Bioorganic & Medicinal Chemistry Letters 22 (2012) 6509–6512

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Camptothecins in tumor homing via an RGD sequence mimetic

Domenico Alloatti, Giuseppe Giannini *, Loredana Vesci, Massimo Castorina, Claudio Pisano, Elena Badaloni, Walter Cabri

Corporate R&D, sigma-tau Industrie Farmaceutiche Riunite S.p.A., via Pontina Km 30.400, I-00040 Pomezia, Italy

ARTICLE INFO

Article history:
Received 8 June 2012
Revised 12 July 2012
Accepted 16 July 2012
Available online 26 July 2012

Keywords:
Tumor homing
Tumor-specific targeting
RGD mimetic and Integrin
Camptothecin–RGD mimetic conjugate
Anticancer drug delivery system

ABSTRACT

A RGD peptide mimetic was conjugated to four camptothecins, with the purpose to improve their therapeutic index. The conjugate derivatives were evaluated against two tumor cell lines, one overexpressing integrins (human ovarian carcinoma, A2780) and a second one with a low integrin expression (human prostate cancer, PC3). The in vitro screening was completed with the adhesion behavior to vitronectin. Compound **8** (ST7456CL1) was selected for the in vivo investigation after stability tests over 24 h, in PBS solution and in rat plasma, and compared to irinotecan. The former showed a prolonged half-life.

Traditional cancer chemotherapy is based on the assumption that rapidly proliferating cancer cells are more likely to be killed than quiescent normal cells. However the main drawback of this approach is that cytotoxic agents have very poor specificity, and lead to systemic toxicity. An ever-increasing knowledge of typical receptors over-expressed by cancer cells allows the exploitation of selective ligands which, properly conjugated with cytotoxic agents, are able to address them selectively to the tumors.

This so-called tumor homing approach could be applied successfully to overcome drug resistance and/or metastasis control.[1,2] Similarly, a delivery system using luteinizing hormone-releasing hormone (LHRH) was described as a targeting moiety for LHRH receptors.[3]

The conjugate should be systemically non-toxic; this means that the linker must be stable in circulation. Upon internalization into the cancer cell the conjugate should be easily cleaved to regenerate the active cytotoxic drug. To achieve effective tumor-specific drug delivery it is important to take advantage of the morphological and physiological differences between malignant and normal tissues. Two main examples are (a) the anaerobic metabolism which lowers tumor cell's pH[4,5] allowing the use of acid-sensitive linkers and (b) tumor cell's huge request of various nutrients for which the cell itself overexpresses tumor-specific receptors that can be used to target cytotoxic warhead. The latter case has been particularly studied using tumor-targeting moieties such as monoclonal antibodies, polyunsaturated fatty acids, hyaluronic acid, small peptides and peptidomimetics.[6]

The highly restricted expression of integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$, overexpressed on tumor endothelial and some epithelial cells, during tumor growth, angiogenesis, invasion, and metastasis present an interesting molecular target for tumor homing approach. That is why among selective receptor-targeting small peptides, integrin-mediated RGD peptides appear to be attractive candidates. The arginine–glycine–aspartic acid (RGD) is a cell adhesion motif present in many proteins of the extracellular matrix (ECM);[7] Through this motif ECM proteins recognize $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrin receptors.

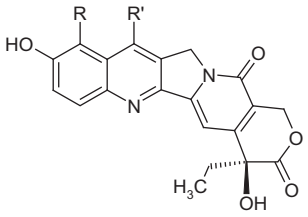
In the present study we investigated the usefulness of an RGD mimetic as a carrier for antitumor drugs of the camptothecin series. Among the different RGD mimetics available in the literature, we focused our attention on **1**, disclosed by Iwama et al.[8] with the aim of linking it to the cytotoxic derivative. We modified **1** by introducing in the side chain a suitable functional group for the attachment of cytotoxic drugs.

Various 10-hydroxy-camptothecin derivatives were evaluated, including SN38 (10-hydroxy-7-ethylcamptothecin), a well-known drug already in clinical use as its prodrug form irinotecan,[9] (see Fig. 1).

The choice of the linker for conjugates with small peptidemimetics was very challenging, its requisites being: stability into the bloodstream, lability into the tumor cell and resistance to plasma peptidases from which it is not shielded by bulky antibodies as it happens for immunoconjugates. Given some previous unpleasant results with linear amides (internal cyclization, followed by release of the two fragments, were frequently observed), we chose

* Corresponding author.
E-mail address: giuseppe.giannini@sigma-tau.it (G. Giannini).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.07.061



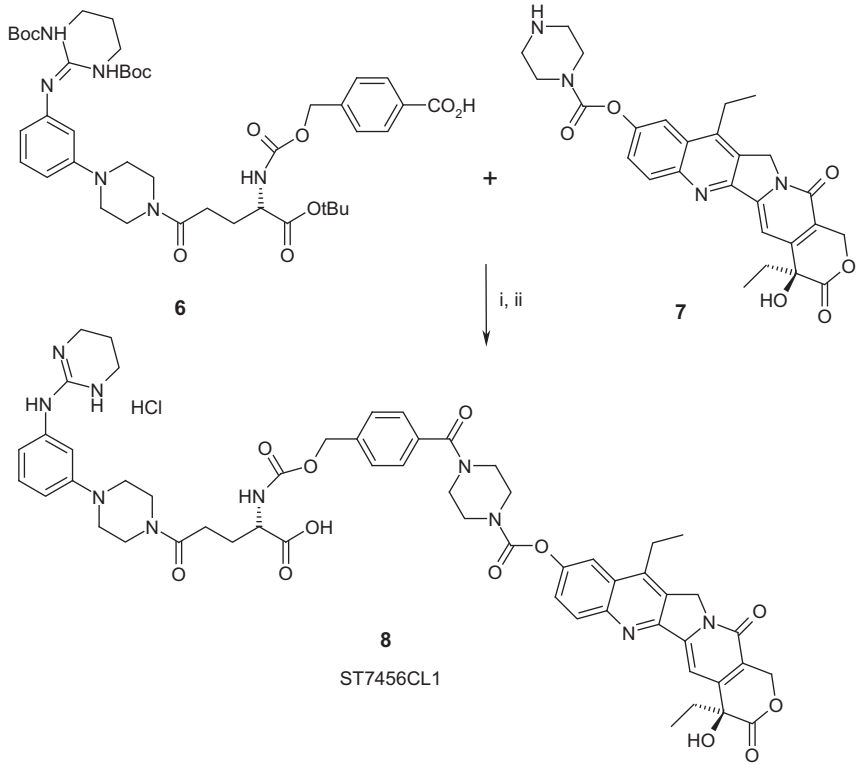
2. R=R' = H 10-hydroxy-Camptothecin

3. R= H; R'= Et SN38

4. R= CH_2NMe_2; R'=H Topotecan

5. R= H; R'= CH=N-O-tBu 10-hydroxy-Gimatecan

Figure 1. 10-hydroxy-camptothecins used in the synthesis of conjugate derivatives.



Scheme 1. Preparation of conjugate **8**; reagents and conditions: (i) PyBOP, DIPEA, Y = 65%; (ii) 4M HCl in Dioxane, *Y* = 45%.

a piperazine carbamate linker in order to prevent a rapid hydrolysis.

To obtain our functionalized mimetic we followed the synthesis disclosed by Iwama et al.,[8] and described, for compound **6** (functionalized mimetic), with only a few minor changes (Supplementary data, Scheme 1a).

The piperazine-carbamate on SN38, **7** was obtained in good yield via a classical *p*-nitrophenylchloroformate protocol followed by TFA-promoted Boc removal (Supplementary data, Scheme 2a).

The two partners were coupled using PyBOP and all protecting groups were removed with 4 N HCl in dioxane to give the desired product **8** in 30% yield over two steps (see Scheme 1).

Compound **6** was selected after exploring some derivatives useful to verify the feasibility of *t* functionalizing the benzyl-carbamic moiety without losing the $\alpha_V\beta_3$-binding properties (Fig. 2). These compounds were synthesized using the same synthetic approach as for the synthesis of **6** (Supplementary data, Scheme 1a).

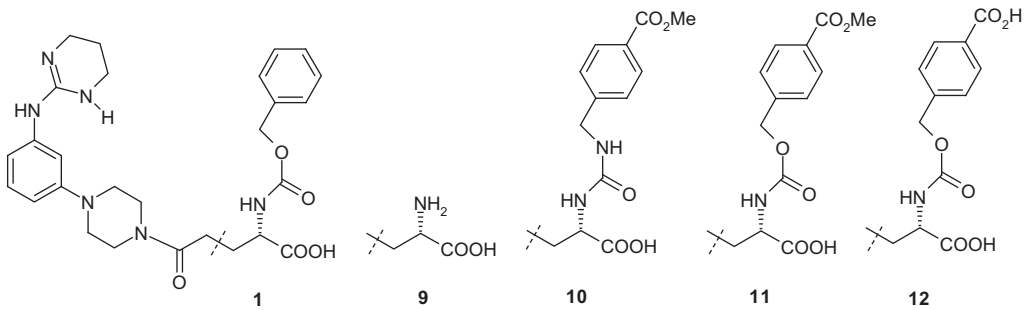


Figure 2. Some RGD mimetic and their $\alpha_V\beta_3$-binding affinity.

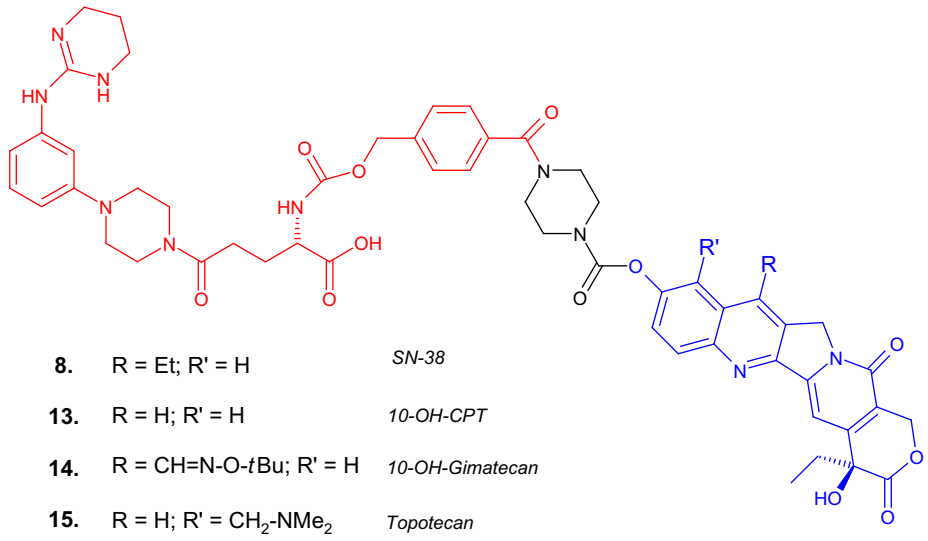


Figure 3. The four conjugate derivatives synthesized.

Table 1
In vitro screening: cytotoxicity, binding and adhesion of CPT derivatives, RGD mimetic and conjugate compounds

Compd	Cytotoxicity IC_{50}, μM		Binding assay IC_{50}, nM		Adhesion to vitronectin IC_{50}, μM	
	PC3	A2780	$\alpha_V\beta_3$	$\alpha_V\beta_5$	PC3	A2780
8	3.4	0.033	1.3	1.0	0.037	0.089
3	0.0026	0.009	/	/	/	/
13	> 1	0.11	2.28	2.05	0.35	0.73
2	0.078	0.004	/	/	/	/
14	> 1	0.067	4.83	0.76	0.13	0.67
5	0.015	0.0004	/	/	/	/
15	> 1	0.57	4.97	0.73	0.54	1.0
4	0.185	0.006	/	/	/	/
12	/	/	0.082	2.20	0.0063	0.017

From this preliminary study it came out that the benzyl moiety was necessary to maintain biological activity and that functionalization in position 4 did not affect binding to integrins. Four conjugate derivatives were synthesized (Fig. 3) and, as first screening, were tested for their cytotoxic activity as well as for the binding to the $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins.

In order to assess the role of the RGD-mimetic in recognizing the tumor cells, these compounds were evaluated against two tumor cell lines, one of which overexpressing integrins (human ovarian carcinoma, A2780) and the second one with a low integrin expression (human prostate cancer, PC3). The in vitro screening was completed with the adhesion behavior to vitronectin (Table 1).

Compound **8** revealed a potent affinity to integrin receptors and a potent ability to inhibit adhesion of vitronectin on tumor cells. Moreover, it showed a different antiproliferative activity on two types of tumor cells (A2780 and PC3, with high and low levels of integrin, 'respectively'). These data prompted us to further investigate the stability profile of **8** in PBS solution and in rat plasma in comparison with irinotecan (Supplementary data, Fig. 1a) over 24 h. The results showed (Fig. 4) a significant increase in $t_{1/2}$ for **8** vs irinotecan in rat plasma (13 h vs 45 min, 'respectively').

On the basis of the in vitro data, compound **8** was chosen for the in vivo investigation. It was delivered intraperitoneally according to the 4 doses over 4 days schedule in nude mice previously inoculated with an intracardiac injection of PC3 tumor cells. This type of implantation enabled the induction of bone metastases expressing $\alpha_V\beta_3$ integrin.[10]

Compound **8**, delivered at 60 mg/kg ip (q4d × 4) revealed to significantly increase the life of span by 34% (P <0.05) and to significantly reduce the area of metastases by 64% compared with vehicle-treated group (Table 2). Moreover, compound **8** given at 55 mg/10 mL/kg ip (q4d × 8) to nude mice implanted with a renal carcinoma overexpressing $\alpha_V\beta_3$ and $\alpha_V\beta_5$[11] showed to significantly inhibit the tumor growth by 39% (Table 3). The data obtained were comparable to those observed with irinotecan.

Overall, these data reveal that this is a feasible approach. However, further studies and synthesis of new derivatives, using different 10-hydroxy-campthothecins as well as different linkers, will be needed to get very potent conjugates presenting a high therapeutic index.

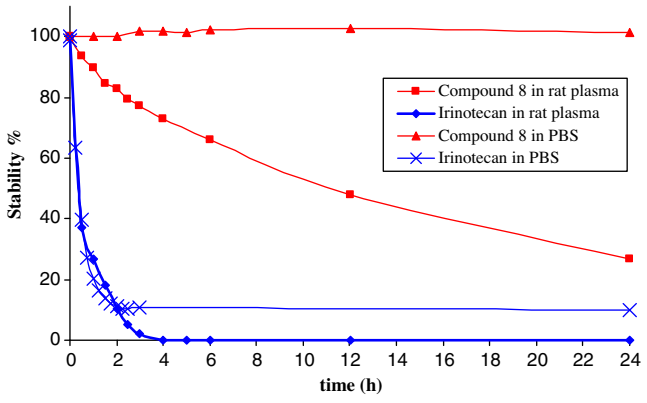


Figure 4. Compound **8** and Irinotecan stability profile in rat plasma and PBS, pH 7.4, T = 37 °C.

Table 2
Antimetastatic activity of compound **8** delivered intraperitoneally (q4d × 4) against bone metastases induced by PC3 prostate ca. xenografted in CD1 nude mice

	Dose[a]	BWL[b] (%)	Lethal toxicity[c]	Area of metastases[d]	MST[e]	ILS[f] (%)
Vehicle[g]	0	0	0/12	6.9 ± 1.2	47	/
8	60	0	0/12	2.5 ± 0.4*	63	34*

[a] Intraperitoneal dose (mg/10 mL/kg) used in each administration.
[b] Maximum BWL percentage due to the drug treatment.
[c] Dead/treated animals.
[d] Area (mm^2 ± SE) evaluated 31 days after tumor injection.
[e] MST: Median survival of time.
[f] ILS%: Increase in life of span.
[g] Vehicle: 10% DMSO
* P <0.05 vs vehicle-treated group (Mann–Whitney test).

Table 3
Antitumor activity of compound **8** delivered intraperitoneally (q4d × 8) against A498 renal carcinoma xenografted in CD1 nude mice

Compd	Dose[a]	BWL[b] (%)	Lethal toxicity[c]	TVI[d] (%)
Vehicle[e]	0	0	0/10	/
8	55	3	0/10	39*

Treatment started 3 days after tumor injection. Efficacy of drugs was evaluated 10 days after the last treatment.
[a] Intraperitoneal dose (mg/10 ml/kg) used in each administration.
[b] Maximum BWL percentage due to the drug treatment.
[c] Dead/treated animals.
[d] TVI percentage versus control mice.
[e] Vehicle: 10% DMSO.
* P <0.05 vs vehicle-treated group (Mann-Whitney test).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.061.

References and notes

1. Eldar-Boock, A.; Joaquin Sanchis, J.; Ruth Lupu, R.; Vicent, M.J.; Satchi-Fainaro, R. AACR (American Association for Cancer Research) 2012, Abs. 5225.
2. Sharkey, R. M.; Govindan, S. V.; Cardillo, T. M.; Goldenberg, D. M. *Mol. Cancer Ther.* **2012**, *11*, 224.
3. Dharap, S. S.; Wang, Y.; Chandna, P.; Khandare, J. J.; Qiu, B.; Gunaseelan, S.; Sinko, P. J.; Stein, S.; Farmanfarmaian, A.; Minko, T. *Proc Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12962.
4. Schornack, P. A.; Gillies, R. J. *Neoplasia* **2003**, *5*, 135.
5. Liu, C. N.; Tadayoni, B. M.; Bourret, L. A.; Mattocks, K. M.; Derr, S. M.; Widdison, W. C.; Kedersha, N. L.; Ariniello, P. D.; Goldmacher, V. S.; Lambert, J. M.; Blattler, W. A.; Chari, R. V. J. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8618.
6. Jaracz, S.; Larisa, J. C.; Kuznetsova, V.; Ojima, I. *Bioorg. Med. Chem. Lett.* **2005**, *13*, 5043.
7. Pierschbacher, M. D.; Ruoslahti, E. *Nature* **1984**, *309*, 30.
8. Iwama, S.; Kitano, T.; Fukuya, F.; Honda, Y.; Sato, Y.; Notako, M.; Morie, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2567.
9. Gollins, S.; Myint, A. S.; Haylock, B.; Wise, M.; Saunders, M.; Neupane, R.; Essapen, S.; Samuel, L.; Dougal, M.; Lloyd, A., et al *J. Clin. Oncol.* **2011**, *1042*, 29.
10. McCabe, N. P.; De, S.; Vasanji, A.; Brainard, J.; Byzova, T. V. *Oncogene* **2007**, *26*, 6238.
11. Korhonen, M.; Sariola, H.; Gould, V. E.; Kangas, L.; Virtanen, I. *Cancer Res.* **1994**, *54*, 4532.