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# Design, Synthesis and Antiproliferative Activity of Tripentones: A New Series of Antitubulin Agents

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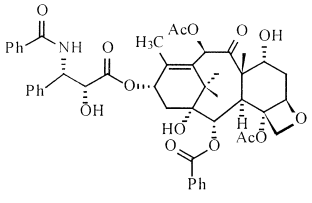
**Abstract**—Structure–activity relationship studies of a new series of tripentones (thieno[2,3-*b*]pyrrolizin-8-ones), led us to prepare several derivatives with antiproliferative activities. The most promising 3-(3-hydroxy-4-methoxyphenyl)thieno[2,3-*b*]pyrrolizin-8-one **20** (leukemia L1210, $IC_{50} = 15$ nM) was shown to be a potent inhibitor of tubulin polymerization. 

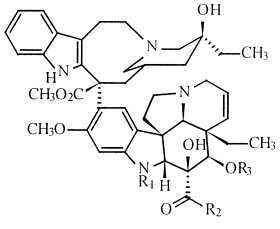
## Introduction

Chemotherapy combined with surgery and/or radiotherapy still has an important role to play in the therapeutic approach of cancer. Cancer cells differ from their normal counterparts in a number of biochemical processes, particularly during the control of cell growth and division. So the mitotic spindle represents an attractive target for cancer therapy. Many natural and synthetic substances are known to interfere with the dynamic assembly of tubulin and prevent the formation of microtubules which are essential for cellular integrity and cell division.[1] Currently, the most useful members of these anti-tubulin agents[2] are natural products such as paclitaxel,[3] vinca alkaloids,[4] colchicine[5] and combretastatin A-4[6] (Fig. 1).

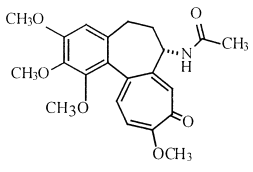
During our investigation towards the synthesis of new potential antitumor agents, we synthesized a series of substituted heterocyclic ketones based on the 'tripentone' skeleton of thieno[2,3-*b*]pyrrolizin-8-ones I.[7] The first tests of the National Cancer Institute led to the discovery of four antiproliferative compounds **1**–**4** with an aryl substituent in position 3. Among them, MR 16924 (NSC 676693) **4** (Fig. 2) displays a remarkable cytostatic activity over all the tested lines with highest



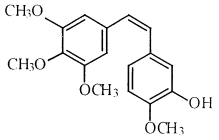
Paclitaxel



Vinca alkaloids



Colchicine



Combretastatin A-4

**Figure 1.** Representative antimitotic compounds which interact with tubulin.

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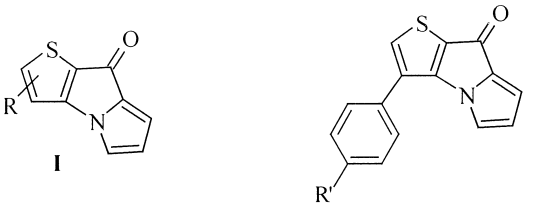
effect against leukemia, central nervous system, ovarian and breast cancers. A selection of these results is presented in Table 1.



**I**

**1** R' = H
**2** R' = Cl
**3** R' = F
**4** R' = $OCH_3$ (MR 16924)

**Figure 2.**

To improve this result and better characterize the pharmacological profile of the series, a first structure–activity relationship studying the influence of the nature and the position of substituents on the phenyl group in position 3 of the tricyclic system was performed. We focused on the synthesis of several hydroxy and alkyloxy derivatives **4**–**21**.

## Chemistry

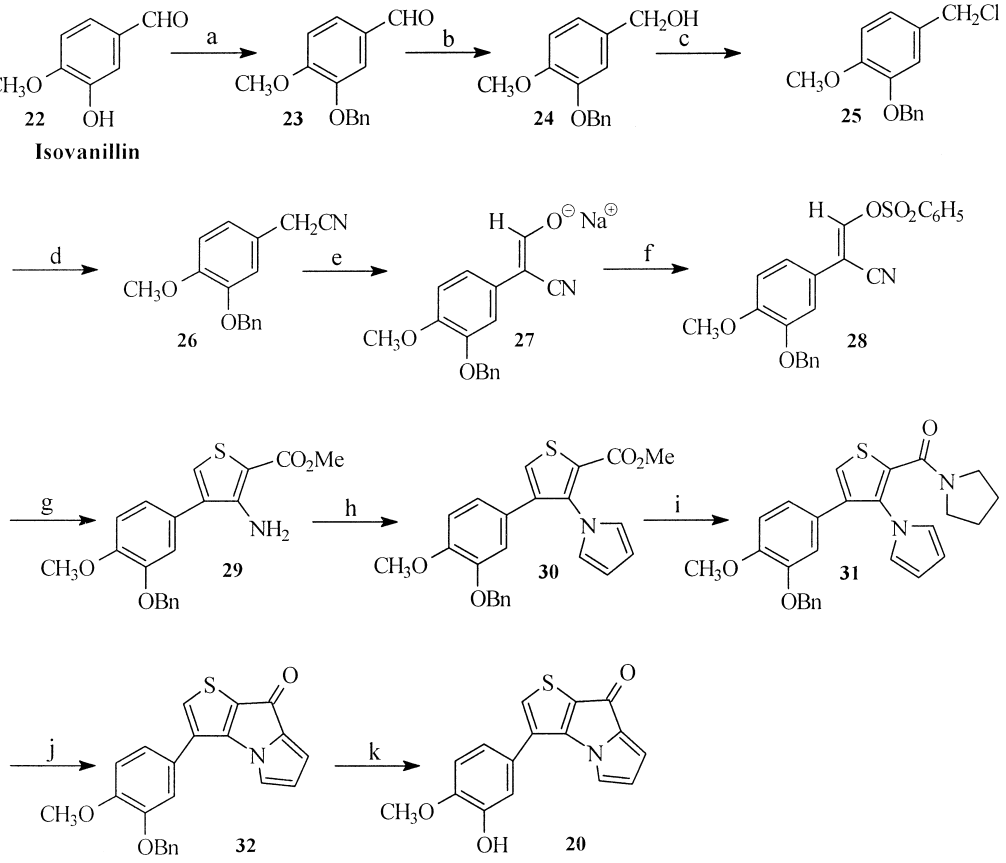
All these compounds were prepared according to the general route described in Scheme 1, starting from suitable arylaldehydes. As an example, the product **20** was synthesized from isovanillin in 12 steps.[8]

After a reaction of benzylation, the isovanillin gave the arylacetonitrile compound **26** in good yields by,

**Table 1.** Compounds **1**–**4**: selected values from the NCI 60 tumoral cell lines panel (cytostatic $\log_{10}$ $GI_{50}$ effects in M)

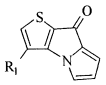
| Cell type | Leukemia | NSCL* | Colon | CNS* | Melanoma | Ovarian | Renal | Prostate | Breast |
|---|---|---|---|---|---|---|---|---|---|
| Cell line | MOLT4 | EKVX | HCC2998 | SF-295 | SK-MEL2 | OVCAR3 | A-498 | DU-145 | HS-578T |
| **1** (NSC 683507) | −4.9 | −5.3 | −4.7 | −4.7 | −4.9 | −4.8 | −5.8 | −4.6 | −5.3 |
| **2** (NSC 683509) | −4.1 | −5.6 | −4.0 | −4.0 | −4.0 | −4.0 | −6.0 | −4.0 | −5.7 |
| **3** (NSC 683508) | −4.6 | −4.6 | −4.6 | −4.6 | −4.5 | −4.3 | −5.8 | −4.1 | −4.7 |
| **4** (NSC 673693) | −7.4 | −6.1 | −6.5 | −7.4 | −6.4 | −7.4 | −6.2 | −6.8 | −7.3 |

NSCL, non small cell lung; CNS, central nervous system.



**Scheme 1.** Reaction conditions and yields: (a) MeOH, $K_2CO_3$, benzyl bromide, reflux, 5 h, 98%; (b) MeOH, $NaBH_4$, rt, 1 h, 90%; (c) dioxane, $SOCl_2$, rt, 1 h, 85%; (d) $CH_3CN$, $(C_2H_5)_4NCN$, rt, 24 h, 70%; (e) dry THF, ethylformate, NaH, reflux, 1 h, 90%; (f) DMF, sulfonylbenzene chloride, rt, 2 h, 50%; (g) MeOH, methyl thioglycolate, NaH, reflux, 2 h, 65%; (h) dioxane, dimethoxyTHF, 4-chloropyridinium hydrochloride, reflux, 2 h, 85%; (i) pyrrolidine, reflux, 2 h 30 min, 80%; (j) (1) $POCl_3$, 80 °C, 1 h 30 min; (2) 10% NaOH, 50 °C, 30 min, 60%; (k) HBr/AcOH 33%, rt, 30 min, 65%.

**Table 2.** In vitro antiproliferative activity and L1210 cell cycle effects of compounds **4**–**21**

|  | $R_1$ | $IC_{50}$ (μM) | Effect on the cell cycle[a] |
|---|---|---|---|
| **5** | Hydrogen | > 100 | NT |
| **6** | Methyl | > 100 | NT |
| **7** | 3,4,5-Trimethoxyphenyl | 36.4 | NT |
| **8** | 4-Hydroxyphenyl | 30.1 | NT |
| **9** | 4-Butoxyphenyl | 29.6 | NT |
| **10** | 3-Hydroxyphenyl | 29 | NT |
| **11** | 4-Propoxyphenyl | 28.1 | NT |
| **12** | Phenyl | 26.7 | NT |
| **13** | 2-Methoxyphenyl | 18.9 | 75% $G_2+M+8N$ at 5 μM |
| **14** | 3,4-Dimethoxyphenyl | 11.7 | 86% $G_2+M+8N$ at 25 μM |
| **15** | 3-Methoxyphenyl | 5.2 | 94% $G_2+M+8N$ at 25 μM |
| **16** | 3,4-Methylenedioxyphenyl | 4.2 | 88% $G_2+M+8N$ at 10 μM |
| **17** | 2-Hydroxyphenyl | 3.7 | 72% $G_1$ at 50 μM |
| **18** | 4-Ethoxyphenyl | 0.43 | 88% $G_2+M+8N$ at 1 μM |
| **19** | 3,4-Dihydroxyphenyl | 0.28 | 86% $G_2+M+8N$ at 0.5 μM |
| **4** | 4-Methoxyphenyl | 0.19 | 83% $G_2+M+8N$ at 0.5 μM |
| **20** | 3-Hydroxy-4-methoxyphenyl | 0.015 | 80% $G_2+M+8N$ at 0.5 μM |
| **21** | 4-Hydroxy-3-methoxyphenyl | 8 | 86% $G_2+M+8N$ at 25 μM |

NT, not tested; $IC_{50}$, drug concentration that inhibits cell growth by 50%.
[a]Percent of untreated cell in the phases of the cycle: 41% (G1); 28% (S); 24% (G2 + M); 1% (8 N).

**Table 3.** Antiproliferative activity of **20** on selected tumoral cell lines

| Cell line | L1210 | P388 | VCR-20 | B16 | DU145 | H-69 | KB 3-1 | KB-A1 | Ovcar-3 | A-2780 |
|---|---|---|---|---|---|---|---|---|---|---|
| $IC_{50}$ (μM) | 0.015 | 0.038 | 0.021 | 0.023 | 0.043 | 0.036 | 0.018 | 0.015 | 0.031 | 0.041 |

successively, a reduction, a chlorination and then a cyanation reaction. By treatment with ethylformate and sodium hydride, **26** formed with 90% yield the corresponding enolate **27**, which is protected by a sulfonylbenzene group to give **28**.[9] This product, by treatment with methylthioglycolate and sodium hydride in tetrahydrofuran, afforded, according to Kirsch's cyclisation,[10] the methyl 3-aminothiophene carboxylate **29**. The amino function of the latter was then reacted with dimethoxytetrahydrofuran in the presence of 4-chloropyridinium hydrochloride in dioxane to give the pyrrole **30** whose methyl carboxylate function by refluxing in excess of pyrrolidine gave the corresponding carboxamide **31**. Cyclization was then performed by action of phosphorus oxychloride to access to the tricyclic ketone **32** with 60% yield. The last deprotective step liberated the phenol function by action of a 33% solution of bromhydric acid in glacial acetic acid to give the tripentone **20**.[11]

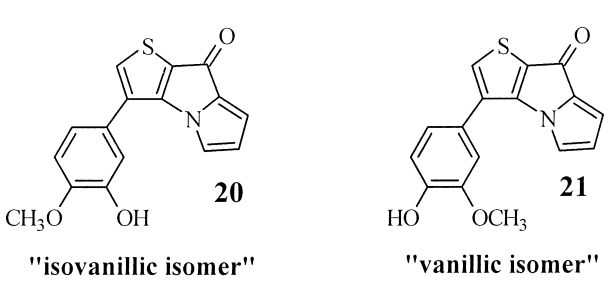



**Figure 3.** Monomethylated analogues.

## Results and Discussion

Compounds **4**–**19** were then evaluated in vitro for their antiproliferative activity against the L1210 leukemia cell line. The results expressed as $IC_{50}$ are reported in Table 2 with their effects on the cell cycle.

Considering these results, it appears that some small structural modifications are responsible for great variations of the $IC_{50}$ values and also that the hydroxy and methoxy series are not really comparable in terms of SAR, but the size of substituents seems to be fundamental and optimal with methoxy in the *para* position. Taking into account the fact that the two best compounds were **4** and **19** derivatives, we investigated the synthesis of 'mixed' compounds bearing in the 3 and 4 positions either hydroxy or methoxy substituents. The result went beyond our expectations because one of the isomers, the isovanillic one **20** ($IC_{50}=0.015$ μM), was about 1000-fold more active than the vanillic one **21** ($IC_{50}=8$ μM) and was the best compound of the series, 10-fold more potent than the parent 4-methoxy derivative **4** (Fig. 3). We actually try to explain this enigmatic difference in activity between **20** and **21**. First X-ray structures were solved for compounds **20** and **14** and it seems that the orientation of the aryl ring could play a key role in the binding interaction with tubulin.

This result for compound **20** was confirmed over a panel of nine tumoral cell lines (Table 3). Flow cytometric studies showed that L1210 cells treated with tripentones

were arrested in the $G_2$/M phases of the cell cycle, with a significant percentage of cells having re-initiated a cycle of DNA synthesis without cell division (8 N DNA content). Compound **20** at 0.5 μM induced the accumulation of 86% of cells at G2/M stage (66% of cells having a DNA content >4 N chromosomes). This effect, observed with the majority of tubulin-interacting drugs,[12] prompted us to perform an inhibitory polymerization tubulin test with deoxypodophyllotoxin as internal reference ($IC_{50} = 2.4\,\mu M$). The compound **20** altered the assembly reaction of microtubules with an $IC_{50} = 2.9\,\mu M$ and without any depolymerization effect.

## Conclusion

We have discovered a new type of antimitotic compounds based on the arylthienopyrrolizinone molecular skeleton. The most promising compound of the series, 3-(3-hydroxy-4-methoxyphenyl)thieno[2,3-*b*]pyrrolizin-8-one **20** has significant human cancer cell growth inhibitory activity in the nanomolar range and interacts with tubulin in the micromolar range. At present, we are trying to specify the type of binding site on the protein with which tripentones interact, and to determine if this mechanism is responsible for all the antiproliferative activity. In vivo studies are also currently under progress.

## Acknowledgements

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11. Selected data for compound **20**: IR (KBr) ν 3419, 1671, 1504, 1210 $cm^{-1}$. $^1H$ NMR ($CDCl_3$) 3.95 (s, 3H), 5.87 (bs, 1H), 6.01 (m, 1H), 6.67 (m, 1H), 6.81 (m, 1H), 6.93 (d, $J = 7.8$ Hz), 6.99 (d, $J = 7.8$ Hz), 7.07 (s, 1H), 7.43 (s, 1H). $^{13}C$ NMR ($CDCl_3$) 56.1; 110.8; 113.2; 114.1; 115.6; 119.7; 121.0; 125.5; 129.4; 134.1; 135.8; 146.1; 146.9; 149.5; 150.7; 174.4. Anal. calcd for $C_{16}H_{11}NO_3S$: C, 64.63; H, 3.72; N, 4.71; found: C, 64.64; H, 4.02; N, 4.48. All compounds were fully characterized by spectroscopic and elemental analysis.
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