Eur. J. Med. Chem. 34 (1999) 663–667

New products

Benzimidazole condensed ring systems. XI. Synthesis of some substituted cycloalkyl pyrido[1,2-a]benzimidazoles with anticipated antineoplastic activity#

El-Sayed A.M. Badawey[a]*, Thomas Kappe[b]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria AR, Egypt*
[b]*Institute für Organische Chemie, Karl Franzens Universität, Graz, Austria*

(Received 30 March 1998; revised 27 July 1998; accepted 7 October 1998)

Abstract – As part of a research project on the synthesis of a number of pyrido[1,2-a]benzimidazole derivatives with possible antineoplastic activity, and as a result of the interesting antineoplastic activity recorded for one such compound (NSC 649900), some new cycloalkylpyrido[1,2-a]benzimidazoles were prepared and evaluated for such activity. Compound (**7c**, NSC 682011) exhibited a good in vitro antineoplastic activity especially against most of the leukaemia cell lines. This compound has been selected by the NCI for further testing in a new in vivo anticancer hollow fibre assay. © 1999 Éditions scientifiques et médicales Elsevier SAS

cycloalkylpyrido[1,2-a]benzimidazoles / antineoplastic agents

1. Introduction

Several years ago we started a research plan to synthesize several pyrido[1,2-a]benzimidazoles (PBIs) as a class of compounds which is inadequately studied for antineoplastic activity [1–4]. In a recent investigation, we found that 1-chloro-2-(2-chloroethyl)-3-methylpyrido-[1,2-a]benzimidazole-4-carbonitrile (NSC 649900, *figure 1*) exhibited good in vitro activity and subpanel disease selectivity especially against leukaemic cell lines; however, it revealed only weak activity against P388 murine leukaemia in vivo [5]. In a search for a cytotoxic candidate with an improved antineoplastic profile, we have replaced the tricyclic PBI ring system in NSC 649900 with other N-heterocycles having a nearly similar substitution pattern; however, the designed compounds were less active [6–8]. This suggested that the PBI ring system may be partly essential for the biological activity. For this reason and for further information concerning the SAR study, we undertook in this report the synthesis of some new cycloalkylpyrido[1,2-a]benzimidazoles in order to assess their potential as anticancer agents. The 11-chloro compound **5** was of particular interest because of its structural similarity to NSC649900, however, its antineoplastic profile did not reveal any improvement in activity. Interestingly, the 5-(p-fluorophenylamino) derivative (**7c**, NSC 682011) showed promising in vitro antineoplastic activity and was selected by the NCI for further testing in a new in vivo anticancer hollow fibre assay. The synthesis of the target compounds is outlined in *figure 2*.

2. Chemistry

Previously, we have described a facile one step synthesis of cyclopentapyrido[1,2-a]benzimidazole, as a new tetracyclic ring system, by fusing 1H-benzimidazole-2-acetonitrile (**1**) with ethyl cylopentanone-2-carboxylate ester at 140 °C in the presence of ammonium acetate [9]. We have now utilized this reaction condition for the synthesis of some cycloalkyl-pyrido[1,2-a]benzimidazoles (**2–4**), namely, the cyclopentapyrido[1,2-a]benzimidazoles (**2** and **3**) and the benzimidazo[1,2-b]isoquinoline (**4**). Thus, reacting **1** with an equimolar quantity of diethyl cyclopentanone-2,5-dicarboxylate in the presence of two equivalents of ammonium acetate at

*Correspondence and reprints

#This work was partly presented in the 6th Ibn Sina International Conference on pure and applied Heterocyclic Chemistry, December, 1997. For part X, see ref. [4]

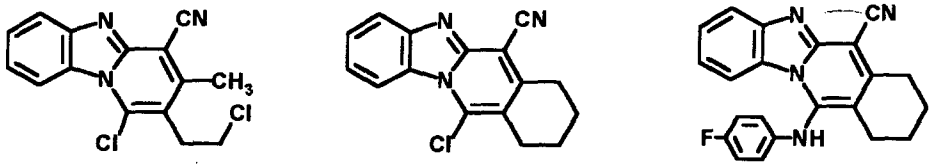

Figure 1. Structures of NSC 649900 and NSC 682011.

140 °C furnished the corresponding ethyl cyclopentapyrido[1,2-a]benzimidazole-3-carboxylate (**2**) in 12% yield. Similarly, the corresponding 1-methyl carboxylate ester (**3**) was obtained in a nearly similar yield by reacting **1** with dimethyl cyclopentanone-2,3-dicarboxylate. Whereas, the benzimidazo[1,2-b] isoquinoline (**4**) was prepared in 67% yield from **1** and ethyl cyclohexanone-2-carboxylate. Next, the N-butyl derivative **6** was obtained by refluxing **4** with tributyl phosphate in the presence of potassium carbonate, while its treatment with phosphorus oxychloride gave the 11-chloro derivative (**5**). Reacting this compound with the selected p-substituted-anilines in dimethylformamide at 80 °C yielded the respective 11-arylamino- derivatives (**7a–c**) in 70–85% yield. Reaction of **5** with sodium azide at room temperature resulted in the corresponding 11-azido com-

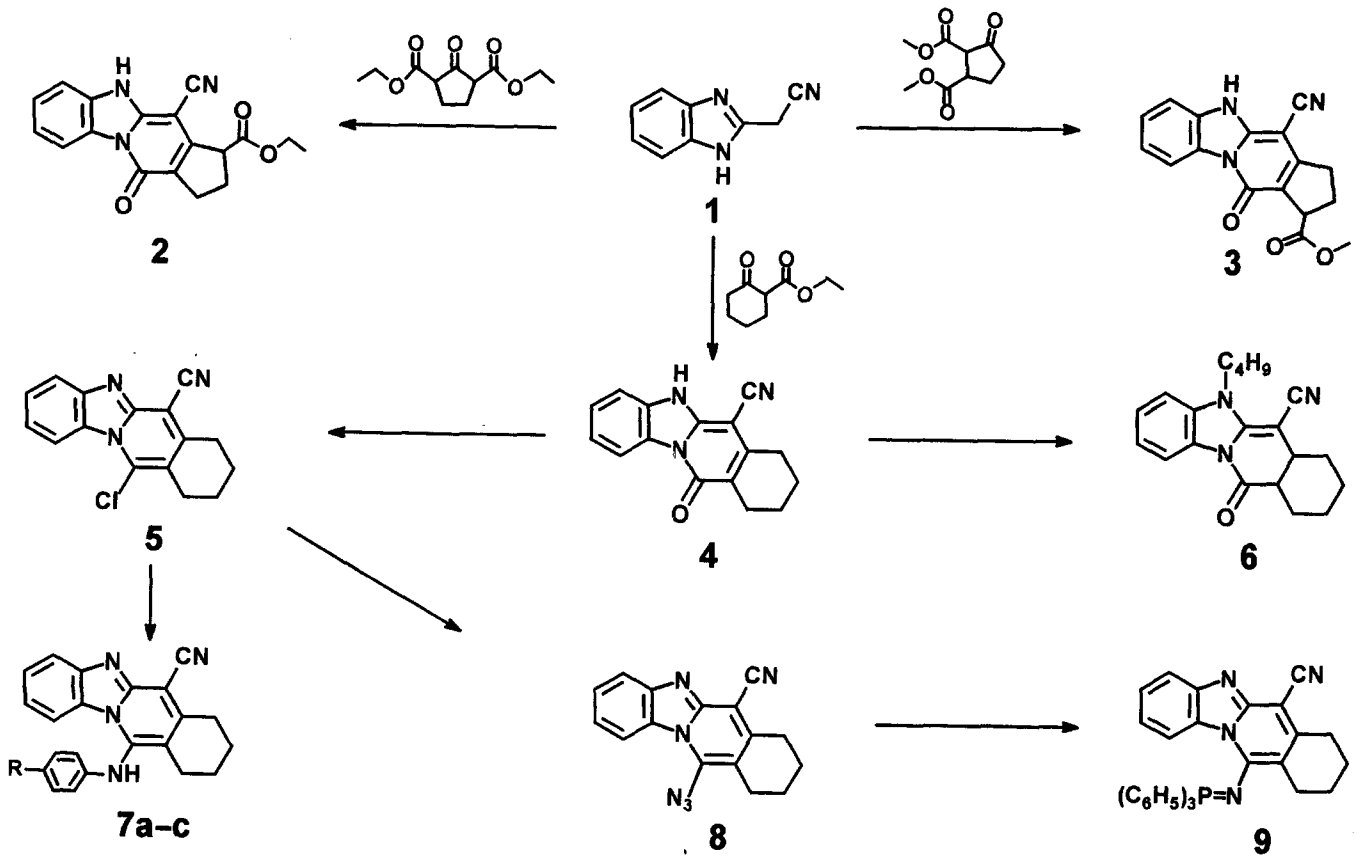

Figure 2. **7a**: R = CH_3, **7b**: R = Cl, **7c**: R = F.

pound **8** which, upon treatment with triphenylphosphine, gave the corresponding 11-triphenyl-phosphoranylideneamino derivative (**9**). A literature survey revealed that compounds **4** and **5** were prepared by Russell in 1995 [10]. However, it is worth mentioning that, although these compounds were prepared according to our previously reported procedure [9], the author did not refer to our leading reference. Surprisingly, we found that some cyclopentapyrido[1,2-a]benzimidazoles which are analogues to compounds **4** and **5** and were reported by us in 1989 [9] were also included in this article.

3. Antineoplastic activity

The prepared compounds were evaluated for their in vitro antineoplastic activity against 60 human cell lines derived from seven clinically isolated cancer types (lung, colon, melanoma, renal, ovarian, brain, and leukaemia) according to the NCI standard protocol [11]. The test results indicated that only the 5-arylamino-tetrahydrobenzimidazo[1,2-b]isoquinolines (**7a–c**) showed significant activity, particularly against the leukaemic cell lines. The p-fluorophenylamio derivative (**7c**, NSC 682011) was the most active candidate in this series, especially against K-562, Molt-4 and RPMI-8226 leukaemic cell lines, KM12 colon cell line, M14 melanoma cell line, ACHN renal cell line and the T-47D breast cell line. As a result of the promising activity recorded for **7c** and as it belongs to a new class which is not adequately studied for antineoplastic activity, this compound was selected by the NCI for further testing in a new in vivo anticancer hollow fibre assay. We can conclude from this result, and other investigations [4–8] that the pyrido[1,2-a]benzimidazole structure is partly essential for the biological activity, and by proper substitution, good cytotoxic candidates can be obtained (e.g., NSC 649900 and NSC 682011, *figure 1*). For this reason, we have synthesized several new pyrido[1,2-a]benzimidazoles which are related to **7c** (NSC 682011) and have similar p-fluorophenylamino substituents and p-fluorophenylaminomethylene or p-fluorophenyazo moieties at position 1 or 2. The test results are underway, and they will be the subject of the next investigation.

4. Results

4.1. Chemistry

Melting points were determined in open-glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 298 spectrophotometer using samples in potassium bromide discs. The ^{1}H-NMR spectra were recorded on a Varian Gemini 200 at 200 MHz using DMSO-d6, unless otherwise stated, with tetramethylsilane as the internal standard. Microanalyses were performed on a Carlo Erba 1106 analyser and are within ± 0.4 of the theoretical percentages.

4.1.1. Ethyl 4-Cyano-2,3,5,11-tetrahydro-11-oxo-1H-cyclopenta[4,5]pyrido[1,2-a]benzimidazole-3-carboxylate **2**

A mixture of 1H-benzimidazole-2-acetonitrile **1** (1.57 g, 10 mmol), diethyl cyclopentanone-2,5-dicarboxylate (2.5 g, 11 mmol) and ammonium acetate (1.7 g, 22 mmol) were heated in an oil bath at 140–150 °C for 30 min. During this period, ethanol and ammonia were liberated, and the reaction mixture was gradually solidified. After cooling, the product was suspended in acetonitrile, filtered and dried; yield: 0.39 g (12%); m.p. > 300 °C (DMF/H_2O); IR ν cm^{-1}: 3 300–2 500 bm, 2 210 s, 1 750 s, 1 670 m, 1 610 w, 1 590 w. ^{1}H-NMR (CF_3COOH): δ 1.4 (t, CH_3CH_2O-), 2.5 (m, 2 H at C-2), 3.3 (2 t, 1 H at C-1 + 2 H at C-3), 4.5 (q, CH_3CH_2O-), 7.8 (bs, 3 Ar*H*), 8.7 (d, 1 Ar*H* at C-6); MS: m/z 321.2, 292, 248.7 (base peak M/E), 220.2. Anal. ($C_{18}H_{15}N_3O_3$) C, H, N.

4.1.2. Methyl 4-Cyano-2,3,5,11-tetrahydro-11-oxo-1H-cyclopenta[4,5]pyrido[1,2-a]benzimidazole-1-carboxylate **3**

This was prepared, similar to **2**, from **1** (1.57 g, 10 mmol), dimethyl cyclopentanone-2,3-dicarboxylate (2.2 g, 11 mmol) and ammonium acetate (1.7 g, 22 mmol); yield: 0.4 g (13%); m.p. = 295–300 °C (DMF); IR ν cm^{-1}: 3 300–2 500 bm, 2 210 s, 1 740 s, 1 670 s, 1 610 w, 1 590 w. ^{1}H-NMR (CF_3COOH): δ 3.3–3.7 (m + t, 4 H at C-1,2 + 1 H at C-3), 3.9 (s, CH_3O-), 7.7 (bs, 3 Ar*H*), 8.7 (d, 1 Ar*H* at C-6). Anal. ($C_{17}H_{13}N_3O_3$) C, H, N.

4.1.3. 5,7,8,9,10,11-Hexahydro-11-oxo-benzimidazo[1,2-b]isoquinoline-6-carbonitrile **4**

This was prepared, similar to **2**, from **1** (7.85 g, 50 mmol), ethyl cyclohexanone-2-carboxylate (9.4 g, 55 mmol) and ammonium acetate (8.5 g, 110 mmol); yield: 8.82 g (67%); m.p. > 300 °C (DMF); IR ν cm^{-1}: 3 250–2 500 bm, 2 210 s, 1 660 s, 1 615 w. ^{1}H-NMR (360 MHz): δ 1.7 (m, 4H, -$CH_2(CH_2)_2CH_2$-), 2.5 & 2.7 (2 t, 4 H, -$CH_2(CH_2)_2CH_2$-), 7.3 (m, 2 Ar*H* at C-8,9), 7.5 (d, 1 Ar*H* at C-10), 8.6 (d, 1 Ar*H* at C-7), 13.3 (s, NH). Anal. ($C_{16}H_{13}N_3O$) C, H, N.

4.1.4. 11-Chloro-7,8,9,10-tetrahydrobenzimidazo[1,2-b]-isoquinoline-6-carbonitrile **5**

Compound **4** (2.63 g, 10 mmol) was refluxed with phosphorus oxychloride (15 mL) for 2 h. The excess phosphorus oxychloride was removed under vacuum, and the residue was treated with ice-water, and neutralized with sodium carbonate. The insoluble was then filtered, washed with water, and dried; yield: 2.7 g (96%); m.p. = 229–231 °C (DMF); IR ν cm^{-1}: 2 210 s, 1 625 m, 1 600 s. ^{1}H-NMR (CF_3COOH): δ 2.2 (m, 4 H, -$CH_2(CH_2)_2CH_2$-), 3.3 & 3.5 (2 t, 4 H, -$CH_2(CH_2)_2CH_2$-), 7.8–8.0 (m, 3 Ar*H*), 9.0 (d, 1 Ar*H* at C-7). Anal. ($C_{16}H_{12}ClN_3$) C, H, N.

4.1.5. 5-Butyl-5,7,8,9,10,11-Hexahydro-11-oxo-benzimidazo[1,2-b]isoquinoline-6-carbonitrile **6**

Compound **4** (1.32 g, 5 mmol) was refluxed with tributyl phosphate (10 mL) for 1 h in the presence of anhydrous potassium carbonate (0.2 g). After cooling and addition of water, the product was filtered, and dried; yield: 1.3 g (81%); m.p. = 154–157 °C (EtOH/H_2O); IR ν cm^{-1}: 3 000–2 800 bw, 2 200 s, 1 660 s, 1 610 w. ^{1}H-NMR ($CDCl_3$): δ 1.0 (t, 3 H, $CH_3(CH_2)_2CH_2$-N), 1.2–1.9 (m, 8 H, $CH_3(CH_2)_2CH_2$-N and $CH_2(CH_2)_2CH_2$-), 2.5 & 2.7 (2 t, 4 H, -$CH_2(CH_2)_2CH_2$-), 4.4 (t, 2 H, $CH_3(CH_2)_2CH_2$-N), 7.0–7.4 (m, 3 Ar*H*), 8.7 (d, 1 Ar*H* at C-7). Anal. ($C_{20}H_{21}N_3O$) C, H, N.

4.1.6. 11-(4-Methylphenylamino)-7,8,9,10-tetrahydrobenzimidazo[1,2-b]isoquinoline-6-carbonitrile **7a**

This was prepared by stirring a solution of **5** (1.4 g, 5 mmol) and p-toluidine (1.07 g, 10 mmol) in dimethylformamide (10 mL) for 4 h at 80 °C. After cooling and addition of water, the precipitate was filtered and dried; yield: 1.5 g (86%); m.p.: 220–221 °C (EtOH); IR ν cm^{-1}: 3 300 bs, 2 210 s, 1 620 w, 1 600 w. ^{1}H-NMR: δ 1.8 (m, 4 H, -$CH_2(CH_2)_2CH_2$-), 2.2 (s, CH_3), 2.7 & 3.1 (2 t, 4 H, -$CH_2(CH_2)_2CH_2$-), 6.6 & 7.0 (dd, 4 Ar*H*, aryl at C-5), 7.2 & 7.5 (2 t, 2 Ar*H* at C-8,9), 7.8 (d, 1 Ar*H* at C-10), 7.9 (d, 1 Ar*H* at C-7), 9.0 (s, NH). Anal. ($C_{23}H_{20}N_4$) C, H, N.

4.1.7. 11-(4-Chlorophenylamino)-7,8,9,10-tetrahydrobenzimidazo[1,2-b]isoquinoline-6-carbonitrile **7b**

This was prepared, similar to **7a**, from **5** (1.4 g, 5 mmol) and p-chloroaniline (1.13 g, 10 mmol); yield: 1.4 g (75%); m.p. = 240–242 °C (EtOH); IR ν cm^{-1}: 3 600 bm, 2 210 s, 1 630 m, 1 590 s. ^{1}H-NMR: δ 1.8 (m, 4 H, -$CH_2(CH_2)_2CH_2$-), 2.7 & 3.2 (2 t, 4 H, -$CH_2(CH_2)_2CH_2$-), 6.7 & 7.3 (dd, 4 Ar*H*, aryl at C-5), 7.2 & 7.5 (2 t, 2 Ar*H* at C-8,9), 7.8 (d, 1 Ar*H* at C-10), 7.9 (d, 1 Ar*H* at C-7), 9.3 (s, NH). Anal. ($C_{22}H_{17}ClN_4$) C, H, N.

4.1.8. 11-(4-Fluorophenylamino)-7,8,9,10-tetrahydrobenzimidazo[1,2-b]isoquinoline-6-carbonitrile **7c**

This was prepared, similar to **7a**, from **5** (1.4 g, 5 mmol) and p-fluoroaniline (1.11 g, 10 mmol); yield: 1.25 g (70%); m.p. = 238–239 °C (EtOH); IR ν cm^{-1}: 3 400 bw, 2 210 s, 1 625 s, 1 600 s. ^{1}H-NMR: δ 1.8 (m, 4 H, -$CH_2(CH_2)_2CH_2$-), 2.7 & 3.1 (2 t, 4 H, -$CH_2(CH_2)_2CH_2$-), 6.7 & 7.1 (dd, 4 Ar*H*, aryl at C-5), 7.2 & 7.5 (2 t, 2 Ar*H* at C-8,9), 7.8 (d, 1 Ar*H* at C-10), 7.9 (d, 1 Ar*H* at C-7), 9.1 (s, NH). Anal. ($C_{22}H_{17}FN_4$) C, H, N.

4.1.9. 11-Azido-7,8,9,10-tetrahydrobenzimidazo[1,2-b]-isoquinoline-6-carbonitrile **8**

The chloro compound **5** (1.4 g, 5 mmol) was treated with sodium azide (0.40 g, 6 mmol) in dimethylformamide (15 mL) and stirred at room temperature for 30 min. After addition of water the insoluble was filtered and dried to yield: 1.3 g (90%) of **8**; m.p. = 155 °C dec. (EtOH); IR ν cm^{-1}: 2 210 s, 2 150 s, 1 630 s, 1 600 s. Anal. ($C_{16}H_{12}N_6$) C, H, N.

4.1.10. 11-Triphenylphosphoranylideneamino-7,8,9,10-tetrahydrobenzimidazo[1,2-b]isoquinoline-6-carbonitrile **9**

To a stirred suspension of **8** (2.3 g, 8 mmol) in benzene (20 mL), a solution of triphenylphosphine (2.62 g, 10 mmol) in benzene (15 mL) was added at room temperature. An immediate clear solution was formed with evolution of nitrogen gas and separation of an orange-red product. After stirring for 1 h, the solid was filtered, washed with benzene, and dried; yield: 4.0 g (96%); m.p. = 265 °C dec. (EtOH); IR ν cm^{-1}: 2 900 w, 2 200 s, 1 620 s, 1 570 s. ^{1}H-NMR: δ 1.2 & 1.5 (2 m, 4 H, -$CH_2(CH_2)_2CH_2$-), 2.0 & 2.9 (2 t, 4 H, -$CH_2(CH_2)_2CH_2$-), 6.7 & 7.3 (2 t, 2 Ar*H* at C-8,9), 7.6–7.9 (m, 15 Ar*H* of $(C_6H_5)_3P$ = N- + 1 Ar*H* at C-10), 8.1 (d, 1 Ar*H* at C-7). Anal. ($C_{34}H_{27}N_4P$) C, H, N.

Acknowledgements

We thank the staff of the National Cancer Institute, Bethesda MD, USA, for the anticancer test reports.

References

[1] Soliman F.S.G., Rida S.M., Badawey E.A.M., Kappe T., Arch. Pharm. 317 (1984) 951–958.

[2] Rida S.M., Soliman F.S.G., Badawey E.A.M., El-Ghazzawi E., Kader O., Kappe T., J. Heterocycl. Chem. 25 (1988) 1087–1093.

[3] Rida S.M., Soliman F.S.G., Badawey E.A.M., Kappe T., J. Heterocycl. Chem. 25 (1988) 1725–1728.

[4] Badawey E.A.M., Kappe T., IL Farmaco 50 (1995) 537–542.

[5] Badawey E.A.M., Kappe T., Eur. J. Med. Chem. 30 (1995) 327–332.

[6] Badawey E.A.M., Kappe T., J. Heterocycl. Chem. 32 (1995) 1003–1006.

[7] Badawey E.A.M., J. Heterocycl. Chem. 33 (1996) 229–233.

[8] Badawey E.A.M., Kappe T., Eur. J. Med. Chem. 32 (1997) 815–822.

[9] Badawey E.A.M., Rida S.M., Soliman F.S.G., Kappe T., Monatsch. Chem. 120 (1989) 73–76.

[10] Russell R.K., VanNievelt C.E., J. Heterocycl. Chem. 32 (1995) 299–306.

[11] Conducted by the National Cancer Institute, Bethesda, Maryland, US.