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Synthesis of indolo[4,3-*bc*]phenanthridine-6,11(2*H*,12*H*)-diones using the Schiff base-homophthalic anhydride cyclization reaction

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Abstract

A novel indolophenanthridine ring system has been synthesized via the Schiff base-homophthalic anhydride cyclization followed by thionyl chloride-mediated dehydrogenation and intramolecular Friedel-Crafts acylation. This adds to the array of heterocyclic systems that are available through the cycloaddition reaction of imines with cyclic dicarboxylic acid anhydrides. The cytotoxicities of the indolophenanthridines were investigated in human cancer cell cultures, and the results documented significant antitumor activity in a variety of human cancer cell lines. This provides a new heterocyclic scaffold for anticancer drug design.

Graphical abstract

Keywords

Indolophenanthridine; Schiff base; homophthalic anhydride; cytotoxicity; anticancer

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Supplemental Data: Supplemental data (complete cytotoxicity data for compounds 8 and 9, ¹H NMR spectra, crystallography of 7ab, and experimental details for the preparation and characterization of 4a, 4b, and 9) can be accessed on the publisher's website.

Introduction

The reaction between Schiff bases and cyclic dicarboxylic acid anhydrides¹ has provided a facile entry to the synthesis of a variety of heterocyclic systems including pyrrolidinones,²⁻⁴ piperidinones,^{5,6} and isoquinolones.⁷ Recent advancements have documented additional applications in heterocyclic chemistry.⁸⁻²⁰ The initial products of the reaction have found use in the synthesis of a variety of naturally occurring protoberberine²¹ and benzophenanthridine alkaloids²² and in the synthesis of the indenoisoquinoline anticancer agents indotecan²³ (LMP400) and indimitecan²³ (LMP776),^{24,25} which have been in Phase I clinical trials at the National Cancer Institute (Figure 1). The present report extends the utility of the reaction to the synthesis of analogues of **1** and **2** based on a novel indolophenanthridine scaffold that has never been reported before. The resulting compounds have been pursued as part of a program to make analogues of the indenoisoquinolines containing additional nitrogen atoms that may be of interest due to their potential anticancer activity.²⁶⁻²⁹

Results and discussion

As shown in Scheme 1, commercially available indole-3-carbaldehyde (**1**) was protected with benzyl, methyl, and benzensulfonyl groups via a phase transfer catalyzed reaction.³⁰ The protected indoles **2a-c** were condensed with 3-bromopropylamine, generated in situ from the commercially available hydrochloride salt, using anhydrous sodium sulfate in chloroform to yield the imines **3a-c**, which were reacted with 3,4-dimethoxyhomophthalic anhydride to provide the isoquinolones **4a-c**.⁷

Only the cis configuration was produced as indicated from the coupling constants ($J = 5-6$ Hz) of the two methine protons in the ¹H NMR spectra of **4a-c**.⁷ Attempts were made to dehydrogenate **4a-c** in thionyl chloride solution and then perform intramolecular Friedel-Crafts acylation on the acid chloride generated in situ (e.g. see **5**), but only the sulfone-protected derivative **4c** yielded the desired product **6**.³¹ None of the isomeric product that would have formed from cyclization at C-2 of the indole was detected. X-ray crystallography indicated that the minor product **7ab** was a 7:3 mixture of **7a** and **7b** (Scheme 1) which co-crystallized with both molecules in a single crystal. We assume that minute amounts of chlorine (Cl₂) present in thionyl chloride chlorinated the indole ring, while chloride generated in the reaction displaced bromide to yield **7b**.

Various reaction conditions were investigated as shown in Table 1 in order to enhance the yield of the product **6** and the trace side product **7ab**. None of the reaction conditions that were investigated increased the yield of **7ab** beyond 2%, but the yield of **6** was increased from 23% to 65% by performing the aluminum-chloride-catalyzed reaction in 1,2-dichloroethane at reflux instead of at 23 °C. The presence of three singlets in the aromatic region of the ¹H NMR spectrum of compound **6** at δ 9.43, 8.74 and 7.84 ppm support the assigned structure while the ¹H NMR spectrum of compound **7ab** showed only two singlets at δ 9.32 and 7.82 ppm. Compound **6** underwent both deprotection and bromide displacement reactions to yield **8** or **9** in one step using morpholine or imidazole in the presence of potassium carbonate and potassium iodide DMSO.

Compounds **8** and **9** were tested for their cytotoxicities in the NCI-60 panel of sixty human cancer cell lines. Table 2 lists representative growth percentages of seven cell lines at 10 μ M concentration of these two compounds relative to control (no drug). The remaining cytotoxicity data are available as Supporting Information. Compound **9** had the best activity against the breast cancer cell line with a 7% decline in viable cancer cells, while compound **8** showed its best activity against the lung cancer cell line with just 9% growth of the cancer cells. The two compounds displayed good activity against melanoma, CNS, and renal cell lines with less than 20% growth at 10 μ M concentration.

Conclusion

The reaction of an indole-containing Schiff base with a substituted homophthalic anhydride provided the key step in the synthesis of a new indolophenanthridine ring system. This adds to the diversity of heterocyclic systems available from the reaction between Schiff bases and homophthalic anhydrides. The cytotoxic activities of two compounds containing this new heterocyclic system were determined in human cancer cell cultures. Both had significant activity against cancer cell lines in the NCI-60 panel. This provides a new scaffold for optimization to produce new anticancer agents.

Experimental

General information

All yields refer to isolated compounds. Unless otherwise stated, chemicals and solvents were of reagent grade and used as obtained from commercial sources without further purification. Melting points were determined using capillary tubes and are uncorrected. ^1H Nuclear magnetic resonance spectroscopy was performed using a 300 MHz spectrometer. Infrared spectra were obtained using an FTIR spectrometer. HPLC analyses were performed on a binary HPLC pump/dual wavelength absorbance detector system, using a 5 μ m C18 reversed phase column with UV detection at 254 and 280 nm. HPLC purities of the two biologically tested compounds were estimated from the major HPLC peak areas, which were $\geq 95\%$ of the combined total peak areas. Compounds **2a-c** were synthesized according to literature procedures.³⁰

2-(3-bromopropyl)-6,7-dimethoxy-1-oxo-3-(1-(phenylsulfonyl)-1*H*-indol-3-yl)-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (**4c**)

Triethylamine (0.585 g, 5.78 mmol) was added to a suspension of 3-bromopropylamine hydrobromide (1.266 g, 5.78 mmol) and anhydrous sodium sulfate (5 g) in chloroform (10 mL) and the mixture was stirred at room temperature for 15 min. Aldehyde **2c** (1.5 g, 5.26 mmol) was dissolved in chloroform (5 mL) and the solution was added slowly to the mixture. The reaction mixture was stirred at room temperature for 48 h and then filtered through a pad of celite. The filtrate was washed with water (2 $\times$ 30 mL) and brine (1 $\times$ 30 mL) and then dried (anhydrous Na_2SO_4) and evaporated to give an orange residue of the crude imine **3c** (2.1 g, 99%), which was used in the following reaction without further purification. The crude imine **3c** (1.3 g, 3.21 mmol) was dissolved in chloroform (5 mL) and the mixture was cooled to 0 $^\circ\text{C}$. The solution was added over a period of 30 min to an ice-

cooled solution of 3,4-dimethoxyhomophthalic anhydride (0.713 g, 3.21 mmol) in chloroform (5 mL). After 2 h at 0 °C, the reaction mixture was stirred at room temperature for another 2 h and then the precipitate was filtered off and washed with chloroform to afford **4c** (0.87 g, 43%) as a white solid: mp 200 °C. IR (thin film) 3650 (br), 2941, 1730, 1697, 1622, 1597, 1577, 1518, 1485, 1467, 1448, 1430, 1367, 1299, 1281, 1228 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.95 (s, 1 H), 8.32 (d, *J* = 3.2 Hz, 1 H), 7.82 (d, *J* = 8.3 Hz, 1 H), 7.68 (t, *J* = 9.1 Hz, 3 H), 7.58 (s, 1 H), 7.51 (dd, *J* = 17.2, 8.0 Hz, 3 H), 7.30 (d, *J* = 6.8 Hz, 1 H), 7.22 (t, *J* = 7.4 Hz, 1 H), 7.15 (d, *J* = 5.9 Hz, 2 H), 5.49 (d, *J* = 5.1 Hz, 1 H), 4.70 (d, *J* = 5.1 Hz, 1 H), 3.87 (s, 3 H), 3.78 (s, 3 H), 3.42 (m, 2 H), 3.07 (m, 1 H), 1.94 (m, 2 H); MALDIMS *m/z* 627/629 [M+H]⁺; HRESIMS *m/z* calculated for C₂₉H₂₇BrN₂O₇SNa, 649.0621, found, 649.0618 [M+Na]⁺.

12-(3-bromopropyl)-8,9-dimethoxy-2-(phenylsulfonyl)indolo[4,3-*bc*]phenanthridine-6,11(2*H*,12*H*)-dione (6) and the crystalline mixture of 6-(3-bromopropyl)-1-chloro-2,3-dimethoxy-11-(phenylsulfonyl)indolo[2',3':4,5]cyclopenta[1,2-*c*]isoquinoline-5,12(6*H*,11*H*)-dione and 6-(3-chloropropyl)-1-chloro-2,3-dimethoxy-11-(phenylsulfonyl)indolo[2',3':4,5]cyclopenta[1,2-*c*]isoquinoline-5,12(6*H*,11*H*)-dione (7ab)

Thionyl chloride (4 mL) was added to the acid **4c** (0.55 g, 0.876 mmol) and the mixture was stirred at room temperature for 5 h. Benzene (15 mL) was added and the solution was evaporated to give a yellow residue of crude **5**. The residue was dissolved in dichloroethane (20 mL) and cooled to 0 °C. Aluminum chloride (0.467 g, 3.51 mmol) was added slowly to the previous solution and the mixture was heated at reflux for 2 h. After the mixture was cooled to room temperature, ice-cooled water was added to quench the reaction and the organic layer was separated, washed with water (2 × 30 mL) and brine (1 × 30 mL) and dried (anhydrous Na₂SO₄). Flash chromatography (SiO₂/CHCl₃) of the residue obtained after evaporation of the organic layer gave **6** (0.34 g, 65%) and **7ab** (10 mg, 2 %) as yellow solids. **6**: mp 260-262 °C (dec). IR (thin film) 3100, 2970, 1645, 1623, 1602, 1530, 1508, 1494, 1463, 1448, 1415 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.43 (s, 1 H), 8.73 (s, 1 H), 8.27 (d, *J* = 8.1 Hz, 1 H), 8.19 (d, *J* = 7.6 Hz, 1 H), 8.10 – 8.02 (m, 2 H), 7.84 (s, 1 H), 7.70 (t, *J* = 7.9 Hz, 1 H), 7.63 (d, *J* = 7.4 Hz, 1 H), 7.55 (t, *J* = 7.5 Hz, 2 H), 4.84 – 4.75 (m, 2 H), 4.12 (s, 3 H), 4.03 (s, 3 H), 3.79 (t, *J* = 6.0 Hz, 2 H), 2.57 (m, 2 H); ESIMS *m/z* 609/607 [M+H]⁺; HRESIMS *m/z* calculated for C₂₉H₂₄BrN₂O₆S, 607.0539, found, 607.0539 [M+H]⁺. **7ab**: mp 123-125 °C; IR (thin film) 2960, 1637, 1622, 1598, 1507, 1495, 1447 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.32 (s, 1 H), 8.49 (d, *J* = 8.3 Hz, 1 H), 8.19 (d, *J* = 7.6 Hz, 1 H), 8.00 (d, *J* = 7.4 Hz, 2 H), 7.82 (s, 1 H), 7.73 (t, *J* = 8.0 Hz, 1 H), 7.65 (d, *J* = 7.4 Hz, 1 H), 7.53 (t, *J* = 7.7 Hz, 3 H), 4.62 (t, *J* = 6.2 Hz, 2 H), 4.10 (s, 3 H), 4.03 (s, 3 H), 2.97 (t, *J* = 5.9 Hz, 2 H), 2.19 – 2.07 (m, 2 H); HRESIMS *m/z* calculated for C₂₉H₂₃BrClN₂O₆S, 641.0149, found, 641.0145, HRESIMS *m/z* calculated for C₂₉H₂₃Cl₂N₂O₆S, 597.0654, found, 597.0647 [M+H]⁺. Single crystal X-ray diffraction data for **7ab** are given in the Supporting Information.

8,9-dimethoxy-12-(3-morpholinopropyl)indolo[4,3-*bc*]phenanthridine-6,11(2*H*,12*H*)-dione (8)

Compound **6** (0.12 g, 0.198 mmol) was dissolved in DMSO (10 mL), and morpholine (0.17 g, 1.98 mmol), KI (0.2 g) and K₂CO₃ (0.3 g) were then added and the mixture was stirred at 90 °C for 16 h. Water (50 mL) was added to the mixture and the solid was extracted with

CHCl₃ (3 × 15 mL). The organic layer was washed with water (2 × 15 mL) and brine (1 × 15 mL) and dried over anhydrous sodium sulfate. Flash chromatography (SiO₂, CHCl₃-MeOH 9:1) of the residue obtained after evaporation of the organic layer gave **8** (0.04 g, 42%) as a yellow solid: mp 273-275 °C (dec). IR (thin film) 3100, 2939, 1651, 1622, 1603, 1532, 1508, 1494, 1449, 1416, 1384, 1309, 1291, 1273, 1254, 1232 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 11.88 (s, 1 H), 9.64 (s, 1 H), 8.55 (s, 1 H), 8.08 (d, *J* = 7.4 Hz, 1 H), 7.76 (s, 1 H), 7.65 (d, *J* = 7.7 Hz, 1 H), 7.45 (t, *J* = 7.7 Hz, 1 H), 4.63 (t, *J* = 7.8 Hz, 2 H), 4.04 (s, 3 H), 3.93 (s, 3 H), 3.78 – 3.71 (m, 4 H), 2.58 (m, 6 H), 2.09 (m, 2 H); MALDIMS *m/z* 474 [M + H]⁺; HRESIMS *m/z* calculated for C₂₂H₂₈N₃O₅ 474.2029, found 474.2022; HPLC purity: 99.8% (C₁₈ reversed phase, MeOH-H₂O, 90:10).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Gonzalez-Lopez M, Shaw JT. Chem Rev. 2009; 109:164. [PubMed: 19140773]
2. Castagnoli N Jr. J Org Chem. 1969; 34:3187. [PubMed: 5811404]
3. Cushman M, Castagnoli N Jr. J Org Chem. 1971; 36:3404. [PubMed: 5132298]
4. Cushman M, Castagnoli N Jr. J Org Chem. 1972; 37:1268.
5. Cushman M, Castagnoli N Jr. J Org Chem. 1973; 38:440. [PubMed: 4687212]
6. Cushman M, Castagnoli N Jr. J Org Chem. 1974; 39:1546. [PubMed: 4833507]
7. Cushman M, Cheng L. J Org Chem. 1978; 43:286.
8. Dar'in D, Bakulina O, Chizhova M, Krasavin M. Organic Lett. 2015; 17:3930.
9. Sampitak P, Krasavin M. Tetrahedron Lett. 2014; 55:2299.
10. Krasavin M, Dar'in D. Tetrahedron Lett. 2016; 57:1635.
11. Ryabukhin SV, Panov DM, Granat DS, Ostapchuk EN, Kryvoruchko DV, Grygorenko OO. ACS Comb Sci. 2014; 16:146. [PubMed: 24479637]
12. Georgieva A, Stanoeva E, Spassov S, Macicek J, Angelova O, Haimova M, Dekimpe N. Tetrahedron. 1991; 47:3375.
13. Georgieva A, Stanoeva E, Karamfilova K, Spassov S, Angelova O, Haimova M, Dekimpe N, Boelens M. Tetrahedron. 1994; 50:9399.
14. Georgieva A, Stanoeva E, Spassov S, Haimova M, Dekimpe N, Boelens M, Keppens M, Kemme A, Mishnev A. Tetrahedron. 1995; 51:8407.
15. Masse CE, Ng PY, Fukase Y, Sanchez-Rosello M, Shaw JT. J Comb Chem. 2006; 8:293. [PubMed: 16676997]
16. Ng PY, Masse CE, Shaw JT. Org Lett. 2006; 8:3999. [PubMed: 16928058]
17. Liu J, Wang Z, Levin A, Emge TJ, Rablen PR, Floyd DM, Knapp S. J Org Chem. 2014; 79:7593. [PubMed: 25036978]
18. Perard-Viret J, Souquet F, Manisse ML, Royer J. Tetrahedron Lett. 2010; 51:96.
19. Guiguemde WA, Shelat AA, Bouck D, Duffy S, Crowther GJ, Davis PH, Smithson DC, Connelly M, Clark J, Zhu FY, Jimenez-Diaz MB, Martinez MS, Wilson EB, Tripathi AK, Gut J, Sharlow ER, Bathurst I, El Mazouni F, Fowble JW, Forquer I, McGinley PL, Castro S, Angulo-Barturen I,

- Ferrer S, Rosenthal PJ, DeRisi JL, Sullivan DJ, Lazo JS, Roos DS, Riscoe MK, Phillips MA, Rathod PK, Van Voorhis WC, Avery VM, Guy RK. *Nature*. 2010; 465:311. [PubMed: 20485428]
20. Dar'in D, Bakulina O, Nikolskaya S, Gluzdikov I, Krasavin M. *RSC Adv*. 2016; 6:49411.
21. Iwasa K, Gupta YP, Cushman M. *J Org Chem*. 1981; 46:4744.
22. Cushman M, Choong TC, Valko JT, Koleček M. *J Org Chem*. 1980; 45:5067.
23. Nagarajan M, Morrell A, Ioanoviciu A, Antony S, Kohlhausen G, Agama K, Hollingshead M, Pommier Y, Cushman M. *J Med Chem*. 2006; 49:6283. [PubMed: 17034134]
24. Cinelli MA, Reddy PVN, Lv PC, Liang JH, Chen L, Agama K, Pommier Y, van Breemen RB, Cushman M. *J Med Chem*. 2012; 55:10844. [PubMed: 23215354]
25. Beck DE, Agama K, Marchand C, Chergui A, Pommier Y, Cushman M. *J Med Chem*. 2014; 57:1495. [PubMed: 24517248]
26. Kiselev E, Dexheimer TS, Pommier Y, Cushman M. *J Med Chem*. 2010; 53:8716. [PubMed: 21090809]
27. Kiselev E, Agama K, Pommier Y, Cushman M. *J Med Chem*. 2012; 55:1682. [PubMed: 22329436]
28. Kiselev E, Empey N, Agama K, Pommier Y, Cushman M. *J Org Chem*. 2012; 77:5167. [PubMed: 22587603]
29. Kiselev E, Sooryakumar D, Agama K, Cushman M, Pommier Y. *J Med Chem*. 2014; 57:1289. [PubMed: 24502276]
30. Jaisankar P, Srinivasan PC. *Synthesis*. 2006; 2413
31. Xiao XS, Morrell A, Fanwick PE, Cushman M. *Tetrahedron*. 2006; 62:9705.

Figure 1.
The structures of the Phase I anticancer drugs indotecan (LMP400) and indimitecan (LMP776).

Scheme 1. ^aSynthesis of 8 and 9

^aReagents and conditions: (a) benzyl bromide, or methyl iodide, or benzenesulfonyl chloride, tetrabutylammonium hydrogensulfate, CH_2Cl_2 , 30% aq NaOH, 23 °C (2 h); (b) Na_2SO_4 , CHCl_3 , 23 °C (48 h); (c) CHCl_3 , 0 °C (2 h), 23 °C (2 h); (d) SOCl_2 , 23 °C (5 h); (e) AlCl_3 , $\text{ClCH}_2\text{CH}_2\text{Cl}$, reflux (2 h); (f) morpholine or imidazole, K_2CO_3 , KI, DMSO, 90 °C (16 h).

Table 1

Optimization of the conversion of **4c** to **6** by thionyl chloride at room temperature followed by aluminum chloride or titanium(IV) chloride under the various conditions listed.

Conditions	% Yield of 6	% Yield of 7ab
AlCl ₃ , DCE, 0 °C	0	0
AlCl ₃ , DCE, 23 °C	23	0
AlCl ₃ , DCE, reflux	65	2
AlCl ₃ , PhNO ₂ , 23 °C	21	0
AlCl ₃ , PhNO ₂ , 120 °C	43	0
TiCl ₄ , DCE, reflux	15	0

Table 2

Growth percentages at 10 μ M concentration of **8** or **9** relative to control

compd	Lung		Colon	CNS	Melanoma		Ovarian	Breast	Renal
	NCT-H460	HT29	U251	LOX IMVI	OVCAR-8	T-47D	786-0		
8	9%	29%	17%	11%	40%	19%	19%		
9	7%	34%	14%	4%	35%	-7%	7%		