Journal of Enzyme Inhibition and Medicinal Chemistry, 2002 VOL. 17 (6), pp. 403–407

Synthesis of New Aromatic Pyrrolo[2,1-*c*][1,4]benzodiazepines and Pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepines as Anti-tumoral Agents

VINCENT LISOWSKI[a], FRÉDÉRIC FABIS[a], ALAIN PIERRÉ[b], DANIEL-HENRI CAIGNARD[b], PIERRE RENARD[b] and SYLVAIN RAULT[a,*]

[a]*Centre d'Etudes et de Recherche sur le Médicament de Normandie-UFR des Sciences Pharmaceutiques 5, rue Vaubénard 14032 Caen cedex, France;* [b]*ADIR, 1 rue Carle Hébert 92415 Courbevoie Cedex, France*

(Received 20 March 2002)

Diazepine analogs of thieno[2,3-*b*]pyrrolizin-8-ones were synthesized by aromatization of 2-hydroxypyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepines. These compounds were evaluated *in vitro* for their antiproliferative activity against the L1210 leukemia cell line. The activity of these compounds was in the micromolar range, the best result being for the mixture of the isomers 5 and 6 which showed a 0.35 μM IC_{50} against cell growth.

Keywords: Thieno[2,3-*b*]pyrrolizin-8-ones; Pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepines; Aromatization; Antiproliferative; Leukemia

INTRODUCTION

The discovery of new anti-tumoral agents remains very important in the therapeutic approach to cancer. Our laboratory very recently described a series of new compounds with antiproliferative activity: the thieno[2,3-*b*]pyrrolizin-8-ones or tripentones which probably interact with tubulin, however, their precise mechanism of action remains unknown. The *in vitro* antiproliferative activity of tripentones against tumoral cells of leukemia L1210 was in the nanomolar range, the best compound being **I** (IC_{50} = 15 nM) (Figure 1).[1] The structure activity relationship studies in the series have demonstrated that the potency of the compound was directly linked with the position of the hydroxy or methoxy group on the aryl substituent.

Other tricyclic heterocyclic compounds, the pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs), are known to recognize and bind to specific sequences of DNA. Extensive studies have been carried out on PBDs[2] and the anti-tumor activity has been demonstrated for many representatives of this series such as DC-81 (Figure 1).[3] Nevertheless, no in-depth study has been reported on aromatic PBDs since their synthesis remains difficult.

We recently described the aromatisation of 2-hydroxypyrrolo[2,1-*c*][1,4]benzodiazepines by using thionyl chloride (Scheme 1).[4] This method prompted us to apply it in the thiophene series and to further synthesize analogues of tripentones **I** in which ring B would be replaced by a diazepine one. Presented in this preliminary communication are the first chemical and biological results from these studies.

MATERIALS AND METHODS

Chemistry

All common chemicals and solvents utilized were reagent grade and purchased from Sigma-Aldrich (Saint Quentin, France) or Acros-Organics (Noisy-Le-Grand, France). Melting points were determined on a Kofler melting point apparatus and are reported uncorrected. Infrared (IR) spectra were obtained in KBr pellets or neat liquid films with a Genesis Series FTIR spectrometer. ^{1}H-NMR spectra were obtained using a JEOL Lambda 400 spectrometer operating at 400 MHz in d_6-DMSO or $CDCl_3$ as solvent. Chemical shifts are expressed as δ values (ppm) relative to Me_4Si as internal standard.

*Corresponding author. Tel.: +33-231936943. Fax: +33-231931188. E-mail: rault@pharmacie.unicaen.fr

ISSN 1475-6366 print/ISSN 1475-6374 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1475636021000005712

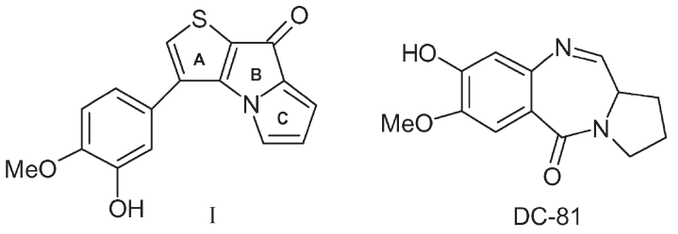


FIGURE 1 Structure of tripentone I and DC-81.

All reactions were monitored by TLC, using 0.25 mm-thick precoated silica gel plates (E. Merck) eluted with ethyl acetate/cyclohexane gradients. Compounds were purified by CC using silica gel 60 as stationary phase and eluted with ethyl acetate/cyclohexane gradients. Literature procedures were used for the preparation of compounds **1a**,[8] **1b–c**,[9,10] **2a**,[5] **3a**.[6]

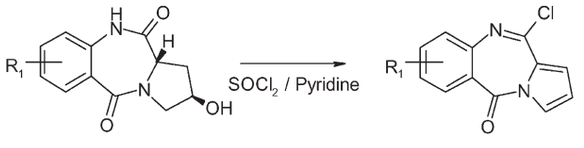
Synthesis

7-(4-chlorophenyl)-2,4-dihydro-1*H*-thieno[3,2-d][1,3]oxazine-2,4-dione **2b**

10 g (37 mmoles) of **1b** were suspended in 100 ml of a solution of potassium hydroxide (3.14 g, 56 mmoles) and 50 ml of ethyl alcohol was added. The mixture was refluxed under microwave irradiation (500 W) for 1 h. The resulting solution was cooled to 0°C and phosgene was gently bubbled until pH 1. After 30 min., the precipitate was filtered and washed successively with water (200 ml), saturated sodium hydrogen carbonate solution (200 ml) and diethyl ether (200 ml). 7.14 g (69%) of anhydride **2b** were obtained as a white solid (mp > 260°C). IR (KBr) (ν: cm^{-1}): 3587 (NH), 1791 (CO), 1724 (CO), 1630, 1470, 1335, 851, 791. ^{1}H NMR (d_6-DMSO; δ: ppm): 7.42 (d, 2H, J = 8.6 Hz, H_{phenyl}), 8.08 (d, 2H, J = 8.6 Hz, H_{phenyl}), 8.18 (s, 1H, $H_{thioph.}$).

7-(3,4-dimethoxyphenyl)-2,4-dihydro-1*H*-thieno[3,2-d][1,3]oxazine-2,4-dione **2c**

Using the same procedure as for **2b**, we obtained from the aminoester **1c** (10 g, 34 mmoles) 5.5 g (55%) of the anhydride **2c** (mp = 220°C). IR (KBr) (ν: cm^{-1}): 3346 (NH), 1788 (CO), 1722 (CO), 1523, 1464, 1264, 1244, 989, 810. ^{1}H NMR (d_6-DMSO; δ ppm): 3.76 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.98 (m, 2H, H_{phenyl}), 7.27 (s, 1H, H_{phenyl}), 8.06 (s, 1H, $H_{thioph.}$).



SCHEME 1 Aromatization of 2-hydroxypyrrolo[2,1-*c*][1,4] benzodiazepines.

3-(4-chlorophenyl)-7-hydroxy-5,5a,6,7,8,10-hexahydro-4*H*-pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4] Diazepine-5,10-dione **3b**

1.1 g (3.9 mmol) of anhydride **2b** and 0.77 g (5.9 mmoles) of *trans*-4-L-hydroxyproline were suspended in a mixture of dioxane (25 ml) and water (25 ml). The reaction mixture was refluxed for 2 h. The solution was evaporated under reduced pressure and the residue taken up in a saturated sodium hydrogen carbonate solution. The solid was filtered and washed with water. Recrystallization in propan-2-ol gave 0.83 g (61%) of **3b** (mp > 260°C). IR (KBr) (ν: cm^{-1}): 3460–3010 (NH and OH), 1686 (CO), 1615, 1424, 1090, 727. ^{1}H NMR (d_6-DMSO; δ ppm): 2.16 (m, 1H, H_{6a}), 2.69 (m, 1H, H_{6b}), 3.39 (d, 1H, J = 12.2 Hz, H_{8a}), 4.03 (d, 1H, J = 12.2 Hz, H_{8b}), 4.41 (m, 2H, H_{5a} and H_7), 4.48 (broad s, 1H, OH), 7.29 (d, 2H, J = 8.3 Hz, H_{phenyl}), 7.38 (d, 2H, J = 8.3 Hz, H_{phenyl}), 8.64 (s, 1H, $H_{thioph.}$).

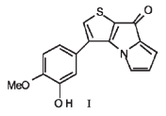
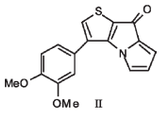
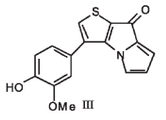
3-(3,4-Dimethoxyphenyl)-7-hydroxy-5,5a,6,7,8,10-hexahydro-4*H*-pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4] Diazepine-5,10-dione **3c**

Using the same procedure as for **3b**, we obtained from the anhydride **2c** (4.5 g, 15 mmoles), 2.02 g (36%) of diazepine **3c** (mp > 260°C). IR (KBr) (ν: cm^{-1}): 3400–3086 (NH and OH); 1691 (CO), 1614, 1461, 1437, 1416, 1259, 1022, 727. ^{1}H NMR (d_6-DMSO; δ ppm): 2.11 (m, 1H, H_{6a}), 2.69 (m, 1H, H_{6b}), 3.33 (d, 1H, J = 11.2 Hz, H_{8a}), 3.69 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.98 (d, 1H, J = 11.2 Hz, H_{8b}), 4.37 (m, 2H, H_{5a} and H_7), 4.43 (broad s, 1H, OH), 6.72 (s, 1H, H_{phenyl}), 6.77 (m, 2H, H_{phenyl}), 7.28 (broad s, 1H, NH), 8.37 (s, 1H, $H_{thioph.}$).

5-Chloro-10*H*-pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepine-10-one **4a**

1.5 ml (18.5 mmoles) of pyridine was added to 150 ml of a cooled solution (0°C) of thionyl chloride. 4 g (17 mmoles) of diazepine **3a** were added and the mixture was refluxed for 2 h. The thionyl chloride was evaporated and the residue taken up in crushed ice. The solid was filtered and washed successively with water and saturated sodium hydrogen carbonate solution. Recrystallization in acetonitrile gave 2.4 g (60%) of **4a** as a yellow solid (mp = 190°C). IR (KBr) (ν: cm^{-1}): 1650 (CO), 1586, 1437, 1316, 1156, 829, 769. ^{1}H NMR ($CDCl_3$; δ ppm):

TABLE I *In vitro* antiproliferative activity

Compound	IC_{50} (μM)
3c	28.60
4b	6.20
4c	2.50
Mixture of **5** and **6**	0.35
	0.015
	11.70
	8.00

IC_{50}: drug concentration that inhibits cell growth by 50%

6.85 (dd, 1H, J = 3.8 and 3.1 Hz, H_7), 7.40 (d, 1H, J = 5.0 Hz, H_3), 7.65 (dd, 1H, J = 3.8 and 1.8 Hz, H_6), 7.82 (d, 1H, J = 5.0 Hz, H_2), 8.38 (dd, 1H, J = 3.1 and 1.8 Hz, H_8). ^{13}C NMR ($CDCl_3$; δ ppm): 114.7, 123.4, 125.0, 126.7, 127.0, 131.6, 134.8, 146.6, 146.8, 156.2. MS (m/z): M^+: 236 (100); $M^+ + 2$: 238 (53).

5-Chloro-3-(4-chlorophenyl)-10*H*-pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepine-10-one **4b**

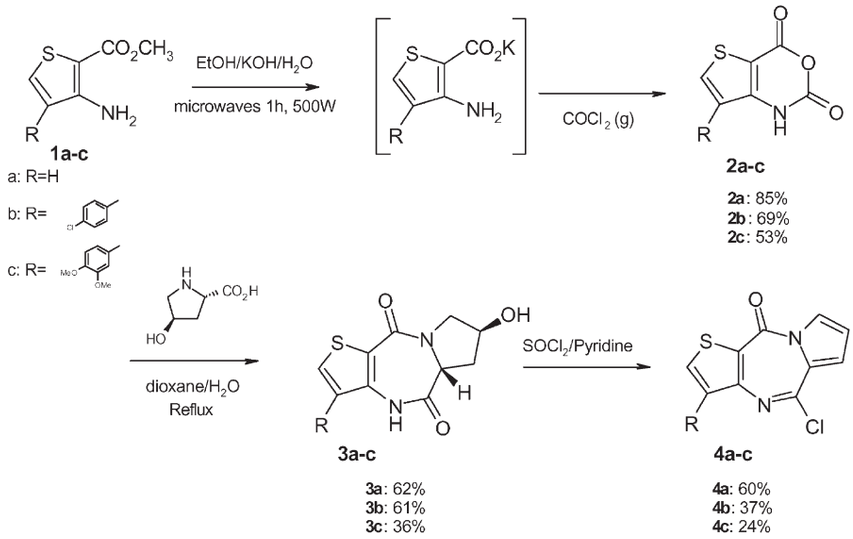
Using the same procedure as for **4a**, we obtained from diazepine **3b** (0.12 g, 0.34 mmole) 0.044 g (37%) of **4b** after recrystallization in cyclohexane (mp = 175°C). IR (KBr) (ν: cm^{-1}): 1668 (CO), 1584, 1455, 1418, 1261, 1094, 1018, 801. ^1H NMR ($CDCl_3$; δ ppm): 6.80 (m, 1H, H_7), 7.37 (d, 2H, J = 8.3 Hz, H_{phenyl}), 7.56 (m, 3H, H_6 and H_{phenyl}), 7.80 (s, 1H, H_2), 8.33 (m, 1H, H_8).

5-Chloro-3-(3,4-dimethoxyphenyl)-10*H*-pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepine-10-one **4c**

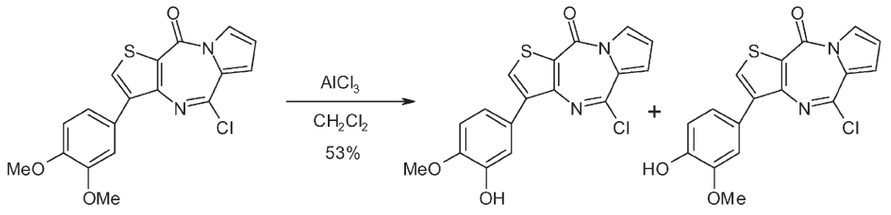
Using the same procedure as for **4a**, we obtained from diazepine **3c** (2 g, 5.4 mmoles) 0.48 g (24%) of **4c** after silica gel chromatography with ethyl acetate/-cyclohexane (1/2) as eluent (mp = 158°C). IR (KBr) (ν: cm^{-1}): 1672 (CO), 1588, 1452, 1412, 1258, 1069, 1024, 832. ^1H NMR ($CDCl_3$; δ ppm): 3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.86 (m, 1H, H_7), 6.99 (m, 2H, H_{phenyl}), 7.27 (s, 1H, H_{phenyl}), 7.62 (m, 1H, H_6), 7.95 (s, 1H, H_2), 8.40 (m, 1H, H_8).

5-chloro-3-(3-hydroxy-4-methoxyphenyl)-10*H*-pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepine-10-one **5** and 5-chloro-3-(4-hydroxy-3-methoxyphenyl)-10*H*-pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepine-10-one **6**

0.4 g (1.1 mmole) of **4c** was dissolved in methylene chloride (30 ml) and excess of aluminium trichloride was added. The brown mixture was refluxed for 1.5 h and cooled to room temperature. The methylene chloride was evaporated and the residue was triturated in crushed ice. The mixture was extracted with ethyl acetate (2 × 50 ml). The organic layer was washed twice with water, dried over magnesium sulfate and the ethyl acetate evaporated. The solid was recrystallized in petroleum ether to give 0.2 g of a mixture of **5** and **6** (53%). ^1H NMR ($CDCl_3$; δ ppm): 3.92 and 3.95 (2s, 3H, OCH_3), 5.59 and 5.76



SCHEME 2 Synthesis of compounds **4a–c**.



SCHEME 3 Monodemethylation of **4c**.

(2 broad s, 1H, OH), 6.85 (m, 1H, H_7), 6.98 (m, 2H, H_{phenyl}), 7.06 (s, 1H, H_{phenyl}), 7.61 (m, 1H, H_6), 7.83 and 7.93 (2s, 1H, H_2), 8.39 (m, 1H, H_8).

Pharmacology

Compounds **3c**, **4b**, **4c**, and the mixture of **5** and **6** were evaluated *in vitro* for their antiproliferative activity against the L1210 leukemia cell line (Table I).[7]

RESULTS AND DISCUSSION

Chemistry

The synthesis of 2-hydroxypyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepines **3a–c** commenced from methyl 3-aminothiophene-2-carboxylate **1a** and methyl 3-amino-4-arylthiophene-2-carboxylates **1b–c**. The ester moiety was hydrolysed in ethanolic potassium hydroxide solution under microwave irradiation and phosgene was added *in situ* to give the thiaisatoic anhydrides **2a–c**.[5]

The anhydrides **2a–c** were then reacted, according to a reported procedure,[6] with *trans*-4-L-hydroxyproline in a mixture of dioxane and water to give the corresponding 2-hydroxypyrrolo[1,2-*a*][thieno[3,2-*e*][1,4]diazepines **3a–c**. The aromatization of diazepines **3a–c** was realized as described in the corresponding benzene series.[4] Thus, refluxing the diazepines **3a–c** in thionyl chloride in the presence of pyridine led to the isolation of the aromatic pyrrolo[1,2-*a*]thieno[3,2-*e*][1,4]diazepines **4a–c** in moderate to good yields (Scheme 2).

Attempts to selectively monodemethylate **4c** using aluminium chloride in methylene chloride in order to synthesize a diazepine analogue of **I**, only led to a mixture of **5** and **6** in an equimolar *ratio* in 53% yield (Scheme 3). An efficient preparative method of separation is now being sought.

Biology

The first biological results confirm our working hypothesis since analogues of **I** present a micromolar antiproliferative activity. **4c** is 10-fold more active than **3c** showing the importance of an aromatic system that is probably quasi-planar. Moreover, **4c** is 4-fold more active than **II** (Table I). These results and the increase in activity of the mixture of **5** and **6** in regard to **4c** show a close correlation with those of the tripentone family.

Nevertheless, in regard to the great difference in activity between **II** and **III**, it would be of interest to separately test compounds **5** and **6**. Should the separation of these compounds prove difficult, the unequivocal synthesis of these compounds would be considered as for the synthesis of **I**.

CONCLUSION

We have synthesized diazepine analogues of thienopyrrolizinones known as antiproliferative compounds. The promising results of this new family and the similarity of the antiproliferative activity between the two families prompt us to continue the exploration of this new pyrrolothienodiazepine series.

References

[1] Lisowski, V., Enguehard, C., Lancelot, J.C., Caignard, D.H., Lambel, S., Leonce, S., Pierre, A., Atassi, G., Renard, P. and Rault, S. (2001) "Design, synthesis and antiproliferative activity of tripentones: a new series of antitubulin agents", *Bioorganic and Medicinal Chemistry Letters* **11**, 2205–2208.

[2] Thurston, D.E. and Bose, D.S. (1994) "Synthesis of DNA-Interactive Pyrrolo[2,1-c][1,4]benzodiazepines", *Chemical Reviews* **94**, 433–465.

[3] Thurston, D.E. (1993) "Advances in the study of pyrrolo[2,1-c][1,4]benzodiazepines (PBD) antitumour antibiotics", Molecular Aspects of Anticancer Drug-DNA Interactions (The Macmillan Press Ltd., London, U.K.), pp 54–88.

[4] Fabis, F., Sopkova-de Oliveira Santos, J., Fouchet-Jolivet, S. and Rault, S. (2001) "An expedient route to aromatic pyrrolo[2,1-*c*][1,4]benzodiazepines and a study of their reactivity", *Tetrahedron Letters* **42**, 5183–5185.

[5] Fabis, F., Jolivet-Fouchet, S., Robba, M., Landelle, H. and Rault, S. (1998) "Thiaisatoic anhydrides: efficient synthesis under microwave heating conditions and study of their reactivity", *Tetrahedron* **54**, 10789–10800.

[6] Jolivet-Fouchet, S., Fabis, F. and Rault, S. (1998) "First direct synthesis of pyrrolo[2,1-c]thieno[3,2-*e*]- or [2,3-*e*][1,4]diazepines, thiophene analogues of pyrrolo[2,1-*c*][1,4]benzodiazepines", *Tetrahedron Letters* **39**, 5369–5372.

[7] Leonce, S., Perez, V., Casabianca-Pignede, M.R., Anstett, M., Bisagni, E., Pierré, A. and Atassi, G. (1996) "*In vitro* cytotoxicity of S16020-2, a new olivacine derivative", *Investigational New Drugs* **14**, 169–180.
[8] Huddleston, P.R. and Barker, J.M. (1979) "A convenient synthesis of 2-substituted 3-hydroxy- and 3-aminothiophenes from derivatives of 2-chloroacrylic acid", *Synthetic Communications* **9**, 731–734.
[9] Rault, S., Lancelot, J.C., Letois, B., Robba, M. and Labat, Y. (1992) "Preparation of 3-mercapto-2-thiophenecarboxylic acid derivatives as intermediates for herbicides. French Patent No 9203732, Mar. 27", *Chemical Abstracts* **123**, 111841.
[10] Kirsch, G., Cagniant, D. and Cagniant, P. (1982) "Synthesis of 3-aminothiophenes from aryl- and hetarylacetonitriles", *Journal of Heterocyclic Chemistry* **19**, 443–444.