Bioorganic & Medicinal Chemistry Letters 20 (2010) 3165–3168

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis and evaluation of progesterone–adenine hybrids as bivalent inhibitors of P-glycoprotein-mediated multidrug efflux

Waël Zeinyeh [a], Ghina Alameh [a], Sylvie Radix [a,*], Catherine Grenot [a], Charles Dumontet [b], Nadia Walchshofer [a,*]

[a] Université de Lyon, Université Lyon 1, ISPB-Faculté de Pharmacie, INSERM U863, 8 avenue Rockefeller, F-69373 Lyon cedex 08, France
[b] Université de Lyon, Université Lyon 1, Faculté de Médecine, Laboratoire de Cytologie analytique, INSERM U590, 8 avenue Rockefeller, F-69373 Lyon cedex 08, France

ARTICLE INFO

Article history:
Received 29 January 2010
Revised 23 March 2010
Accepted 25 March 2010
Available online 28 March 2010

Keywords:
Multidrug resistance
P-Glycoprotein
ABCB1
Bivalent ligand
Multivalent ligand
Progesterone
Adenine

ABSTRACT

Steroidal bivalent ligands were designed on the basis of the described closer proximity of the ATP-site and the putative steroid-binding site of P-glycoprotein (ABCB1). The syntheses of seven progesterone–adenine hybrids were described. Their abilities to inhibit P-glycoprotein-mediated daunorubicin efflux in K562/R7 human leukemic cells overexpressing P-glycoprotein were evaluated versus progesterone.

Chemotherapeutics are the most effective treatment for metastatic cancer, but their efficacy is limited by the multidrug resistance (MDR) phenotype which results from multifactor mechanisms.[1] Drug efflux is a significant contributor for this phenotype in cancer cells, wherein drug-extrusion pumps belonging to the ABC superfamily of proteins are overexpressed.[2] One of these human ATP-dependent membrane transporters is P-glycoprotein (Pgp) which allows the efflux of a wide variety of structurally and functionally unrelated compounds.[3] Therefore, inhibition of Pgp may improve chemotherapeutic treatments and numerous studies[4] have focused on the development of Pgp modulators. Whereas the more hydrophilic steroids are transported by Pgp (e.g., cortisol), the more hydrophobic ones function as inhibitors (e.g., progesterone which is always considered as the reference among steroidal inhibitors of Pgp although it exhibits a low affinity to this protein).[5] Until now, some progesterone derivatives have been described as inhibitors of Pgp-mediated antitumor drugs efflux.[6–8] Moreover, it has been postulated that the steroid-binding region would be adjacent to the ATP-binding site of Pgp.[9] If this could be confirmed, it would be interesting to build on this feature to lead to more potent and selective Pgp inhibitors.

We decided to design and synthesize progesterone–adenine hybrids as potential bivalent ligands which may bind simultaneously to these two sites in the Pgp molecule (Fig. 1). Indeed, bivalent ligands can be used as valuable tools to confirm the proximity of the targeted binding sites,[10] owing to the binding-affinity and selectivity improvements generally recorded with these compounds compared to their monovalent counterparts.[11] The synthesis of dimeric compounds targeting substrate-binding sites of Pgp has already been reported,[12] but to our knowledge, heterobivalent compounds

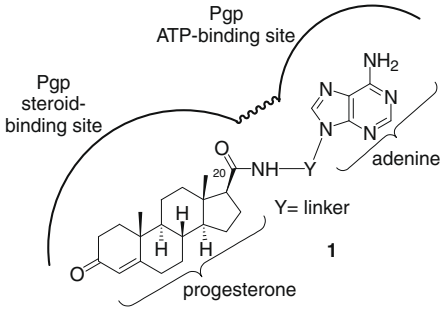
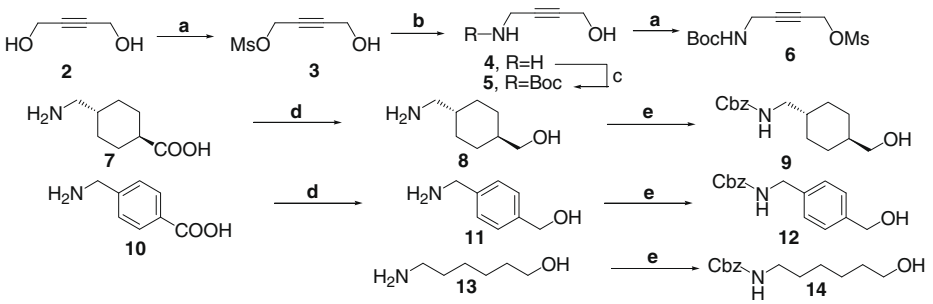


Figure 1. Structure of targeted bivalent compounds **1**.

* Corresponding authors. Tel.: +33 478777253; fax: +33 478777158 (S.R.); tel.: +33 478777006; fax: +33 478777158 (N.W.).
E-mail addresses: sylvie.radix@univ-lyon1.fr (S. Radix), nadia.walchshofer@univ-lyon1.fr (N. Walchshofer).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.085



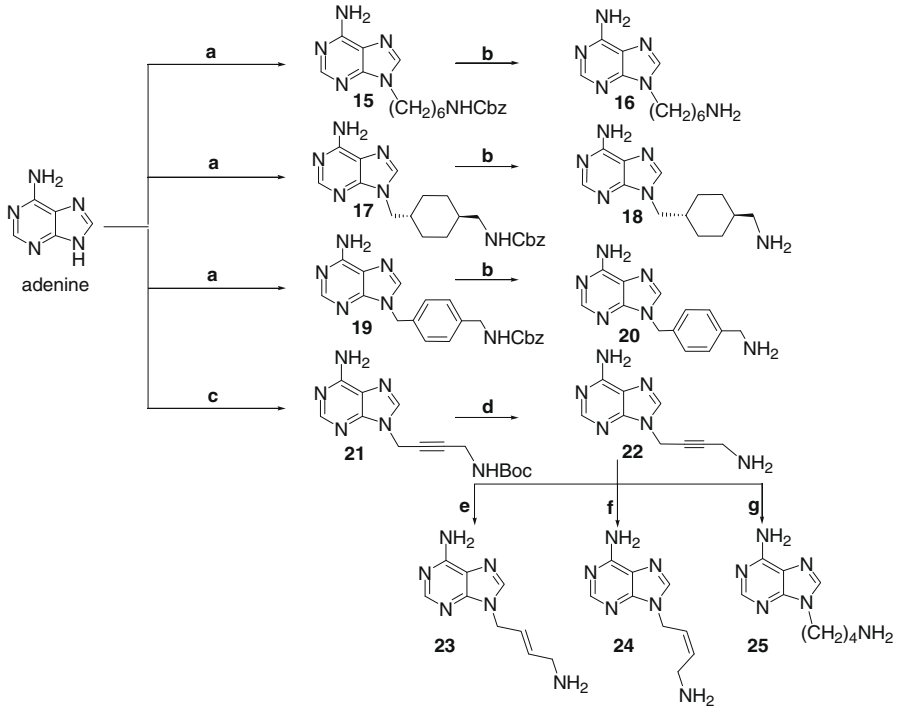
Scheme 1. Reagents and conditions: (a) MsCl, NEt_3, THF, 0 °C to rt, overnight (45% for **3**; 76% for **6**); (b) NH_4OH, rt, 1 h then Dowex (76%); (c) Boc_2O, NEt_3, THF, rt, overnight (73%); (d) $LiAlH_4$, THF, 0 °C to reflux, 3 h (70% for **8**; 28% for **11**); (e) Na_2CO_3, CbzCl, H_2O, 0 °C to rt, overnight (70% for **9**; 90% for **12**; 79% for **14**).

targeting ATP-binding site and/or steroid-binding region have never been described. In order to evaluate our strategy, we chose, in a first attempt, to synthesize the most readily accessible C20-substituted progesterone derivatives (with a NH group instead of the methyl group in the C21 position), and rather short-length linkers with different conformational flexibilities. We report here the synthesis of compounds **1** and their abilities to inhibit the Pgp-mediated daunorubicin efflux in K562/R7 human leukemic cells overexpressing P-glycoprotein.

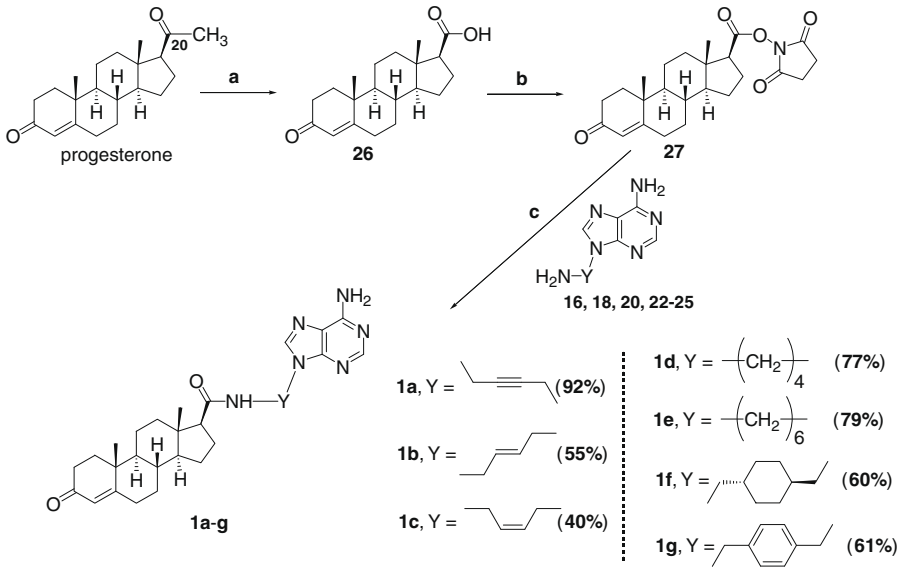
Syntheses of protected-amino linkers **6**, **9**, **12**, and **14**, starting from commercially available compounds **2**, **7**, **10**, and **13**, are described in Scheme 1. Monomesylation of diol **2** afforded mesylate **3**. Treatment of **3** with NH_4OH,[13] followed by *N*-Boc-protection[14] and methanesulfonylation of the remaining hydroxy function, provided the linker **6**. Reduction of carboxylic acids **7** and **10** in presence of $LiAlH_4$ gave alcohols **8** and **11**, respectively.[15] Cbz protection of aminoalcohols **8**, **11**, and **13** led to linkers **9**, **12**, and **14**. Then, amino derivatives **14**, **9**, and **12** were allowed to react with adenine using the Mitsunobu method, producing compounds **15**, **17**, and **19** (Scheme 2). Cbz deprotection of these products was achieved by catalytic hydrogenation (H_2, Pd/C) to afford free amino compounds **16**, **18**, and **20**. As Mitsunobu reaction failed with **5**, we used classical adenine N-alkylation conditions with mesylate **6** to provide derivative **21**. Alkyne **22**, easily obtained by Boc deprotection of **21**, was used as a common key precursor of amino compounds **23**, **24**[16] and **25**. Reduction of **22** by $LiAlH_4$ led to *trans*-alkene **23**. Catalytic hydrogenation of the same alkyne using Lindlar catalyst yielded *cis*-alkene **24**, while the use of Pd on charcoal provided **25**.

At the same time, progesterone was converted to its 17β-carboxylic acid analog **26** by the haloform reaction using sodium hypobromite (Scheme 3).[17] The activated ester **27**, obtained by treatment of acid **26** with *N*-hydroxysuccinimide,[18] was then allowed to react with the adenine derivatives **16**, **18**, **20** or **22–25** to afford the corresponding bivalent compounds **1a–g** in average to good yields (cf. Supplementary data).

The efficiency of progesterone–adenine hybrids to inhibit Pgp-mediated cytotoxic drug efflux was evaluated by measuring the intracellular accumulation of daunorubicin in the K562/R7 human leukemic resistant cell line overexpressing Pgp (Table 1). Progesterone, used as a positive control, was found to improve daunorubicin accumulation in K562/R7 cells by 25%. On the other hand,



Scheme 2. Reagents and conditions: (a) PPh_3, DIAD, THF, **14** or **9** or **12** (41% for **15**, 49% for **17**, 18% for **19**); (b) H_2, Pd/C, MeOH or MeOH/AcOEt (100% for **16**, 71% for **18**, 77% for **20**); (c) K_2CO_3, DMF, **6** (75%); (d) (i) aq HCl; (ii) Dowex 1 × 8 ($^-$OH form) (80%); (e) $LiAlH_4$, THF (29%); (f) H_2, Lindlar Pd, quinoline, MeOH (67%); (g) H_2, Pd/C, MeOH (90%).



Scheme 3. Reagents and conditions: (a) (i) NaOH, Br_2, *t*-BuOH, 0 °C, 3 h; (ii) Na_2SO_3, rt, overnight (57%); (b) *N*-hydroxysuccinimide, THF, rt, overnight (55%); (c) $(i\text{-Pr})_2$NEt, DMF, overnight, rt (**1a–f**) or 35 °C (**1g**).

Table 1
Capacity of the synthesized derivatives to improve daunorubicin accumulation in K562/R7 human leukemic resistant cells[19]

Compounds[a]	Daunorubicin accumulation (% progesterone) [b]
1a	61.2 (±8)
1b	72.2(±6.7)
1c	86.2 (±2.6)
1d	78.0 (±8.7)
1e	110. 9 (±13.7)
1f	98. 9 (±6.2)
1g	98.1 (±0.5)

[a] Compounds were tested at a 10 μM concentration.[19]
[b] Accumulation of daunorubicin (1 μM) in the presence of progesterone (10 μM) was considered as 100% . Daunorubicin accumulation in the presence of the tested compounds was expressed as percent of the accumulation in the presence of progesterone. Standard deviation is given in brackets.

hybrid-compounds cytotoxicity was tested on sensitive K562 cells which express a very low amount of Pgp. In all cases, cell survival after 24 h incubation in the presence of 10 μM of progesterone or compounds **1a–g** was found comprised between 80% and 100% of untreated control cells (data not shown). These first synthesized progesterone–adenine hybrids showed only a low capacity to inhibit Pgp-mediated drug efflux. Compared to progesterone, bivalent compounds with the shortest linkers (**1a–d**) caused a small decrease in activity while hybrids with longer middle chains (**1e–g**) did not lead to dramatic changes in inhibitory potency whatever the conformational rigidity of the spacers.

Despite the low Pgp-inhibition activity of compounds **1e–g**, the correct positioning of the progesterone moiety in the steroid-binding site was evidenced by an inhibitory capacity similar to progesterone. However, since no improvement of their inhibitory efficiency was noticed, their adenine part does not seem to be properly positioned in the ATP-binding pocket. Therefore, longer linkers should be carried out at the C20-position of progesterone. On the other hand, the points of attachment on the two moieties are crucial for the design of bivalent ligands. A very recent in silico docking study has predicted that the adenine moiety of ATP and the A/B rings of steroids should bind to vicinal regions within Pgp.[9b] Thus, the evaluation of hybrids whose linker arms are attached, for example, at the C2- or C7-position of progesterone should also be considered in further studies.

Acknowledgments

We thank Dr. Luc Rocheblave (UCB Lyon 1) for his helpful monitoring of cell culture and Dr. Ph. Lawton (UCB Lyon 1) for a careful reading of the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.085.

References and notes

1. (a) Fojo, T.; Bates, S. *Oncogene* **2003**, *22*, 7512; (b) Lage, H. *Cell. Mol. Life Sci.* **2008**, *65*, 3145.
2. (a) Eckford, P. D. W.; Sharom, F. J. *Chem. Rev.* **2009**, *109*, 2989; (b) Szakacs, G.; Paterson, J. K.; Ludwig, J. A.; Booth-Genthe, C.; Gottesman, M. M. *Nat. Rev. Drug Disc.* **2006**, *5*, 219.
3. Matsson, P.; Pederson, J. M.; Norinder, U.; Bergström, C. A. S.; Artusson, P. *Pharm. Res.* **2009**, *26*, 1816.
4. (a) Baumert, C.; Hilgeroth, A. *Anticancer Agents Med. Chem.* **2009**, *9*, 415. and references therein; (b) Ramahete, C.; Molnar, J.; Mulhovo, S.; Rosario, V. E.; Ferreira, M.-J. U. *Bioorg. Med. Chem.* **2009**, *17*, 6942; (c) Viale, M.; Cordazzo, C.; Corimelli, B.; De Torero, D.; Castagnola, P.; Aiello, C.; Severi, E.; Petrillo, G.; Cianfriglia, M.; Spinelli, D. *J. Med. Chem.* **2009**, *52*, 259.
5. (a) Barnes, K. M.; Dickstein, B.; Cutler, G. B.; Fojo, T.; Bates, S. E. *Biochemistry* **1996**, *35*, 4820; (b) Li, Y.; Wang, Y.-H.; Yang, L.; Zhang, S.-W.; Liu, C.-H.; Yang, S.-L. *J. Mol. Struct.* **2005**, *733*, 111.
6. (a) Yang, C. P.; Cohen, D.; Greenberger, L. M.; Hsu, S. I.; Horwitz, S. B. *J. Biol. Chem.* **1990**, *265*, 10282; (b) Perez-Victoria, F. J.; Conseil, G.; Munoz-Martinez, F.; Perez-Victoria, J. M.; Dayan, G.; Marsaud, V.; Castanys, S.; Gamarro, F.; Renoir, J. M.; Di Pietro, A. *Cell. Mol. Life Sci.* **2003**, *60*, 526.
7. Leonessa, F.; Kim, J.-Y. K.; Ghiorghis, A.; Kulawiec, R. J.; Hammer, C.; Talebian, A.; Clarke, R. J. *J. Med. Chem.* **2002**, *45*, 390.
8. Li, J.; Xu, L.-Z.; He, K.-L.; Guo, W.-J.; Zheng, Y.-H.; Xia, P.; Chen, Y. *Breast Cancer Res.* **2001**, *3*, 253.
9. (a) Dayan, G.; Jault, J. M.; Baubichon-Cortay, H.; Baggetto, L. G.; Renoir, J. M.; Baulieu, E. E.; Gros, P.; Di Pietro, A. *Biochemistry* **1997**, *36*, 15208; (b) Mares-Sámano, S.; Badhan, R.; Penny, J. *Eur. J. Med. Chem.* **2009**, *44*, 3601.
10. (a) Yeager, A. R.; Finney, N. S. *J. Org. Chem.* **2004**, *69*, 613; (b) Meltzer, P. C.; Kryatova, O.; Pham-Huu, D.-P.; Donovan, P.; Janowsky, A. *Bioorg. Med. Chem.* **2008**, *16*, 1832.
11. (a) Halazy, S.; Perez, M.; Fourrier, C.; Pallard, I.; Pauwells, P. J.; Palmier, C.; John, G. W.; Valentin, J.-P.; Bonnafous, R.; Martinez, J. *J. Med. Chem.* **1996**, *39*, 4920; (b) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754; (c) Meyer, S. C.; Shomin, C. D.; Gaj, T.; Ghosh, I. *J. Am. Chem. Soc.* **2007**, *129*, 13812; (d) Bérubé, M.; Poirier, D. *Can. J. Chem.* **2009**, *87*, 1180.
12. (a) Sauna, Z. E.; Andrus, M. B.; Turner, T. M.; Ambudkar, S. V. *Biochemistry* **2004**, *43*, 2262; (b) Chan, K. F.; Zhao, Y. Z.; Chow, T. W. S.; Yan, C. S. W.; Ma, D. L.; Burkett, B. A.; Wong, I. L. K.; Chow, L. M. C.; Chan, T. H. *ChemMedChem* **2009**, *4*,

594; (c) Namanja, H. A.; Emmert, D.; Pires, M. M.; Hrycyna, C. A.; Chmielewski, J. *Biochem. Biophys. Res. Commun.* **2009**, *388*, 672; (d) Pires, M. M.; Emmert, D.; Hrycyna, C. A.; Chmielewski, J. *Mol. Pharmacol.* **2009**, *75*, 92; (e) Pires, M. M.; Hrycyna, C. A.; Chmielewski, J. *Biochemistry* **2006**, *45*, 11695.
13. Juenge, E. C.; Spangler, P. *J. Org. Chem.* **1964**, *29*, 226.
14. Spiegel, D. A.; Schroeder, F. C.; Duvall, J. R.; Schreiber, S. L. *J. Am. Chem. Soc.* **2006**, *128*, 14766.
15. Haddenham, D.; Pasumansky, L.; DeSoto, J.; Eagon, S.; Singaram, B. *J. Org. Chem.* **2009**, *74*, 1964.
16. Krištafor, V.; Raić-Malić, S.; Cetina, M.; Kralj, M.; Šuman, L.; Pavelić, K.; Balzarini, J.; De Clercq, E.; Mintas, M. *Bioorg. Med. Chem.* **2006**, *14*, 8126.
17. Ashford, S. W.; Grega, K. C. *J. Org. Chem.* **2001**, *66*, 1523.
18. Hoyte, R. M.; Rosner, W.; Johnson, I. S.; Zielinski, J.; Hochberg, R. B. *J. Med. Chem.* **1985**, *28*, 1695.
19. Compte, G.; Daskiewicz, J.-P.; Bayet, C.; Conseil, G.; Viorney-Vanier, A.; Dumontet, C.; Di Pietro, A.; Barron, D. *J. Med. Chem.* **2001**, *44*, 763.