Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 49 (2008) 3276–3278
Tetrahedron Letters

The synthesis of new, selected analogues of the pro-apoptotic and anticancer molecule HA 14-1

Danielle Grée [a], Samuel Vorin [a], Vijay L. Manthati [a], Frédéric Caijo [a], Guillaume Viault [a], Florence Manero [b,c], Philippe Juin [b,c], René Grée [a,*]

[a] Université de Rennes1, Chimie et Photonique Moléculaires, CNRS UMR 6510, Avenue du Général Leclerc, 35042 Rennes Cedex, France
[b] U 892 INSERM, Département de Recherche en Cancérologie, 9 quai Moncousu, F-44035 Nantes Cedex 01, France
[c] Faculté de Médecine, Université de Nantes, 9 quai Moncousu, F-44035 Nantes Cedex 01, France

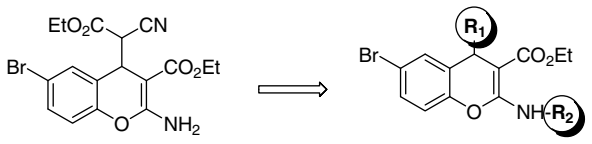
Received 4 February 2008; revised 5 March 2008; accepted 17 March 2008
Available online 19 March 2008

Abstract

A new and versatile strategy has been developed towards HA 14-1 analogues, selectively modified on position 4 and/or on the primary amine function. An important aspect was the appropriate selection of the phenol protective group in the 5-bromosalicylaldehyde, allowing the isolation of the key intermediate the 2*H*-benzopyrane-2-imine **2′**.

Keywords: HA 14-1; Benzopyrane; Anticancer; Bcl-2

Apoptosis is a cell-death process that functions as a barrier to cancer initiation, and on which the efficiency of many currently used anticancer therapies relies.[1,2] It is critically regulated by the Bcl-2 family of proteins. Overexpression of anti-apoptotic Bcl-2 members in cancer cells is understood to promote their survival in the face of oncogene-induced pro-apoptotic signals, and their resistance against therapy.[3,4] In this context, the discovery of small molecules which antagonise the aberrant survival conferred to cancer cells by Bcl-2 homologues appears as an attractive strategy in the area of cancer research. Over a dozen of Bcl-2 inhibitory molecules have been reported in the last 10 years and some of them are already in preclinical and clinical studies.[3] The 4*H*-chromene derivative HA 14-1 has been discovered by using computer screening strategies.[5] This compound has demonstrated promising pro-apoptotic activity against cancer cells both in vitro and in vivo, as a single agent or in combination with chemo- or radiotherapy.[6–8] However, many questions remain open concerning the mechanism of action of this molecule.[8] This is due, at least in part, to the very limited structure–activity relationship available in these series. To the best of our knowledge, only analogues modified on the aromatic ring have been reported.[9] The biological tests have shown that the bromine in position 6 could be replaced by some alkyl or aryl groups.[9] As part of our studies in this area, it appeared important to us to develop synthetic strategies allowing the preparation of new analogues of HA 14-1. In particular, modulations on the 'upper part' of the molecule (the cyanoester substituent) and the free amino group appeared very attractive (Fig. 1). The purpose of this Letter



HA 14-1 New Analogues

Fig. 1. HA 14-1 and designed analogues.

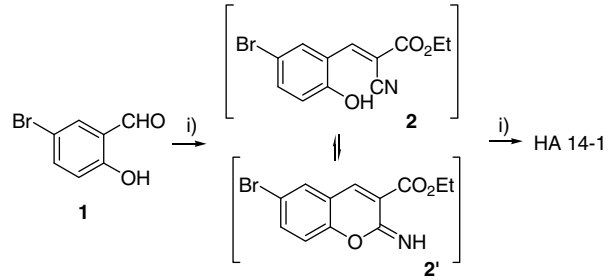
* Corresponding author. Tel.: +33 (0)2 23 23 57 15; fax: +33 (0)2 23 23 65 98.
E-mail address: rene.gree@univ-rennes1.fr (R. Grée).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.03.070

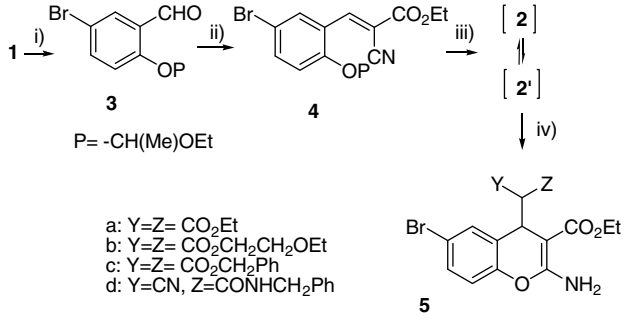
is to present the strategies introduced towards this goal and the preparation of a few new, selected, HA 14-1 analogues.

The synthesis of HA 14-1 is easily performed in 86% yield by the condensation of 5-bromosalicylaldehyde **1** with two molecules of cyanoacetate in the presence of molecular sieves.[10] Various types of catalysts and conditions were used to prepare 4*H*-chromenes of this type.[11] The synthesis of HA 14-1 is a consecutive two-step process: Cope–Knoevenagel condensation leading to intermediate **2**, followed by Michael addition of a second molecule of cyanoacetate on the cyclic isomer **2′** affording the final product (Scheme 1). It has been reported, for a few salicylaldehyde derivatives containing a second phenolic group, that it was possible to stop at the first stage and isolate the corresponding 2*H*-benzopyrane-2-imines intermediates.[12] This is not the case when starting from bromo derivative **1**. Whatever the reaction conditions, the condensation of **1** with 1 equiv of cyanoacetate afforded a 1:1 mixture of starting material **1** and the HA 14-1 molecule. In that case, the second step appears to occur faster than the first one.

Therefore, in order to prepare new HA 14-1 analogues, it was necessary to develop an alternative strategy with the temporary protection of the phenol group. For that purpose, the use of ethylvinylether was found to be the most suitable. The synthesis of a first series of analogues is described in Scheme 2.



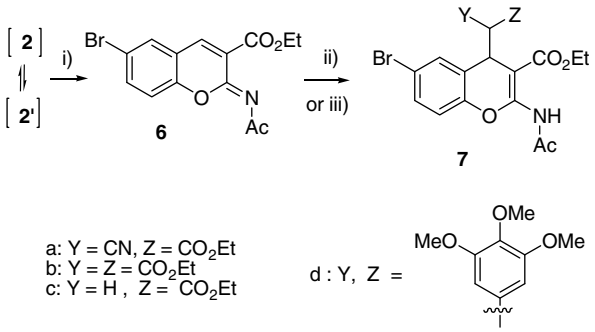
Scheme 1. Reagents and conditions: (i) ethylcyanoacetate, EtOH, 3 Å Mol. Sieves, rt, overnight.



Scheme 2. Reagents and conditions: (i) ethylvinylether (6.3 equiv), camphorsulfonic acid (0.2 equiv), THF, −10 °C, 4 h then Et_3N and K_2CO_3 (90%); (ii) ethylcyanoacetate (1.1 equiv), EtOH, 3 Å mol. Sieves, rt, overnight (65%); (iii) Amberlyst 15, 4 Å mol. Sieves, CH_2Cl_2, rt, 5 h (80%); (iv) general procedure: malonate or cyanoacetamide (1.1 equiv), EtOH, 4 Å mol. Sieves, piperidine (cat), rt, 5 h, **5a** (70%), **5b** (79%), **5c** (81%), **5d** (73%).

The protection of bromophenol **1** with ethylvinylether afforded salicylaldehyde derivative **3** in 90% yield. Condensation with 1 equiv of cyanoacetate gave the desired electrophilic alkene **4** in 65% yield. Deprotection using Amberlyst 15 in CH_2Cl_2 at rt afforded the desired key intermediate (**2**–**2′**) isolated in 80% yield as a pale yellow crystalline powder.[13] The heterocyclisation occurred only on the nitrile group. Extensive NMR studies indicated that this compound was present as an equilibrium mixture of the open form (**2**) and the closed 2*H*-benzopyrane-2-imine form (**2′**).[14] This equilibrium is strongly shifted towards the close form in $CDCl_3$ and towards the open form in DMSO-d_6. In agreement with the literature data on similar compounds,[15] particularly representative are the chemical shifts at 102.2 ppm characteristic of the $C{=}(CN)CO_2Et$ carbon in **2** and at 131.2 pm for the benzopyrane-2-imine form **2′**. On this intermediate, various types of malonate derivatives reacted readily to afford the desired HA 14-1 analogues **5a**–**c** in good to excellent yields (70–81%).[16] In the same manner the cyanoacetamide anion, selected as a representative example of a dissymmetric system, afforded the desired adduct **5d** in 73% yield.

This strategy, taking advantage of an isolated intermediate **2′**, offers another very attractive possibility through the possible modulations on the primary amine group.[17] As a first step towards this goal, the reaction of **2′** with Ac_2O afforded the desired derivative **6** in 93% yield (Scheme 3).[18] *N*-Chloro and hydrazino derivatives of 2*H*-benzopyrane-2-imines are known,[12] but very few *N*-Ac representatives have been reported to date.[19] Reaction of **6** with the cyanoacetate anion afforded, smoothly and in 82% yield **7a**, the *N*-acetyl analogue of HA 14-1.[20] In a similar way, the diester derivative **7b** was also obtained in 76% yield by addition of the malonate anion. This intermediate **6** proved to have both a good stability and a good reactivity. In particular, it was possible to perform cuprate additions on **6**. For instance, reaction with the ethylacetate derived cuprate afforded compound **7c** in 55% yield.[21] On the other hand, the HA 14-1 analogue **7d** with an aromatic group in



Scheme 3. Reagents and conditions: (i) Ac_2O (75 equiv), 4 Å mol. Sieves, 40 °C, 12 h; (ii) malonate or cyanoacetamide (1.1 equiv), EtOH, 4 Å mol. sieves, piperidine (cat), rt, 5 h, **7a** (82%), **7b** (76%); (iii) EtOAc or 5-bromo-1,2,3-trimethoxybenzene (2.3 equiv) THF, −80 °C, LDA or BuLi (2.1 equiv), after 20 min CuI (1.1 equiv), and after 1 h at −35 °C addition of **6** at −80 °C for 2 h, **7c** (55%), **7d** (71%).

position 4 was also easily prepared in 71% yield by cuprate addition.[22]

Therefore, this *N*-acetylimine derivative **6** appears to be a very versatile intermediate towards HA 14-1 analogues modified both on the amino function and on the 'upper part' of this molecule.

In conclusion, this strategy involving the temporary protection of the phenol group proved to be very fruitful. It allowed an easy synthesis of the two key intermediates **2′** and **6.** These derivatives should allow the preparation of a large number of the HA 14-1 analogues modified on the two positions required for Structure–Activity Relationships. Such syntheses, as well as the biological evaluation of the corresponding analogues, are under active study in our groups.

Acknowledgements

We thank Laboratoires Servier for support of this research and we thank Drs. O. Geneste, B. Pfeiffer, L. Oliver and F. Vallette for fruitful discussions. We thank the Ligue contre le Cancer (Comité d'Ille et Vilaine) for fellowships to V.L.M and G.V. Furthermore, P.J. is supported by Région Pays de la Loire (CIMATH network), ARC (Ref. 3875) and Institut de Recherche Servier.

References and notes

1. Schmitt, C. A.; Lowe, S. W. *J. Pathol.* **1999**, *187*, 127–137.
2. Lowe, S. W.; Cepero, E.; Ewan, G. *Nature* **2004**, *432*, 307–315.
3. Juin, P.; Geneste, O.; Raimbaud, E.; Hickman, J. A. *Biochim. Biophys. Acta* **2004**, *1644*, 251–260 and references cited therein.
4. Letai, A. *Trends Mol. Med.* **2005**, *11*, 442–444.
5. (a) Wang, J.-L.; Liu, D.; Zhang, Z.-J.; Shan, S.; Han, X.; Srinivasula, S. M.; Croce, C. M.; Alnemri, E. S.; Huang, Z. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7124–7129; (b) Huang, Z.; Hui, D.; Han, X.; Zhang, Z.; Wang, J. PCT Int. Appl. WO 2000/004901; *Chem. Abstr. 132*, 117566.
6. (a) Manero, F.; Gautier, F.; Gallenne, T.; Cauquil, N.; Grée, D.; Cartron, P.-F.; Geneste, O.; Grée, R.; Vallette, F. M.; Juin, P. *Cancer Res.* **2006**, *66*, 2757–2764; (b) Fesik, S. W. *Nature Rev. Cancer* **2005**, *5*, 876–885 and references cited therein.
7. Oliver, L.; Mahe, B.; Grée, R.; Vallette, F. M.; Juin, P. *Leukemia Res.* **2007**, *31*, 859–863.
8. Doshi, J. M.; Tian, D.; Xing, C. *Mol. Pharm.* **2007**, *4*, 919–928 and references cited therein.
9. Doshi, J. M.; Tian, D.; Xing, C. *J. Med. Chem.* **2006**, *49*, 7731–7739.
10. Yu, N.; Aramini, J. M.; Germann, M. W.; Huang, Z. *Tetrahedron Lett.* **2000**, *41*, 6993–6996.
11. (a) Roudier, J. F.; Foucaud, A. *Synthesis* **1984**, 159–160; (b) Curini, M.; Epifano, F.; Chimichi, S.; Montanari, F.; Nocchetti, M.; Rosati, O. *Tetrahedron Lett.* **2005**, *46*, 3497–3499.
12. Volmajer, J.; Toplak, R.; Leban, I.; Le Marechal, A. M. *Tetrahedron* **2005**, *61*, 7012–7021.
13. The choice of ethylvinylether as a protective group proved to be important in that case since, after the deprotection step using Amberlyst, the key intermediate **2**–**2′** could be isolated *directly* as a powder and used for further synthesis. In fact, this compound **2**–**2′** could never be purified by chromatography technics affording only hydrolysis and/or decomposition products.
14. Main spectral data of **2** and **2′**, based on extensive 1D and 2D experiments: Compound **2**: ^{1}H NMR (DMSO-d_6, 500 MHz): $\delta = 8.49$ (s, 1H); 8.20 (d, $J = 2.5$ Hz, 1H); 7.61 (dd, $J = 8.9$ Hz, $J = 2.5$ Hz, 1H); 6.98 (d, $J = 8.9$ Hz, 1H); 4.31 (q, $J = 7.1$ Hz, 2H); 1.30 (t, $J = 7.1$ Hz, 3H) ppm. ^{13}C NMR (DMSO-d_6, 125.77 MHz): $\delta = 162.4$; 158.4; 147.9; 138.0; 130.5; 120.7; 119.2; 116.1; 110.9; 102.2; 62.9; 14.4 ppm. Compound **2′**: ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 9.68$ (br s, 1H); 8.02 (s, 1H); 7.58 (dd, $J = 8.7$ Hz, $J = 2.3$, 1H); 7.54 (d, $J = 2.3$ Hz, 1H); 7.09 (d, $J = 8.7$ Hz, 1H); 4.40 (q, $J = 7.2$ Hz, 2H); 1.42 (t, $J = 7.2$ Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125.77 MHz): $\delta = 163.6$; 158.5; 153.5; 140.5; 136.6; 131.2; 119.4; 118.1; 115.9; 62.1; 14.1 ppm. HRMS: M^+. ($C_{12}H_{10}NO_3{}^{79}Br$): calcd 294.9844; Found: 294.9851 (2 ppm).
15. O'Callaghan, C. N.; McMurry, T. B. H.; O'Brien, J. E. *J. Chem Soc., Perkin Trans. 2* **1998**, 425–429.
16. Main spectral data of **5a**: ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.50$ (d, $J = 2.4$ Hz, 1H); 7.31 (dd, $J = 8.6$ Hz, $J = 2.4$ Hz, 1H); 6.85 (d, $J = 8.6$ Hz, 1H); 6.41 (br s, $-NH_2$); 4.66 (d, $J = 3.8$ Hz, 1H); 4.23 (q, $J = 7.0$ Hz, 2H); 4.22 (q, $J = 7.0$, 2H); 4.01–3.91 (m, 2H); 3.75 (d, $J = 3.8$ Hz, 1H); 1.31 (t, $J = 7.0$ Hz, 3H); 1.28 (t, $J = 7.1$ Hz, 3H); 1.06 (t, $J = 7.1$ Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 168.5$; 168.5; 167.9; 161.8; 149.9; 131.9; 131.1; 124.9; 117.3; 116.5; 75.1; 61.6; 61.1; 58.8; 58.6; 34.3; 14.5; 14.4; 13.8 ppm. HRMS: calcd for $C_{12}H_{11}NO_3{}^{79}Br$: 295.99223; found: 295.9913 (6 ppm).
17. In our hands, all reactions designed to protect or modify the primary amino group, *and performed directly on HA 14-1*, yielded only decomposition products.
18. The crude product, obtained in 93% yield after removal under vacuum of the excess of reagents, is pure (by NMR control) and can be used directly for the next step. Main spectral data of **6**: ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.99$ (s, 1H), 7.67–7.60 (m, 2H), 7.11 (d, $J = 9.3$ Hz, 1H), 4.40 (q, $J = 7.1$ Hz, 2H), 2.36 (s, 3H), 1.41 (t, $J = 7.1$ Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.9, 153.0, 147.4, 144.1, 141.5, 137.4, 136.5, 131.8, 121. 9, 118.2, 117.5, 62.7, 26.3, 14.6 ppm.
19. Ukhov, S. V.; Kons'hin, M. E.; Odegova, T. F. *Pharm. Chem. J.* **2001**, *35*, 364–365.
20. Main spectral data of **7a**: Mixture of two diastereoisomers: 1st dia: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.86 (s, 1H), 7.38 (dd, $J = 8.8$, 2.2 Hz, 1H), 7.22 (d, $J = 2.2$ Hz, 1H), 7.07 (d, $J = 8.8$ Hz, 1H), 4.69 (d, $J = 3.6$ Hz, 1H), 4.23 (q, $J = 7.3$ Hz, 2H), 3.82 (d, $J = 3.6$ Hz, 1H), 2.23 (s, 3H), 1.30 (t, $J = 7.3$ Hz, 3H) ppm. 2nd dia: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.91 (s, 1H), 7.51 (d, $J = 2.2$ Hz, 1H), 7.41 (dd, $J = 8.7$, 2.2 Hz, 1H), 7.03 (d, $J = 8.7$ Hz, 1H), 4.59 (d, $J = 4.4$ Hz, 1H), 4.04 (q, $J = 7.2$ Hz, 2H), 3.61 (d, $J = 4.4$ Hz, 1H), 2.21 (s, 3H), 1.11 (t, $J = 7.2$ Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz,) δ (mixture of diastereoisomers) 167.6, 167.5, 167.3, 167.1, 164.4, 164.1, 156.9, 156.8, 149.2, 149.0, 132.8, 132.6, 131.2, 130.9, 122.0, 121.1, 119.1, 118.7, 118.3, 118.1, 115.1, 114.6, 81.9, 81.3, 63.4, 63.1, 61.5, 61.4, 46.2, 45.2, 36.4, 36.3, 25.7, 25.6, 14.3, 14.2, 14.0, 13.7 ppm.
21. For another method to obtain analogues with a monoester chain in position 4 see: Curini, M.; Rosati, O.; Marcotullio, M. C.; Montanari, F.; Campagna, V.; Pace, V.; Cravotto, G. *Eur. J. Org. Chem.* **2006**, 746–751.
22. Some 4-aryl-4*H*-chromenes are also known as potent apoptosis inducers, see: Kemnitzer, W.; Drewe, J.; 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.; Kasibhatla, S.; Cai, S. X. *J. Med. Chem.* **2004**, *47*, 6299–6310.