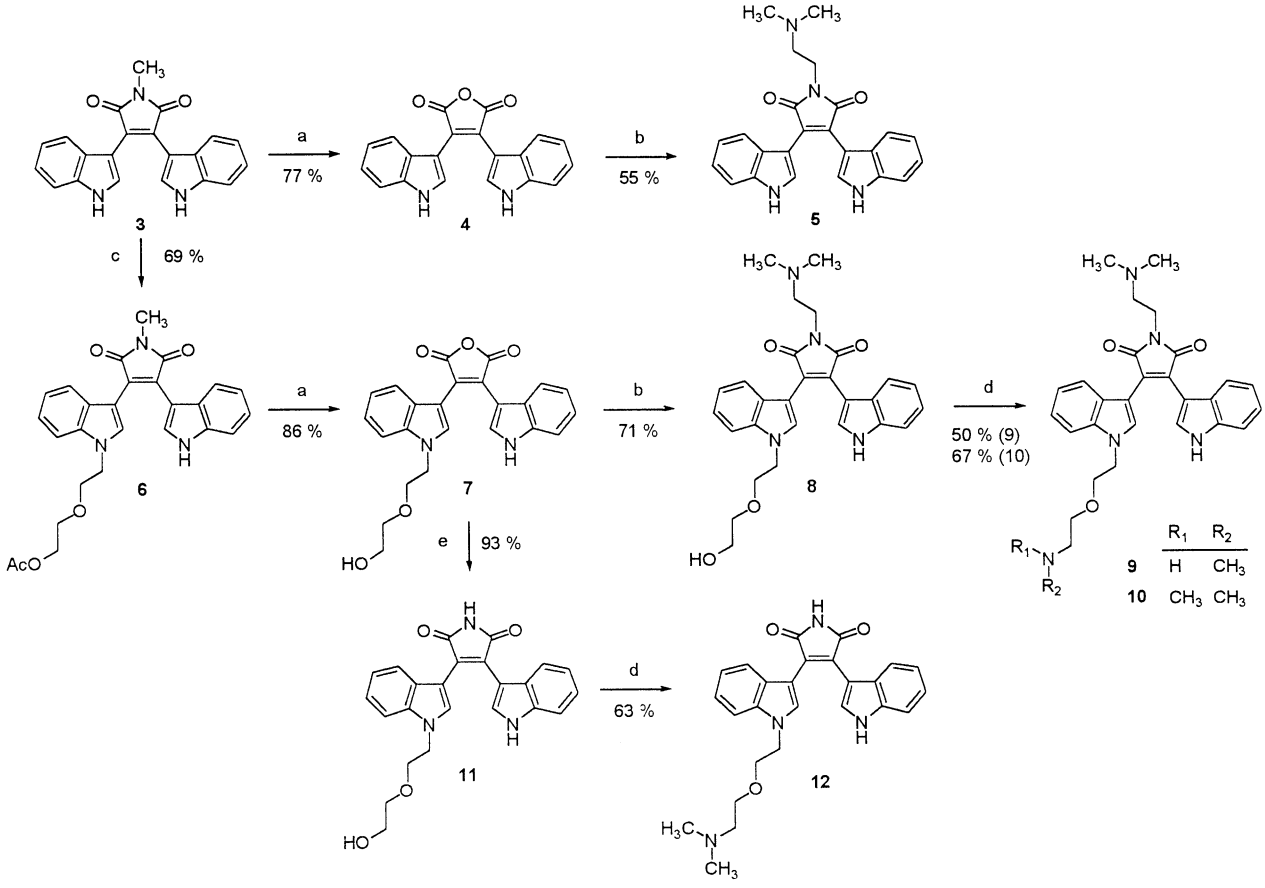
Additionally, this basic residue may increase water solubility.

In accordance with the above, we have synthesized a series of novel bisindolylmaleimides **(I)** indicated in Figure 2. Further, antiproliferative activities (HUVECs and three tumor cell lines) of these compounds were investigated.

Chemistry

Starting imide **3** was synthesized from *N*-methyl-2,3-dibromomaleimide[9] and indolylmagnesium bromide, following a procedure similar to the one described by Faul et al.[10] Basic treatment of **3** led to anhydride **4**,[11] direct precursor of **5**.

To introduce the side chain, imide **3** was treated with cesium carbonate and the corresponding iodine derivative. Hydrolysis of imide **6** led to anhydride **7**. Derivatives with a basic moiety in imidic nitrogen, were formed by treatment of **7** with *N,N*-dimethylethylendiamine. Then, alcohol **8** was converted to its mesylate and later substituted by the correspondant amine, leading to compounds **9** and **10**. To obtain non-substituted bisindolylmaleimide **11**, imidic NH was introduced from anhydride **7,** following the procedure described by Davis et al.[12] Together with NH introduction, silylation



Scheme 1. (a) (i) KOH 5 N, EtOH, rt; (ii) HCl 2 N; (b) *N,N*-dimethylethylendiamine (2 equiv), DMF, 80 °C; (c) Cs_2CO_3 (0.5 equiv), $I(CH_2)_2O(CH_2)_2OAc$ (0.5 equiv), DMF, 60 °C; (d) (i) MsCl, py, CH_2Cl_2, rt; (ii) $(R_1)(R_2)NH$, THF, rt; (e) (i) HMDS/MeOH, DMF, rt; (ii) TBAF, THF, rt.

Table 1. Antiproliferative activities of the bisindolylmaleimides

Compds	HUVECs IC_{50} (μM)	LoVo[a] IC_{50} (μM)	DLD-1[a] IC_{50} (μM)	ST-486[b] IC_{50} (μM)
5	30	>100	>100	>100
8	7	7	40	10
9	50	>100	>100	20
10	25	>100	>100	30
11	2	20	6	2
12	20	>100	>100	10
Suramin	500	—	—	—
Staurosporine	0.004	0.001	0.009	0.007

[a]Human colon cancer cell line.
[b]Burkit lymphoma cell line.

of the hydroxyl group was also observed, but treatment with TBAF led to **11**, which was converted to the corresponding amine **12** via its mesylate (Scheme 1).

Biological Evaluation

The results of human umbilical vein endothelial cells (HUVECs) proliferation[13] are summarized in Table 1. Antiproliferative activities were measured by MTT assay.[14] Compound **11** had been described by Sasaki et al.[15] as a cdc2 inhibitor, but it has not been tested in an antiproliferative assay. Compounds **9**, **10** and **12** were tested as their corresponding hydrochloride salts. Suramin was used as standard compound.[7]

All the compounds were less active but more selective towards HUVECs proliferation (except in the Burkit lymphoma cell line) than staurosporine. Alcohols **8** and **11** showed better antiproliferative activity on HUVECs than correspondant amines **5**, **9**, **10** and **12**. However, these amines were more selective with respect to tumor cell lines. No decrease in the activity was observed when imide nitrogen was substituted with a dimethylaminoethyl chain. This can be observed comparing **8** with **11**, and **10** with **12**. It seems that imidic NH is not essential for antiproliferative activity and that it can be replaced by an aminoalkyl group. Previously, it has been observed that imidic NH plays an important role in PKC inhibition of many bisindolylmaleimides.[16] This suggest that our compounds may act through a different mechanism.

In conclusion, we have developed some bisindolylmaleimides with better selectivity than staurosporine. Two water-soluble derivatives, **10** and **12**, possessing a dimethylaminoalkoxy side chain in their structure, were the most active among non-alcoholic analogues. Further studies on the mechanism of action of these compounds and development of structure-related derivatives are in progress.

Acknowledgements

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