Bioorganic & Medicinal Chemistry Letters 18 (2008) 5571–5575

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of 4-aryl-2-oxo-2*H*-chromenes as a new series of apoptosis inducers using a cell- and caspase-based high-throughput screening assay

William Kemnitzer [a], Songchun Jiang [a], Hong Zhang [a], Shailaja Kasibhatla [a], Candace Crogan-Grundy [a], Charles Blais [b], Giorgio Attardo [b], Real Denis [b], Serge Lamothe [b], Henriette Gourdeau [b], Ben Tseng [a], John Drewe [a], Sui Xiong Cai [a,*]

[a] *Epicept Corporation, Inc. 6650 Nancy Ridge Drive, San Diego, CA 92121, USA*
[b] *Shire Biochem Inc., 275 Armand-Frappier Blvd., Laval, Que., Canada H7V 4A7*

ARTICLE INFO

Article history:
Received 16 July 2008
Revised 26 August 2008
Accepted 2 September 2008
Available online 6 September 2008

Keywords:
Apoptosis inducers
Anticancer agents

ABSTRACT

As a continuation of our efforts to discover and develop the apoptosis inducing 4-aryl-4*H*-chromenes as potential anticancer agents, we explored the removal of the chiral center at the 4-position and prepared a series of 4-aryl-2-oxo-2*H*-chromenes. It was found that, in general, removal of the chiral center and replacement of the 2-amino group with a 2-oxo group were tolerated and 4-aryl-2-oxo-2*H*-chromenes exhibited SAR similar to 4-aryl-2-amino-4*H*-chromenes. The 4-aryl-2-oxo-2*H*-chromenes with a *N*-methyl pyrrole fused at the 7,8-positions were highly active with compound **2a** having an EC_{50} value of 13 nM in T47D cells. It was found that an OMe group was preferred at the 7-positon. 7-NMe_2, 7-NH_2, 7-Cl and 7,8 fused pyrido analogs all had low potency. These 4-aryl-2-oxo-2*H*-chromenes are a series of potent apoptosis inducers with potential advantage over the 4-aryl-2-amino-4*H*-chromenes series via elimination of the chiral center at the 4-position.

Apoptosis, or programmed cell death, is the process for eliminating excessive cells that may threaten tissue homeostasis and organ morphogenesis. Unlike necrotic cell death, apoptosis involves a series of precisely regulated events, including condensation of the nucleoplasm and cytoplasm, chromosomal DNA fragmentation, and the formation of apoptotic bodies, which are rapidly recognized and eliminated by phagocytes.[1] The mechanism of apoptosis has been intensively studied over the past decade and two pathways have been identified, both involving a cascade of initiator and effector caspases.[2] Caspase-3 is the main executioner of apoptosis by cleaving multiple protein substrates in cells, leading to irreversible cell death.[3] Defects in the apoptotic machinery is one of the mainstays of cancers that leads to uncontrollable tumor cell growth, as well as tumor resistance to chemotherapeutic treatment.[4] Not surprisingly, many of the clinically useful cytotoxic agents are known to induce apoptosis in cancer cells[5] and intense efforts are ongoing to identify apoptosis inducers.[1,6] We have been interested in the discovery and development of apoptosis inducers as potential anticancer agents,[7] and have therefore developed a cell-based high throughput-screening technology for apoptosis inducers using our proprietary fluorescent caspase-3 substrate.[8]

We have reported the discovery and structure–activity relationships (SAR) of 4-aryl-4*H*-chromenes as a new series of potent apoptosis inducers using our cell- and caspase-based Anti-cancer Screening Apoptosis Program (ASAP) HTS assays. These compounds were found to be tubulin inhibitors binding at or near the binding site of colchicine, with vascular disrupting activity and high in-vivo activity in several anticancer animal models.[9,10] From our screening hit 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxy-phenyl)-4*H*-chromene (**1a**) (Chart 1), we have identified 2-amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-7-dimethylamino-4*H*-chromene (**1b**) as a lead compound.[11] Additional SAR studies showed that cyclization of the 7,8-positions into a ring structure led to potent compounds, such as 2-amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,7-dihydropyrrolo[2,3-*h*]chromene.[12] Introduction of a methyl group at the 7-position of the pyrrolo ring led to highly potent compounds such as

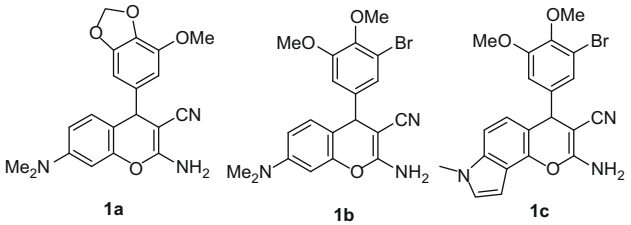


Chart 1.

* Corresponding author. Tel.: +1 858 202 4006; fax: +1 858 202 4000.
E-mail address: scai@epicept.com (S.X. Cai).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.09.011

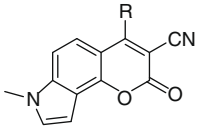
2-amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,7-dihydro-7-methylpyrrolo[2,3-*h*]chromene (**1c**)[13] with EC_{50} values in the 1–2 nM range (Table 1).

The 4-aryl-2-amino-3-cyano-4*H*-chromenes were prepared in general via a one-pot reaction of substituted arylaldehyde and aryl alcohol with malononitrile[11] and isolated as racemic mixtures. To simplify the structure, we explored the removal of the chiral center at the 4-position. In addition, we have found that the 2-amino group can be replaced by other functional groups.[14] We therefore designed and synthesized a series of 4-aryl-2-oxo-2*H*-chromenes via removing the chiral center at the 4-position and replacing the 2-amino group with a 2-oxo group. The 4-aryl-2-oxo-2*H*-chromenes also were chosen because it is known that coumarin derivatives are an important family of active compounds with a wide range of pharmacological properties.[15] Herein we wish to report the synthesis and SAR of 4-aryl-2-oxo-2*H*-chromenes as a novel series of potent apoptosis inducers.

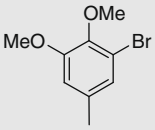
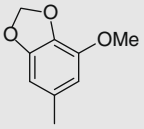
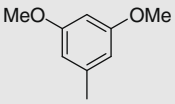
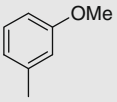
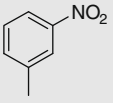
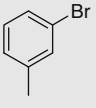
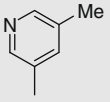
Compounds **2a**–**2g** were prepared from the previously reported 2-amino-3-cyano-7-methyl-4*H*-pyrrolo[2,3-*h*]chromenes (**5a**–**g**)[13] in two steps (Scheme 1). Oxidation of **5a**–**5g** using DDQ at room temperature rapidly converted the 2-amino group to the 2-imino group together with a double bond at the 3,4-postitions to produce compounds **6a**–**6g** in good yield. The 2-imino group was then hydrolyzed under acidic conditions to give the 2-aryl-2-oxo-2*H*-chromenes **2a**–**2g** in good yield.[16] Compound **3e** was prepared similarly in two steps starting from 2-amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-pyrido[3,4-*h*]chromene.[12]

Compounds **3a**, **4a** and **4b** were prepared using a different method (Scheme 2). The 2-cyano-acrylic acid ethyl ester intermediates (**7a**–**7c**) were synthesized first from the appropriate aryl

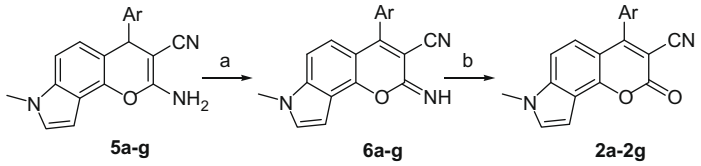
Table 1
SAR of the 4-aryl group of 4-aryl-3-cyano-7-methyl-2-oxo-2*H*-pyrrolo[2,3-*h*]chromenes in caspase activation assay



2a-2g

Entry	R	EC_{50} (μM)[a]		
		T47D	HCT116	SNU398
1a	NA	0.073 ± 0.006[b]	ND[d]	ND
1b	NA	0.019 ± 0.004[b]	0.009 ± 0.0004[b]	0.019 ± 0.0007[b]
1c	NA	0.002 ± 0.0003[c]	0.003 ± 0.0004[c]	0.002 ± 0.0004[c]
2a		0.013 ± 0.001	0.010 ± 0.001	0.006 ± 0.0007
2b		0.027 ± 0.003	0.027 ± 0.002	0.017 ± 0.0002
2c		0.024 ± 0.003	0.020 ± 0.002	0.013 ± 0.001
2d		0.043 ± 0.005	0.020 ± 0.002	0.017 ± 0.0002
2e		0.043 ± 0.005	0.013 ± 0.001	0.0095 ± 0.0002
2f		0.024 ± 0.005	0.014 ± 0.001	0.0096 ± 0.0006
2g		0.019 ± 0.002	0.016 ± 0.001	0.014 ± 0.002

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).
[b] Data from Ref. 11.
[c] Data from Ref. 12.
[d] ND, not determined.



Scheme 1. Conditions: (a) DDQ, CH_2Cl_2, molecular sieves, rt; (b) 10% HCl/H_2O, rt, or 10% HCl/H_2O, MeOH, rt.

aldehydes with ethyl cyanoacetate similar to published procedures for benzylidene formation.[11] Reaction of **7a**–**7c** with 3-methoxyphenol and sodium hydride in either THF or toluene at reflux afforded the compounds **3a**, **4a** and **4b**. In a similar manner compounds **3b** and **3c** were prepared from the 3-(3-bromo-4,5-dimethoxyphenyl)-2-cyano-acrylic acid ethyl ester intermediate, followed by reaction with the appropriate aryl alcohol and ^{t}BuOK in THF. Compound **3d** was prepared in two steps (Scheme 3) from the previously prepared 4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-2,7-diamino-4*H*-chromene (**8**).[17] Reaction of **8** with DDQ resulted in the imino-2*H*-chromene (**9**), which was followed by reaction with *t*-butylnitrite and copper (II) chloride to give **3d** in a yield of 19% for the two steps.

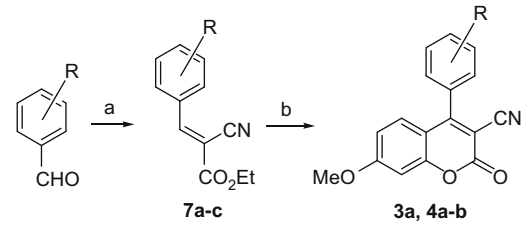
The apoptosis inducing activity of these 4-aryl-2-oxo-2*H*-chromenes was measured by our proprietary cell- and caspase-based HTS assay[18] in human breast cancer cells T47D, human colon cancer cells HCT116 and hepatocellular carcinoma cancer cells SNU398. The results are summarized in Tables 1 and 2. Starting from our current lead compound **1c** we explored the SAR of the 4-aryl group of 4-aryl-2-oxo-2*H*-chromenes. Changing the 2-position amino group to an oxo group and removing the chiral center at the 4-position by incorporating a double bond at the 3,4-positions was found to be well tolerated (Table 1). Compound **2a**, with a 3-bromo-4,5-dimethoxyphenyl group at the 4-position, was very potent, having an EC_{50} value of 13 nM in T47D cells, 10 nM in HCT116 cells, and 6 nM in SNU398 cells (Table 1). Compound **2a** was only sixfold less potent than **1c** in T47D cells. The 3,4-methylenedioxo-5-methoxyphenyl analogue (**2b**) also was highly active and was twofold less potent than **2a** in T47D cells. The 3,5-dimethoxyphenyl analogue (**2c**) and 3-methoxyphenyl analogue (**2d**) were also highly potent and were only 2- to 3-fold less potent than **2a**. We also explored other mono-substitutions at the 3-position, such as a nitro (**2e**) and a bromo (**2f**). These compounds were found to be highly active, and were only 2- to 3-fold less potent than **2a**. Similar to the reported SAR for 7-methyl-4*H*-pyrrolo[2,3-*h*]chromenes, the 5-substituted 3-pyridyl analogue (**2g**) was also highly potent with an EC_{50} value approaching that of **2a**.

We then explored the SAR of the 7-position of the 4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-2-oxo-2*H*-chromenes (Table 2). Substitution at the 7-position with a methoxy group (**3a**) was found to be highly active, with an EC_{50} value of 41 nM in T47D cells. Surprisingly, substitution at the 7-position with either an amino group (**3b**) or a dimethylamino group (**3c**) resulted in a significant reduction in activity. This is in stark contrast to the SAR of 4-aryl-4*H*-chromenes where a 7-NH_2 substitution maintains potency and a 7-NMe_2 group, as in lead compound **1b**, enhances potency.[13] Compound **3b** is >40-fold less active than **1b**, while **3c** is >14-fold less active than **1b**. The dramatic difference in potency of **3c** when compared to **1b** indicates that the 7-position SAR of 4-aryl-2-oxo-2*H*-chromenes is different from that of 4-aryl-4*H*-chromenes. Further support for the change in SAR can be seen in the 7-Cl analogue (**3d**), which is >500-fold and >350-fold less potent than **2a** and **1b**, respectively. Compound **3e**, with a six-membered fused ring at the 7,8-positions, also resulted in a large reduction of potency.

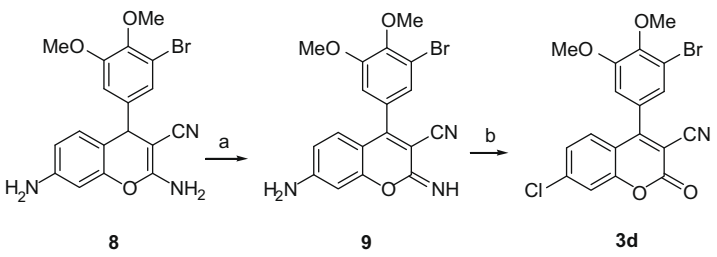
We also explored substitution at the 4-phenyl group with a methoxy group at the 7-position (Table 2). Changing from the trisubstituted 4-(3-bromo-4,5-diemthylphenyl) (**3a**) to the 3,5-dimethoxyphenyl (**4a**) resulted in a twofold increase in potency compared to **3a**. The 3-methoxy group analogue (**4b**) also was highly potent, with similar potency to that of **4a**. It is interesting that the 3,5-disubstituted analogue **4a** was more potent than the 3,4,5-trisubstituted analogue **3a**, indicating a subtle change in the 4-phenyl group SAR from 4-aryl-4*H*-chromenes to 4-aryl-2-oxo-2*H*-chromenes.

The activities of the 4-aryl-2-oxo-2*H*-chromenes toward the colon cancer cells HCT116 and the hepatocellular carcinoma cancer cells SNU398 roughly paralleled activity toward T47D cells. In general, HCT116 was slightly less sensitive to these compounds than T47D cells in this assay as indicated by the slightly higher EC_{50} values. In contrast, SNU398 was slightly more sensitive to these compounds than T47D cells in this assay as indicated by the lower EC_{50} values.

Selected compounds were also tested by the traditional cell growth inhibition assay (GI_{50}) to confirm that the active compounds can inhibit tumor cell growth. The growth inhibition assays

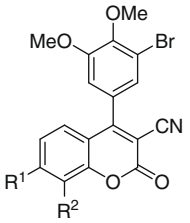


Scheme 2. Conditions: (a) ethyl acetoacetate, EtOH, piperidine, rt; (b) 3-methoxyphenol, NaH, THF or toluene, reflux.

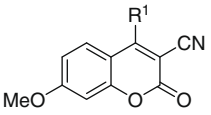


Scheme 3. Conditions: (a) DDQ, CH_2Cl_2, molecular sieves, rt; (b) *t*-BuONO, THF, $CuCl_2$, 0° C.

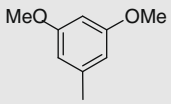
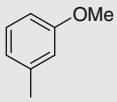
Table 2
SAR of 7,8-positions and 4-aryl group of 4-aryl-2-oxo-2*H*-chromenes in caspase activation assay



3a-3e



4a-4b

Entry	R^1	R^2	EC_{50} (μM)[a]		
			T47D	HCT116	SNU398
3a	OMe	H	0.042 ± 0.003	0.041 ± 0.003	0.026 ± 0.002
3b	NH_2	H	0.82 ± 0.09	0.99 ± 0.16	0.49 ± 0.03
3c	NMe_2	H	0.28 ± 0.05	0.40 ± 0.06	0.26 ± 0.07
3d	Cl	H	6.9 ± 0.5	9.4 ± 0.9	5.5 ± 0.2
3e	CH=NCH=CH	CH=NCH=CH	3.0 ± 0.6	4.8 ± 0.9	2.5 ± 0.5
4a		NA[b]	0.017 ± 0.0001	0.018 ± 0.0001	0.015 ± 0.0002
4b		NA	0.053 ± 0.003	0.059 ± 0.0005	0.029 ± 0.0009

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).
[b] NA, not applied.

in T47D, HCT116 and SNU398 cells were run in a 96-well microtiter plate as described previously.[18] The GI_{50} are summarized in Table 3. Compound **2a** was found to be highly active with GI_{50} values of 0.3–0.9 nM in the three cell lines tested. Similar to what was observed in the caspase activation assay, compound **2b** was found to be less active than **2a** in the GI_{50} assay. Compounds **3b** and **3c**, which had low activity in the caspase activation assay, were also found to have low activity in the GI_{50} assay.

In conclusion, we have explored the removal of the 4-position chiral center in the 4-aryl-4*H*-chromenes and synthesized a series of 4-aryl-2-oxo-2*H*-chromenes having modifications at the 4-aryl, 7- and 8-positions. Converting the 2-amino group to a 2-oxo group and removing the chiral center at the 4-position with a 3,4 double bond led to a series of highly potent apoptosis inducers. It was found that the 3-cyano-2-oxo-7-methyl-2*H*-pyrrolo[2,3-*h*]chromenes with 3,4,5-trisubstituted, 3,5-disubstituted and 3-monosubstituted phenyl group at the 4-position all are highly active. Similar to what had been found for 2-amino-3-cyano-7-methyl-4*H*-pyrrolo[2,3-*h*]chromenes, **2a** and **2g** were found to be the most potent analogues in the caspase activation assay with EC_{50} values of 13 and 19 nM in T47D cells, respectively. Compound **2a** also was highly active in the growth inhibition assay with a GI_{50} value of 0.7 nM in T47D cells. Differences in the SAR did arise however, when we looked at substitutions at the 7-position. It was found that a 7-OMe group was relatively well tolerated, while 7-NH_2, 7-NMe_2 and 7-Cl all resulted in significant reduction of potency. With an OMe group at the 7-position, modifications of the 4-phenyl group were well tolerated and led to highly active analogues. The high activity of 4-aryl-2-oxo-2*H*-chromenes demonstrate that a chiral center at the 4-position of 4-aryl-4*H*-chromenes is not essential for inducing apoptosis and provides an alternative series of potent apoptosis inducers with a simpler structure compared to the 4-aryl-4*H*-chromenes.

Table 3
Inhibition of Cell Growth of 4-Aryl-2-oxo-2*H*-chromenes

Entry	GI_{50} (μM)[a]		
	T47D	HCT116	SNU398
2a	0.0007 ± 0.0002	0.0009 ± 0.0004	0.0003 ± 0.0001
2b	0.025 ± 0.005	0.026 ± 0.006	0.0079 ± 0.0027
3b	0.63 ± 0.08	0.63 ± 0.13	0.44 ± 0.02
3c	0.62 ± 0.18	0.46 ± 0.11	0.15 ± 0.01

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).

References and notes

1. (a) Jana, S.; Paliwal, J. *Curr. Med. Chem.* **2007**, *14*, 2369; (b) Reed, J. C. *Nat. Clin. Pract. Oncol.* **2006**, *3*, 388; (c) Mehlen, P.; Puisieux, A. *Nat. Rev. Cancer* **2006**, *6*, 449; (d) Reed, J. C. *Nat. Rev. Drug Discov.* **2002**, *1*, 111; (e) Reed, J. C.; Tomaselli, K. J. *Curr. Opin. Biotechnol.* **2000**, *11*, 586.
2. Zimmermann, K. C.; Green, D. R. *J. Allergy Clin. Immunol.* **2001**, *108*, S99.
3. (a) Salvesen, G. S. *Essays Biochem.* **2002**, *38*, 9; (b) Thornberry, N. A. *Chem. Biol.* **1998**, *5*, R97.
4. (a) Hunter, A. M.; LaCasse, E. C.; Korneluk, R. G. *Apoptosis* **2007**, *12*, 1543; (b) Evan, G. I.; Vousden, K. H. *Nature* **2001**, *411*, 342; (c) Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57; (d) Reed, J. C. *J. Clin. Oncol.* **1999**, *17*, 2941.
5. (a) Herr, I.; Debatin, K. M. *Blood* **2001**, *98*, 2603; (b) Rich, T.; Allen, R. L.; Wyllie, A. H. *Nature* **2000**, *407*, 777.
6. (a) Reed, J. C. *Blood* **2005**, *106*, 408; (b) Reed, J. C. *Cancer Cell* **2003**, *3*, 17.
7. Cai, S. X.; Drewe, J.; Kasibhatla, S. *Curr. Med. Chem.* **2006**, *13*, 2627.
8. Cai, S. X.; Zhang, H. Z.; Guastella, J.; Drewe, J.; Yang, W.; Weber, E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 39.
9. Kasibhatla, S.; Gourdeau, H.; Meerovitch, K.; Drewe, J.; Reddy, S.; Qiu, L.; Zhang, H.; Bergeron, F.; Bouffard, D.; Yang, Q.; Herich, J.; Lamothe, S.; Cai, S. X.; Tseng, B. *Mol. Cancer Ther.* **2004**, *3*, 1365.
10. Gourdeau, H.; Leblond, L.; Hamelin, B.; Desputeau, C.; Dong, K.; Kianicka, I.; Custeau, D.; Bourdeau, C.; Geerts, L.; Cai, S. X.; Drewe, J.; Labrecque, D.; Kasibhatla, S.; Tseng, B. *Mol. Cancer Ther.* **2004**, *3*, 1375.
11. Kemnitzer, W.; Kasibhatla, S.; Jiang, S.; Zhang, H.; Wang, Y.; Zhao, J.; Jia, S.; Herich, J.; Labreque, D.; Storer, R.; Meerovitch, K.; Bouffard, D.; Rej, R.; Denis,

R.; Blais, C.; Lamothe, S.; Attardo, G.; Gourdeau, H.; Tseng, B.; Drewe, J.; Cai, S. X. *J. Med. Chem.* **2004**, *47*, 6299.
12. Kemnitzer, W.; Drewe, J.; Jiang, S.; Zhang, H.; Zhao, J.; Crogan-Grundy, C.; Xu, L.; Lamothe, S.; Gourdeau, H.; Denis, R.; Tseng, B.; Kasibhatla, S.; Cai, S. X. *J. Med. Chem.* **2007**, *50*, 2858.
13. Kemnitzer, W.; Drewe, J.; Jiang, S.; Zhang, H.; Crogan-Grundy, C.; Labreque, D.; Bebenick, M.; Attardo, G.; Denis, R.; Lamothe, S.; Gourdeau, H.; Tseng, B.; Kasibhatla, S.; Cai, S. X. *J. Med. Chem.* **2008**, *51*, 417.
14. Kemnitzer, W.; Jiang, S.; Wang, Y.; Kasibhatla, S.; Crogan-Grundy, C.; Bubenick, M.; Labreque, D.; Denis, R.; Lamothe, S.; Attardo, G.; Gourdeau, H.; Tseng, B.; Drewe, J.; Cai, S. X. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 603.
15. Jacquot, Y.; Laios, I.; Cleeren, A.; Nonclercq, D.; Bermont, L.; Refouvelet, B.; Boubekeur, K.; Xicluna, A.; Leclercq, G.; Laurent, G. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 2269.
16. Experimental procedure for the synthesis of 3-cyano-7-methyl-4-(3-nitrophenyl)-2-oxo-2*H*-pyrrolo[2,3-*h*]chromene (**2e**). (A) 3-Cyano-2-imino-7-methyl-4-(3-nitrophenyl)-2*H*-pyrrolo[2,3-*h*]chromene (**6e**). A mixture of 2-amino-3-cyano-7-methyl-4-(3-nitrophenyl)-4*H*-pyrrolo[2,3-*h*]chromene (0.031 g, 0.090 mmol), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.023 g, 0.10 mmol), molecular sieves (4 Å, 0.100 g) in dichloromethane (5 mL) was stirred at room temperature for approximately 2 h. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (10 mL), and dried over $MgSO_4$, filtered and concentrated to yield 0.031 g (100 %) of **6e** as a yellow solid; ^{1}H NMR (DMSO-d_6) 8.75 (s, 1H), 8.49–8.44 (m, 2H), 8.04 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.94 (t, *J* = 8.1 Hz, 1H), 7.51 (d, *J* = 3.0 Hz, 1H), 7.29 (d, *J* = 9.0 Hz, 1H), 6.73–6.68 (m, 2H), 3.82 (s, 3H). (B) 3-cyano-7-methyl-4-(3-nitrophenyl)-2-oxo-2*H*-pyrrolo[2,3-*h*]chromene (**2e**). A solution of 3-cyano-2-imino-7-methyl-4-(3-nitro-phenyl)-2*H*-pyrrolo[2,3-*h*]chromene (**6e**, 0.031 g, 0.090 mmol) in 10% HCl (3 mL) and methanol (5 mL) was stirred at room temperature for 7 h. The reaction mixture was neutralized with 10% NaOH (1.5 mL). The yellow precipitate was collected by vacuum filtration, washed with water, and dried *in vacuo* to give 0.024 g (77%) of **2e** as yellow a solid; ^{1}H NMR (acetone-d_6) 8.58–8.52 (m, 2H), 8.11 (dt, *J* = 7.2, 0.9 Hz, 1H), 8.03 (dt, *J* = 7.8, 0.9 Hz, 1H), 7.53 (d, *J* = 3.3 Hz, 1H), 7.49 (dd, *J* = 8.7, 0.6 Hz, 1H), 7.05 (d, *J* = 8.7 Hz, 1H), 6.94 (dd, *J* = 3.3, 0.9 Hz, 1H), 3.97 (s, 3H).
17. Kemnitzer, W.; Kasibhatla, S.; Jiang, S.; Zhang, H.; Zhao, J.; Jia, S.; Xu, L.; Crogan-Grundy, C.; Denis, R.; Barriault, N.; Vaillancourt, L.; Charron, S.; Dodd, J.; Attardo, G.; Labreque, D.; Lamothe, S.; Gourdeau, H.; Tseng, B.; Drewe, J.; Cai, S. X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4745.
18. Cai, S. X.; Nguyen, B.; Jia, S.; Guastella, J.; Reddy, S. J.; Tseng, B.; Drewe, J.; Kasibhatla, S. *J. Med. Chem.* **2003**, *46*, 2474.