

SYNTHESES AND ANTITUMOR ACTIVITY OF CIS-RESTRICTED COMBRETASTATINS: 5-MEMBERED HETEROCYCLIC ANALOGUES

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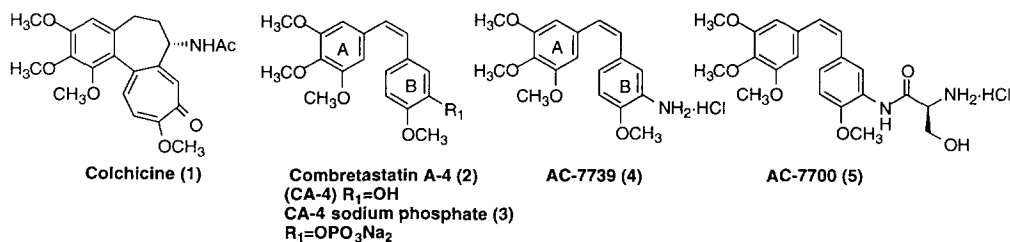
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ABSTRACT: A series of cis-restricted combretastatin analogues with 5-membered heterocycles were synthesized and their inhibitory activity against microtubule assembly and cytotoxic activity against the colon 26 adenocarcinoma cancer cell line were evaluated. Some of the heterocyclic analogues showed potent antitubulin activity and cytotoxicity. Compounds **16** and **35** showed marked tumor growth suppression in the colon 26 murine tumor model. © 1998 Elsevier Science Ltd. All rights reserved.

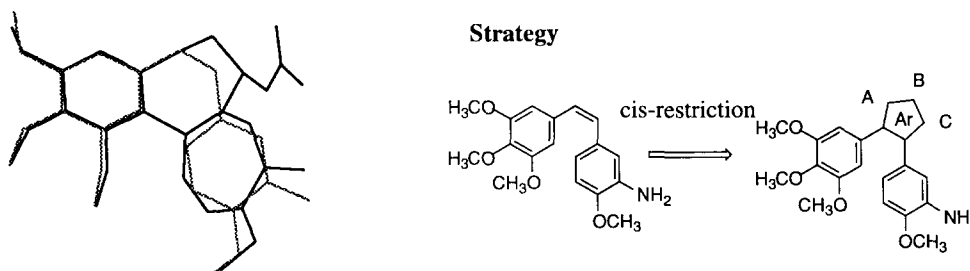
INTRODUCTION: Combretastatin A-4 (CA-4) (**2**) is one of the most potent antimitotic agents derived from *Combretum caffrum* and binds to tubulin on the colchicine binding site.¹ It shows strong cytotoxicity against a variety of human cancer cells, including multi-drug resistant cancer cell lines.² However, the low water-solubility of CA-4 limited its efficacy *in vivo* and a water soluble sodium phosphate prodrug of CA-4 (**3**) was evaluated for clinical application.³ We recently synthesized a series of CA-4 derivatives to improve water-solubility and obtained a new antimitotic agent AC-7739 (**4**) and its serine prodrug AC-7700 (**5**) (**Figure 1**).⁴ These compounds show marked tumor growth suppression against the colon 26 murine tumor model. Additionally, we found that these compounds exert potent antitumor activity by reducing blood flow at the tumor site, resulting the hemorrhagic necrosis within tumor tissue.⁵

Figure 1



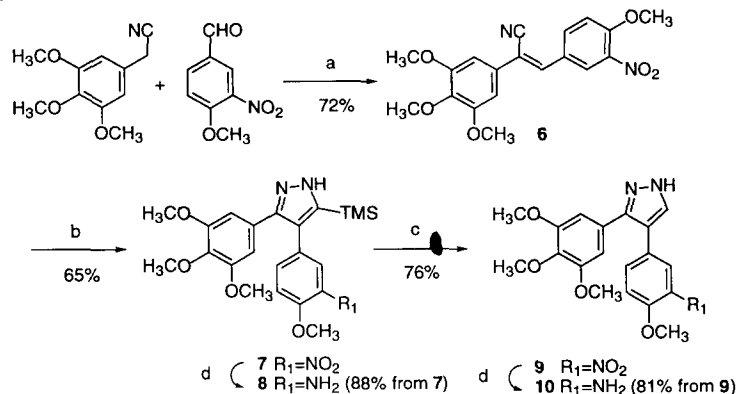
AC-7739 (**4**) and colchicine (**1**) have common structural features: 1) the 3,4,5-trimethoxy phenyl ring and 2) two aromatic rings located in appropriate chiral torsion, suggesting they bind to the colchicine binding site with the same orientation. By superimposition of these compounds, it seemed that the olefinic position of AC-7739 could be replaced with bulky groups without loss of activity (**Figure 2**).^{6,7} Cis combretastatin analogues are prone to isomerize to trans-forms during storage and administration. The trans-form of these compounds show dramatic reduction in antitubulin activity and antitumor activity. Thus, we attempted to introduce 5-membered heterocycles in place of the olefinic position of AC-7739 to fix the conformation in cis-forms in order to obtain more potent compounds *in vitro* as well as *in vivo* tumor models. (**Figure 2**).⁸

Figure 2



Superimposition of colchicine (**1**) and AC-7739 (**4**)

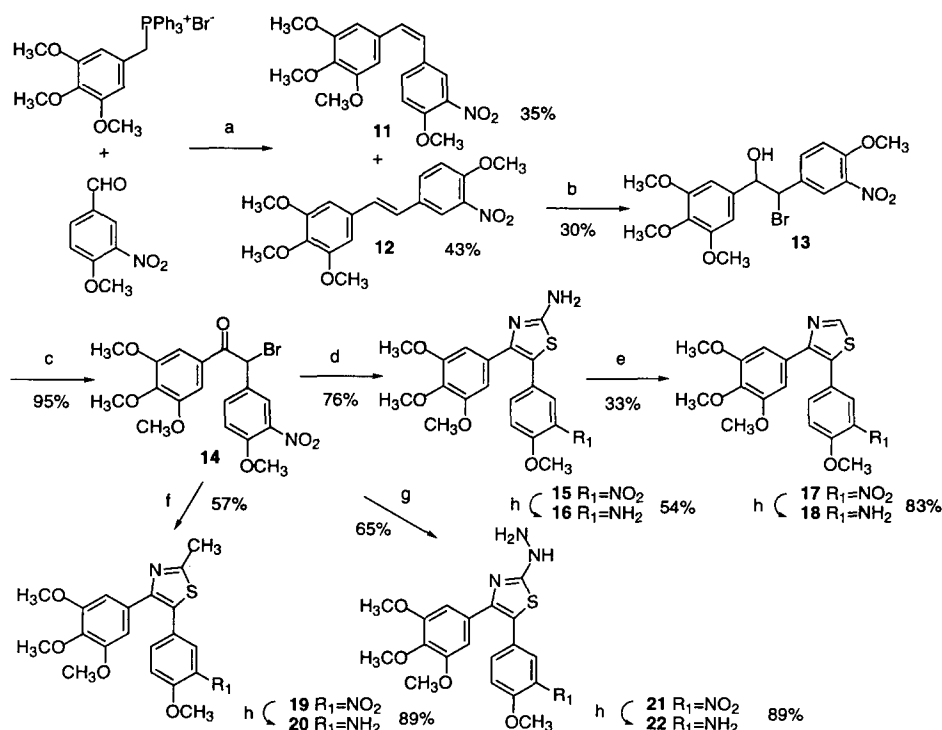
Scheme 1



a) 1N NaOHaq, trimethyloctylammonium chloride, CH_2Cl_2 , rt.
 b) Lithium trimethylsilyl diazomethane, THF, -78°C . c) 10% KOHaq, EtOH, reflux.
 d) Zn, AcOH, rt.

Scheme 1 shows the synthesis of pyrazole compounds. Phenylacetonitrile and nitrobenzaldehyde were condensed in aqueous NaOH to give *Z*-acrylonitrile **6**, which was then treated with lithium trimethylsilyl diazomethane to give pyrazole **7** in 65% yields.⁹ A TMS group on pyrazole **7** was removed by aqueous 10% KOH to give **9**. The nitro groups on the B ring of **7** and **9** were reduced by Zn/AcOH to give corresponding aniline derivatives **8** and **10**, respectively.

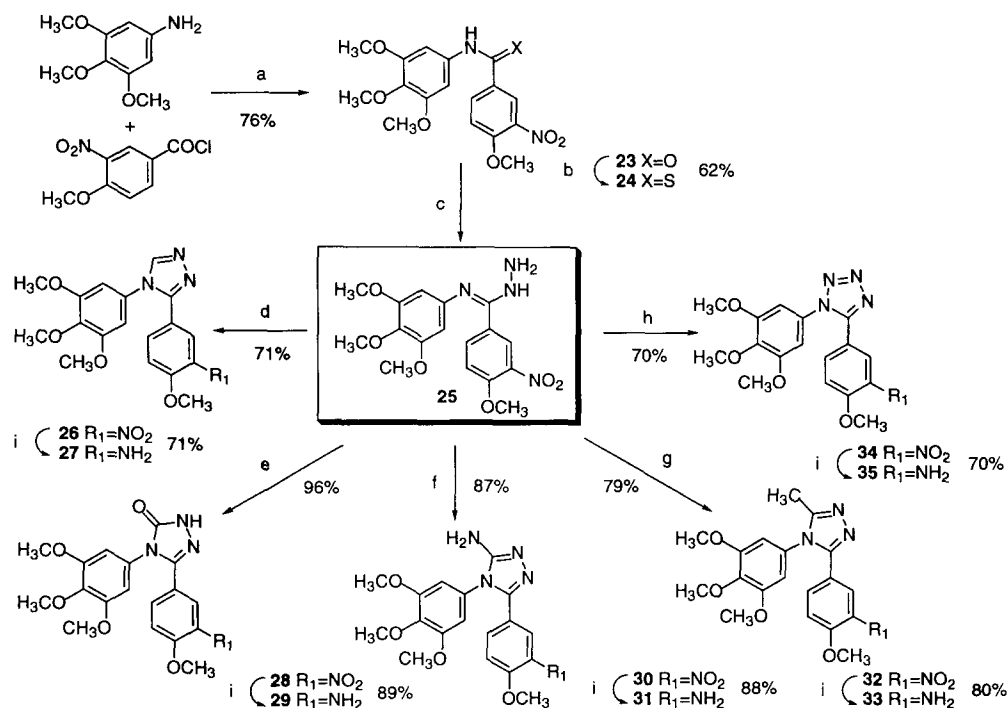
Scheme 2



Scheme 2 shows the synthesis of a series of thiazole analogues.¹⁰ Condensation of phosphonium bromide and 4-methoxy-3-nitrobenzaldehyde gave approximately 1:1-mixture of (*E*), (*Z*)-stilbenes. (*E*)-stilbene (**12**) was purified by crystallization.⁴ Obtained **12** was then converted to bromohydrin by NBS- H_2O in a 30% yield. Bromohydrin **13** was oxidized by DMSO-TFAA to give bromoketone **14**, which was condensed with thiocarbamoyl compounds in the presence of Na_2CO_3 in DMF to give the corresponding 2-substituted thiazole derivatives (**15**, **19** and **21**). The amino group of thiazole **15** was converted to diazonium salt then reduced by H_3PO_2 to give thiazole **17**. The nitro group of **15**, **17**, **19** and **21** was reduced to amino group by Zn/AcOH to give anilines **16**, **18**, **20** and **22**, respectively.

Scheme 3 shows the synthesis of a series of triazole and tetrazole analogues.¹¹ Amide **23** was converted to thioamide **24** by Lawesson's Reagent. Compound **24** was condensed with hydrazine hydrate to give hydrazone **25**. The hydrazone **25** was reacted with trimethyl orthoformate or trimethyl orthoacetate in the presence of H_2SO_4 to give triazole **26** and 5-methyltriazole **32**, respectively. Compound **25** was reacted with BrCN or NaNO_2 to give 5-amino triazole **30** or tetrazole **34**, respectively. Compound **25** was also reacted with ethyl chloroformate to give carbamate, which was then cyclized by NaOMe to give **28**. The nitro group of **26**, **28**, **30**, **32** and **34** was reduced with Zn/AcOH to give amino compounds **27**, **29**, **31**, **33** and **35**, respectively.

Scheme 3



Results and Discussion

The biological activities of synthesized compounds are shown in **Table 1**. Tubulin polymerization inhibitory activity of these compounds was tested using bovine brain tubulin.¹² Cytotoxic activity was tested against the colon 26 adenocarcinoma cancer cell line.¹³ Antitumor activity was tested in the colon 26 murine tumor model.¹⁴

Pyrazole **10** showed potent antimitotic (IC_{50} $3\mu\text{M}$) and cytotoxic activity (IC_{50} 8.4nM), while TMS-pyrazole **8** did not show any activity. All thiazole compounds other than **20** showed potent antitubulin activity and cytotoxicity. Low cellular permeability of **20** is one possible explanation for its weak cytotoxicity. A hydrazino compound **22** showed potent cytotoxicity (IC_{50} 21.2nM) suggesting that insertion of a bulky group in this position is possible. In the case of triazole compounds, only **27** showed moderate antimitotic and weak cytotoxic activity. Compounds **29**, **31** and **33** which have substituents on the triazole ring lost their activities (antitubulin: IC_{50} $>10\mu\text{M}$, cytotoxicity: IC_{50} $>3000\text{nM}$). Tetrazole compound **35** showed potent antitubulin activity (IC_{50} $2\mu\text{M}$) as well as cytotoxicity (IC_{50} 7.2nM).

Table 1. Biological Activities of Cis-restricted Combretastatins.

Compd No.	anti-tubulin ^{a)}	Cytotoxicity ^{b)}	Antitumor Activity ^{c)}	
	IC ₅₀ (μM)	IC ₅₀ (nM)	route	IR (%) (dose) ^{d)}
8	>10	>3000	-	nt. e)
10	3	8.4	iv	0 (20mg/kg)
16	1	57.5	iv	75 (40mg/kg)
18	3	14.5	iv	66 (160mg/kg)
20	2	2040	iv	24 (40mg/kg)
22	3	21.1	iv	55 (80mg/kg)
27	4	840	iv	47 (160mg/kg)
29	>10	>3000	-	nt.
31	>10	>3000	-	nt.
33	>10	>3000	-	nt.
35	2	7.2	sc	89 (160mg/kg)
4 (AC-7739)	1	2.8	iv	73 (40mg/kg)
cisplatin	-	-	iv	64 (5mg/kg)

a) Tubulin polymerization was determined as shown in ref 12. b) Drug concentration required to inhibit the growth of colon 26 cells by 50%, see ref 13. c) Tumor growth suppression was determined as shown in note 14. d) Maximum tolerated dose of each compound was administered.

e) nt.; not tested.

From these results, it is demonstrated that five membered heterocycles are tolerable as a linker of the two aromatic ring and the overall conformation of the heterocyclic compounds is similar to those of AC-7739 and colchicine.

The position of substituents on the heterocyclic ring seemed to be critical to antitubulin activity and cytotoxic activity. In the case of triazole compounds, substituents as small as NH₂, CH₃ and carbonyl groups on position A of the 5-membered ring lost antimitotic and cytotoxic activity. TMS-Pyrazole **8**, which is substituted on position C, also lost its activity. Substituents on position A or C of the 5-membered ring seemed to decrease compound activity. Hydrazine **22**, which is substituted on position B of the 5-membered ring, retained its activity, suggesting that only this position is allowed for substitution (Figure 2).

Compounds with potent cytotoxicity *in vitro* were further evaluated in the colon 26 murine tumor model. The maximum tolerated dose of each compound was administered iv or sc. Compound **16** showed potent tumor suppression (IR=75%, 40mg/kg) comparable to that of AC-7739. Tetrazole **35** showed marked tumor suppression with IR=89% at an elevated dose of 160mg/kg.

SUMMARY: We have synthesized a series of cis-restricted combretastatins with 5-membered heterocycles to obtain compounds which are stable in terms of isomerization. Some of the heterocyclic analogues showed potent antitubulin and cytotoxic activity. Among these, thiazole **16** and tetrazole **35** showed potent antitumor activity *in vivo*. The antitumor activity of **16** was comparable to that of AC-7739 at the same dose. Currently, synthesis of other types of heterocyclic analogues of AC-7739 are under way in our laboratory.

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REFERENCE AND NOTES

1. Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. *Can. J. Chem.* **1982**, *60*, 1374. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209. Hamel, E.; Lin, C. M. *Biochem. Pharmacol.* **1983**, *32*, 3864. Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984.
2. McGown, A. T.; Fox, B. W. *Cancer Chemother. Pharmacol.* **1990**, *26*, 79. El-Zayat, A. A. E.; Degen, D.; Drabek, S.; Clark, G. M.; Pettit, G. R.; Von Hoff, D. D. *Anti-Cancer Drugs* **1993**, *4*, 19.
3. Pettit, G. R.; Temple, C. Jr.; Narayanan, V. L.; Varma, R.; Simpson, M. J.; Boyd, M. R.; Renner, G. A.; Bansal, N. *Anti-Cancer Drug Des.* **1995**, *10*, 299.
4. Ohsumi, K.; Nakagawa, R.; Fukuda, Y.; Hatanaka, T.; Morinaga, Y.; Nihei, Y.; Ohishi, K.; Suga, Y.; Akiyama, Y.; Tsuji, T. *J. Med. Chem.* **1998**, *41*, 3022.
5. Nihei, Y.; Suga, Y.; Morinaga, Y.; Ohishi, K.; Suzuki, M.; Okano, A.; Ohsumi, K.; Nakagawa, R.; Tsuji, T.; Akiyama, Y.; Tsuruo. Proceedings of the American Association for Cancer Research, *39*, # 1143, **1998**. Nihei, Y.; Suzuki, M.; Okano, A.; Tsuji, T.; Akiyama, Y.; Saito, S.; Hori, K.; Sato, Y. Proceedings of the American Association for Cancer Research, *39*, # 324, **1998**.
6. McGown, A. T.; Fox, B. W. *Anti-Cancer Drug Des.* **1989**, *3*, 249.
7. Superimposition was calculated by Molecular Modeling System SYBYL, Version 6.2; Tripos Associates, Inc.
8. Since we started this study, the synthesis of structure-restricted combretastatin analogues was reported. Shirai, R.; Okabe, T.; Iwasaki, S. *Heterocycles* **1997**, *46*, 1457.
9. Aoyama, T.; Inoue, S.; Shioiri, T. *Tetrahedron Lett.* **1984**, *25*, 433.
10. Ife, R. J.; Leach, C. A.; Pope, A. J.; Theobald, C. J. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 543.
11. Meguro, K.; Kuwada, Y. *Chem. Pharm. Bull.* **1973**, *21*, 2375. Fray, M. J.; Bull, D. J.; Cooper, K.; Parry, M. J.; Stefaniak, M. H. *J. Med. Chem.* **1995**, *38*, 3524.
12. Hamel, E.; Lin, C. M. *Biochemistry* **1984**, *23*, 4173. Muzaffar, A.; Brossi, A.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1990**, *33*, 567.
13. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589. Mossman, T. *J. Immunol. Meth.* **1983**, *65*, 55.
14. Fragments of colon 26 tumor (5mg) were inoculated sc into CD2F1 mice. Test compounds were given iv or sc on days 7, 11 and 15. On day 21, the tumor weights were measured. The inhibition ratio was evaluated as $(1-T/C) \times 100$ (%) (T; the mean tumor weight of the treated group, C; the mean tumor weight of the control group). Each group consisted of 5 mice.