Synthesis and biological activity of *meso*-tetrakis (2,10-dioxo-2*H*, 10*H*-pyrano [2,3-*f*] chromene-9-yl) porphyrins

V. Naveen Kumar [a], Y. Thirupathi Reddy [a], P. Narasimhareddy [a], B. Rajitha[a]*, and E. De Clercq [b]

[a]*Department of Chemistry, National Institute of Technology, Warangal, India*
[b] *Rega Institute of Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium*
E-mail: rajitabhargavi@yahoo.com

Abstract

Synthesis of *meso*-tetrakis (2, 10-dioxo-2*H*, 10*H*-pyrano[2,3-*f*] chromene-9-yl) porphyrins are synthesized directly by reaction of pyrrole with substituted 4-methyl-2,10-dioxo-2*H*, 10*H*-pyrano[2,3-*f*]chromene-9-carbaldehydes in dichloromethane / acid media. The aldehyde's molar ratio was controlled to optimize the synthesis and purification of the desired porphyrins. This new series of porphyrins was characterized by TLC, Mass Spectrometry (FAB mass), ^{1}H NMR, UV and IR

Keywords: Porphyrins, chromene, TFA

Introduction

The synthesis of porphyrins has gained special attention in recent years because of its importance in bioorganic and bioinorganic chemistry[1] and its application in biomedical sciences. Porphyrin type compounds have been actively investigated as sensitizing drugs for application in cancer diagnosis and treatment using photodynamic therapy (PDT). The *meso*-tetraphenyl porphyrins offer attractive features in this context and have been used in a wide variety of model studies.[2,3]

Recently, the combination of porphyrins with another large organic molecules has become an interesting route to new materials.[4-7] Here we describe the synthesis of porphyrins substituted at the meso positions by heteroaryl chromenes, wherein both chromone and coumarin moieties are present at meso positions. Earlier, we reported[8] the synthesis of simple porphyrins substituted with chromene-4-ones in the meso position, from our laboratories. Coumarins have attracted intense interest in recent years because of their diverse pharmacological[9-11] properties including anticancer activity.[12-16] In the present work, we reported a study on cytotoxic and antiviral activities, in vitro, against tumor cell lines and viruses. This paper aims at elucidating the

preliminary structure activity relationships by simple chemical structural modifications and probing structural requirement for the marked antitumor activity of these compounds.

Results and Discussion

Treatment of 7-hydroxy coumarin (**I**) and acetic anhydride under reflux conditions afforded 7-acetoxycoumarin (**2**), which on Fries rearrangement furnished 7-hydroxy-8-acetylcoumarin(**3**).The Vilsmeier reaction[17] of compound (**3**) with DMF and $POCl_3$ afforded 2,10-dioxo-2*H*, 10*H*-pyrano[2,3-*f*]chromene-9-carbaldehydes(**4**). As per the Lindsey's procedure[18] condensation of an equimolar mixture of 2,10-dioxo-2*H*,10*H*-pyrano[2,3-*f*]chromene-9-carbaldehydes (**4a-f**) with pyrrole in TFA at room temperature as shown in scheme 1, gave the porphyrinogens, that after addition of DDQ and reflux afforded the meso-tetrakis (2,10-dioxo-2*H*, 10*H*-pyrano[2,3-*f*]chromene-9-yl) porphyrins (**6a-f**) in 25-26% overall yields. Purple colored porphyrins of high purity resulted by flash chromatography using chloroform and methanol (95:5) as eluent. The structures of the porphyrins were confirmed by various spectroscopic techniques. The UV-Vis spectra of the free base porphyrins were recorded at $2x10^{-5}$ mol concentrations in methanol. The more intense B band, around 425nm and four much less intense Q bands are observed around 450-600nm. Briefly for **6a,** the Soret band is prominent as a single absorption at 422 nm.

The IR spectra of all the compounds showed a broad band at 3448 cm^{-1}, and bands at 1630 (the chromone carbonyl stretching), 1720 (the lactone carbonyl stretching), 2950, 2830 (aliphatic str), 940 cm^{-1}(ω- porphyrin macrocyclic bending). The FAB mass spectra confirmed the composition and the complete structures were assigned by detailed ^1H NMR experiments. For **6a**, pyrrole β-CH protons appear as a singlet at 9.12 ppm. The C-2H protons of chromene-4-one are shown as a singlet at 8.0 ppm. The two inner NH protons resonate at –2.5 ppm.

Biological results

Antitumor activity

The compounds **6a-f** were screened for antitumor activity against L1210 murine leukemia cells as well as human T-lymphocyte cells molt 4/C8 and CEM/0 and the results are portrayed in Table 1. Etoposide was used as a standard drug.

Antitumor experiments were carried out as described in the literature.[19-21] In simple porphyrin **6a** antitumor activity was lower in murine leukemia cells and higher in human T-lymphocyte cells. Remarkable enhancement of antitumor activity was observed when a halogen group is located in position 3 of the coumarin (**6c**). The compound (.**6f**) displayed intermediate cytotoxic activity, and others (**6b, 6e**) only had marginal or no activity.

Antiviral activity

Compounds **6** were evaluated for their antiviral activity against HEL, Hela and Vero cell cultures. The cytotoxicity was verified in mock-infected HEL, Hela and Vero cells. The antiviral activity assays were based on inhibition of virus-induced cytopathicity in the above mentioned cultures. The activity of these compounds was compared with that of standard Brivudin, Ribavirin, Acyclovir and Ganciclovir. Briefly, the confluent cell culture in 96 well microliter plates was inoculated with 100 CC1D50 of virus, ICC1D50 being the virus dose required to infect 50% of the cell cultures. After 1h virus adsorption period, the residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations, i.e. 400, 200 and 100 ug/ml, of the test compounds. Viral cytopathicity was recorded as soon as reached completion in the control virus in treated cell cultures.

Table 1. Inhibitory effects on the proliferation of murine leukemia cells (L1210/0) and human T-Lymphocyte cells (Molt 4/C8, CEM/0)

Compound	IC_{50} (ug/ml)[a]		
	L1210/0	Molt4/C8	CEM/0
6a	15 ± 2	5.5± 0.6	5.5±1.1
6b	102 ±2	≥200	110±9
6c	6.0±1.6	8.7±1.2	4.6±0.7
6d	15±1	6.6±1.2	8.6±4.9
6e	45 ±1	19±4	20±4
6f	72±5	28±1	23±1
Etoposide	60±13	25±3	100±25

[a] 50% inhibitory concentration.

As shown in tables 2, 3 and 4, some compounds (**6b, 6d**) showed significant antiviral activities while others (**6f, 6a**) displayed medium antiviral activities. Compounds (**6c, 6e**) only had marginal or no antiviral activities against HEL, Hela and Vero cell cultures.

Table 2. Cytotoxicity and antiviral activity of compounds (6a-f) in HEL cell cultures

Compound	Minimum Cytotoxic concentration[a] (ug/ml)	Minimum Inhibitory concentration[b] (ug/ml)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2(G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK KOS ACV^r
6a	≥80	>80	>80	>80	>80	>80
6b	16	>3.2	>3.2	>3.2	>3.2	>3.2
6c	400	>80	>80	>80	>80	>80
6d	16	>3.2	>3.2	>3.2	>3.2	>3.2
6e	200	>40	>40	>40(120)	>40	>40
6f	≥3.2	>3.2	>3.2	>3.2	>3.2	>3.2
Brivudin	>400	0.0256	80	1.92	>400	16
Ribavirin	>400	>400	240	48	48	>400
Acyclovir	>400	0.384	0.384	>400	>400	48
Ganciclovir	>100	0.0192	0.096	>100	>100	0.48

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table 3. Cytotoxicity and antiviral activity of compounds (**6a-f)** in Vero cell cultures

Compound	Minimum Cytotoxic concentration[a] (ug/ml)	Minimum Inhibitory concentration[b] (ug/ml)				
		Para influenza3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
6a	80	>16	>16	>16	>16	>16
6b	16	>3.2	>3.2	>3.2	>3.2	>3.2
6c	≥400	>400	>400	>400	>400	>400
6d	≥3.2	>3.2	>3.2	>3.2	>3.2	>3.2
6e	>200	>200	120	>200	120	>200
6f	80	>16	>16	>16	>16	>16
Brivudin	≥400	>400	>400	>400	>400	>400
(S)-DHPA	>400	80	240	240	>400	240
Ribavirin	>400	48	48	48	240	48

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table 4. Cytotoxicity and antiviral activity of compounds (**6a-f**) in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a] (ug/ml)	Minimum Inhibitory concentration[b] (ug/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytical virus
6a	80	>16	>16	>16
6b	16	>3.2	>3.2	>3.2
6c	400	>80	>80	>80
6d	40	>8	>8	>8
6e	40	>8	>8	>8
6f	400	>80	>80	>80
Brivudin	≥400	>400	>400	>400
(S)-DHPA	>400	240	>400	48
Ribavirin	>400	9.6	48	9.6

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Experimental Section

General Procedures. Melting points were determined in open capillaries and are uncorrected. UV-Vis spectra were recorded on a Shimadzu UV-160A UV-Vis-NIR spectrophotometer using methanol as solvent. IR spectra were recorded as KBr discs using a Shimadzu FTIR model 8010 spectrophotometer, ^{1}H NMR spectra were recorded on a Varian FT 200 MHz instrument using $CDCl_3$ and d_6-DMSO as solvent TMS as an internal standard. FABS mass spectra were recorded on YG Micromass 7070H (F1 or C1) auto spectrometer. The C, H and N analysis of compounds was performed on a Carlo Erba Model EA1108 CHNS-O elemental analyzer. Porphyrins were purified by flash column chromatography using 230-400 mesh silica gel (Aldrich make).

General procedure for the synthesis of *meso*-tetrakis (2, 10-dioxo-2*H*, 10*H*-pyrano [2,3-*f*] chromene-9-yl) porphyrin (6a). To a solution of 4-methyl-2, 10-dioxo-2*H*, 10*H*-pyrano[2,3-*f*] chromene-9-carbaldehyde (4a, 4 mmol) in dichloromethane (150 ml) anhydrous TFA (4 mmol) via syringe and pyrrole (4 mmol, drop wise) were added. The reaction mixture was stirred in an inert atmosphere. After 1h, DDQ (200mg) was added to the reaction mixture, the flask was immersed in a water bath, pre-heated to 45°C, and the solution was refluxed for 1h. Then dichloromethane was distilled off and the crude product was purified by flash chromatography using chloroform: methanol (95:5) as eluent to afford **6a** as a dark purple solid: Yield: 26%; m.p. >300^{o}C; ^{1}H NMR (DMSO-d_6): 9.12 (s, 8H, pyrrole C-H), 8.32 (d, 4H, C-8H, J = 9Hz), 8.01 (s, 4H, C-2H), 7.29 (d, 4H, C-10H, J = 9 Hz), 6.70 (d, 4H, C-9H, J = 9 Hz), 6.25 (d, 4H, C-7H, J =

9Hz), –2.52 (s, 2H, N-H); FABMS: m/z, 1159 (M^+); Anal. Calcd for $C_{68}H_{30}N_4O_{16}$: C, 70.46; H, 2.59; N, 4.83. Found (%): C, 70.48; H, 2.55; N, 4.85.

Compounds **6b-f** were prepared similarly.

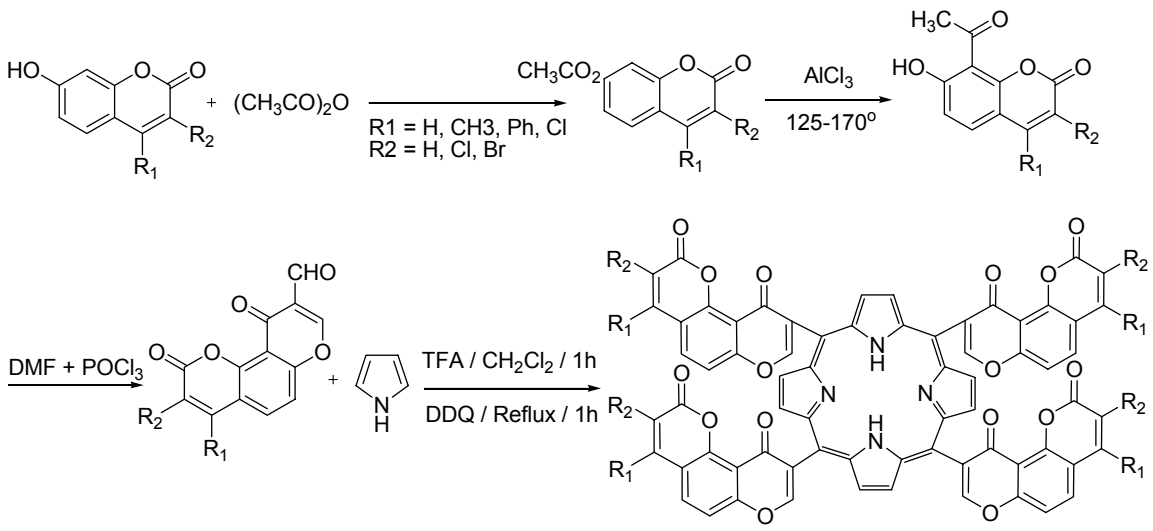
***meso*-Tetrakis(4-methyl-2,10-dioxo-2*H*,10*H*-pyrano[2,3-*f*] chromene-9-yl) porphyrin 6b.** Yield: 28%; m.p. >300°C; ^{1}H NMR (DMSO-d_6): δ 9.12 (s, 8H, pyrrole C-H), 8.02 (s, 4H, chromene C-2H), 7.25 (d, 4H, C-10H, J = 9 Hz), 6.80(d, 4H, C-9H, J = 9 Hz), 6.02 (s, 4H, C-7 of coumarin), 3.03 (s, 12H, CH_3), –2.34 (s, 2H, porphyrin N-H); FABMS: m/z, 1215 (M^+); Anal. Calcd for $C_{72}H_{38}N_4O_{16}$ (%): C, 71.16; H, 3.13; N, 4.61. Found (%): C, 71.10; H, 3.18; N, 4.63.

***meso*-Tetrakis (3-chloro-4-methyl-2, 10-dioxo-2*H*, 10*H*-pyrano[2,3-*f*] chromene-9-yl) porphyrin 6c.** Yield: 28%; m.p. >300°C; ^{1}H NMR (DMSO-d_6): δ 9.12 (s, 8H, pyrrole C-H), 8.03 (s, 4H, chromene C-2H), 7.25 (d, 4H, C-10H, J = 9 Hz), 6.90 (d, 4H, C-9H, J = 9 Hz), 3.20(s, 12 H, CH_3), -2.35 (s, 2H, porphyrin N-H); FABMS: m/z, 1353 (M^+); Anal. Calcd for $C_{72}H_{34}Cl_4N_4O_{16}$ (%): C, 63.90; H, 2.51; N, 4.14. Found (%): C, 63.94; H, 2.55; N, 4.19.

***meso*-Tetrakis (3-bromo-4-methyl-2, 10-dioxo-2*H*, 10*H*-pyrano[2,3-*f*] chromene-9-yl) porphyrin 6d.** Yield: 26%; m.p. >300°C; ^{1}H NMR (DMSO-d_6 & $CDCl_3$): δ 9.12 (s, 8H, pyrrole C-H), 8.05 (s, 4H, chromene C-2H), 7.35 (d, 4H, C-10H, J = 9 Hz), 6.90 (d, 4H, C-9H, J = 9 Hz), 3.12 (s, 12 H, CH_3), –2.31 (s, 2H, porphyrin N-H); FABMS: m/z 1531 (M^+); Anal. Calcd for $C_{72}H_{34}Br_4N_4O_{16}$ (%): C, 56.47; H, 2.22; N, 3.66. Found (%): C, 56.45; H, 2.26; N, 3.68.

***meso*-Tetrakis(2,10-dioxo-4-phenyl-2*H*,10*H*-pyrano[2,3-*f*] chromene-9-yl) porphyrin 6e.** Yield: 25%; m.p. >300°C; ^{1}H NMR ($CDCl_3$ & DMSO-d_6), δ 9.12 (s, 8H, pyrrole C-H), 8.01 (s, 4H, chromene C-2H), 7.29-7.35 (m, 20-H, Ar-H), 7.25 (d, 4H, C-10H, J = 9 Hz), 6.90 (d, 4H, C-9H, J = 9 Hz), 6.40 (s, 4H, C-7 of coumarin), –2.53 (s, 2H, porphyrin N-H); FABMS: m/z, 1463 (M^+); Anal. Calcd for $C_{92}H_{46}N_4O_{16}$ (%): C, 75.51; H, 3.14; N, 3.83. Found (%): C, 75.55; H, 3.11; N, 3.87.

***meso*-Tetrakis (4-chloro-2,10-dioxo-4-phenyl -2*H*, 10*H*-pyrano[2,3-*f*] chromene-9-yl) porphyrin 6f.** Yield: 27%; m.p. >300°C; ^{1}H NMR (DMSO-d_6): δ 9.12 (s, 8H, pyrrole C-H), 8.01 (s, 4H, chromene C-2H), 7.15 (d, 4H, C-10H, J = 9 Hz), 6.50 (d, 4H, C-9H, J = 9 Hz), 6.48 (s, 4H, C-7 of coumarin), –2.51(s, 2H, porphyrin N-H); FABMS: m/z, 1297 (M^+); Anal. Calcd for $C_{68}H_{26}Cl_4N_4O_{16}$ (%): C, 62.96; H, 2.00; N, 4.32. Found (%): C, 62.90; H, 2.04; N, 4.36.



Compd	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
R_1	H	CH_3	CH_3	CH_3	Ph	Cl
R_2	H	H	Cl	Br	H	H

Scheme 1

Acknowledgements

The authors greatly acknowledge the UGC (F.12-18/2003 (SR) Dt30\3\2003) for the sanction of major research project.

References

1. (a) Lindsey, J.S.; Montanari, F.; Casella, L., Eds. *Metalloporphyrins-Catalyzed Oxidations*, Academic: The Netherlands, 1994.(b) Milgron, L.R. *The Colours of Life*, Oxford University Press: New York, 1997.
2. Lindsey, J. S.; Mauzerall, D. C.; Linschitz, H. *J. Am. Chem. Soc*. **1983**, *105*, 6528.
3. Bogatskii, A. V.; Zhilina, Z. I. *Russ. Chem. Rev*. **1982**, *51*, 592.
4. Tashiro, K.; Aida, T.; Zheng, J. Y.; Kinbara, K.; Saigo, K.; Sakamotao, S.;
5. Yamaguchi, K. *J. Am. Chem. Soc*. **1999**, *121*, 7090.
6. Petritsch, K.; Friend, R. II.; Lux, A.; Rozenderg, G.; Moraui, S. C.; Ilolmes, A. B. *Synth. Met*. **1999**, *102*, 1776.
7. Shanmugathasan, S.; Edwards, C. B.; Boyle, R. W. *Tetrahedron* **2000**, *56*, 1025.
8. Nyman, E. S.; Hynninen, P. H. *J. Photochem. Photobiol. B Biology* **2004**, *73*, 1.

9. Narasimha Reddy, P.; Thirupathi Reddy, Y.; Amaravathi, M.; Kanakalingeswara Rao M.; Rajitha, B. *Heterocyclic Communications* **2004**, *10*, 4.
10. Kostova, I.; *Curr. Med. Chem-Anti-Cancer Agents* **2005**, *5***,** 29.
11. Kostova, I.; Raleva, S.; Genova, P. Argirova, R. *Bioinorganic Chemistry and Applications* **2006,** *2006*, 68274.
12. Budzisz, E.; Brzezinska, E.; Krajwska, U.; Rozalski, M. *Eur. J. Med. Chem*. **2003**,*38*, 597.
13. Winkelman, J.; Rubenfeld, S.; McAffe, J. *J. Nuclear Medicine* **1964**, *5*, 462.
14. Kubat, P.; Mosinger, J. *J. Photochem. Photobiol*. A. **1996**, *96* (1), 93.
15. Zenkerich, E.; Sagun, E.; Knyukshtol, V.; Andrei, M.; Olga, E.; Kaymond, B.; Songa, S. P. *J. Photochem. Photobiol* B. **1996**, *33*, (2), 171.
16. Kawamura-Konishi, Y.; Neya, S.; Norika, F.; Horuo, S.; *Biochem. Biophys, Res Commun.* **1996**, *225*, (2), 537.
17. Tazaki, Y.; Furune, H. *Cancer Chemotherapy Reports*. **1961**, *13*, 41
18. Lacova, M.; Loos, D.; Furdik, M.: Matulova, M. Synthesis, Reaction and Theoretical Study of 3-Formylchromones, *First International Electronic Conference on Synthetic Organic Chemistry* (ECSOC-I), September 1-30 (1997), Switzerland. Lindsey, J. S.; Schreiman, I.C.; Hsu, H. C.; Kearney, P. C.; Marguerettaz, A. M. *J. Org. Chem.* **1987**, *52*, 827.
19. Folkman, J.; Engl, N. *J. Med. Chem*. **1971,** *285*, 1181.
20. Gasti, G.; Hermann, T.; Steurer, M.; Zmijia, J.; Gunsilius, E.; Unger, C.; Kraft, A.*Oncology*. **1997,** *54***,** 177.
21. Nguyen-Hai, N.; Yong, K.; Young-Jae, Y.; Hwanmook, K.; Byung-Zun, A. *J. Med. Chem*. **2003**, *38***,** 179.