Bioorganic & Medicinal Chemistry Letters 22 (2012) 1850–1853

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of sulfonyl acrylonitriles as novel inhibitors to peritoneal carcinomatosis

Craig A. Zificsak [a,*,†], Yi Shen [b,†], Joseph G. Lisko [a], Jay P. Theroff [a], Xuegang Lao [b], Oana Bollt [b], Xiufen Li [b], Bruce D. Dorsey [a], Scott K. Kuwada [b]

[a] Worldwide Discovery Research, Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380, USA
[b] Department of Medicine, University of Hawaii, Honolulu, HI 96813, USA

ARTICLE INFO

Article history:
Received 29 November 2011
Revised 20 January 2012
Accepted 23 January 2012
Available online 30 January 2012

Keywords:
Peritoneal carcinomatosis
BAY 11-7085

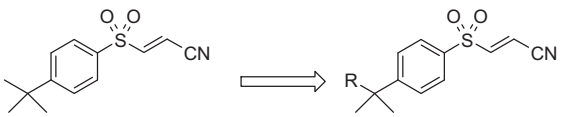
ABSTRACT

The vast majority of cancer patients die from metastasis, the process by which cancer cells spread to secondary tissues through body fluids. Peritoneal carcinomatosis is a type of metastasis in which cancer cells gain access to the intra-abdominal cavity and then implant in the peritoneum, the thin tissue that lines the abdominal wall and internal organs. Unfortunately, peritoneal carcinomatosis can occur following surgical resection of intra-abdominal malignancies. We previously reported proapoptotic activity of (2*E*)-3-[[4-(1,1-dimethylethyl)phenyl]sulfonyl]-2-propenenitrile (BAY 11-7085, **1**) on colon and pancreatic cancer cells during adhesion and demonstrated that this compound could significantly inhibit peritoneal carcinomatosis in mice.[1,2] In order to determine the chemical basis of the anti-metastatic properties of BAY 11-7085, a series of analogs were synthesized and evaluated for their ability to induce apoptosis in pancreatic and ovarian cancer cells during adhesion to mesothelial cells, which line the surface of the peritoneum. The co-culture assay results were validated using a murine peritoneal carcinomatosis model. These analogs may greatly benefit patients undergoing surgical resections of colorectal, pancreatic, and ovarian cancers depending on their tolerability.

Peritoneal carcinomatosis, a uniformly fatal form of metastasis, results from the peritoneal implantation of cancer cells, which are shed from intra-abdominal cancers or dislodged during surgical resection. At the time patients with abdominal malignancies undergo resection of their tumors, seeding of the peritoneal cavity and the circulatory system can occur.[3,4] Perhaps the best evidence for the iatrogenic spread of cancer cells to the peritoneum is from studies demonstrating seeding of tracts created in the abdominal wall to allow laparoscopic resection of intra-abdominal cancers.[5]

The small molecule BAY 11-7085 (**1**, Fig. 1), a known NF-κB inhibitor, was shown to induce apoptosis of colon and pancreatic cancer cells during cell adhesion and reduce colon and pancreatic cancer cell implantation in a murine peritoneal seeding model.[1,2] The molecular mechanism of BAY 11-7085 relates to its ability to inhibit the expression of c-FLIP$_L$ (FLICE Inhibitory Protein) which restores death receptor signaling in cancer cells.[2] c-FLIP$_L$ is commonly overexpressed in cancer cells of many types and inhibits the recruitment of caspase 8 to activated death receptors of the TNFα superfamily.[6–8] Many types of cancer cells paradoxically express death receptors which mediate pleiotropic effects, such as proliferation, despite what their name implies.[9,10] Mesothelial cells lining the peritoneum express the cognate ligands of these death receptors, which activate death receptor-mediated signaling.[2] Inhibition of c-FLIP allows activated death receptors to recruit caspase 8 and 10, which in turn initiate the cleavage of downstream 'executioner' caspases that effect apoptosis. Although restoration of death receptor-mediated apoptosis has been demonstrated in cancer cell cultures, we were the first to demonstrate this occurs in a juxtacrine fashion when cancer cells come into contact with normal human mesothelial cells.[2]

As a first study, we designed analogs of **1** wherein a methyl of the *t*-butyl group is replaced by a carboxylic acid, or alkyl, aryl or benzyl amide. An in vitro model of peritoneal tumor implantation was utilized in which pancreatic or ovarian cancer cells in suspension were allowed to adhere to confluent monolayers of mesothelial cells. The cancer cells were transduced with a constitutively expressing firefly



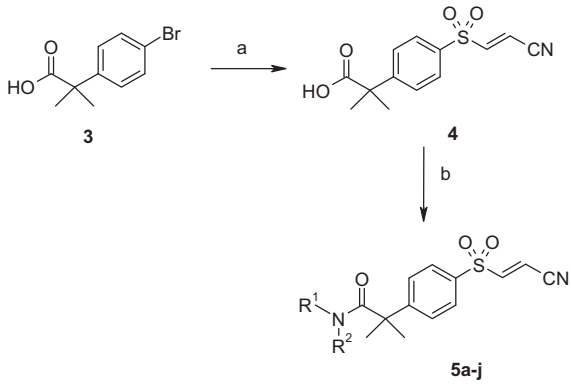
1
BAY 11-7085

2
R = CONR^{1}R^2

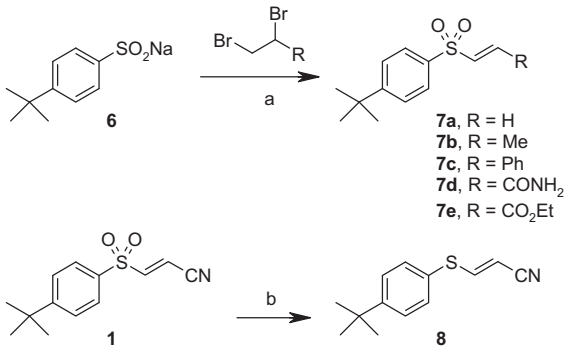
Figure 1. BAY 11-7085 (**1**) and novel inhibitors **2**.

* Corresponding author. Tel.: +1 610 738 6745; fax: +1 610 738 6643.
E-mail address: czificsa@cephalon.com (C.A. Zificsak).
† These co-authors contributed equally to this paper.

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.01.085

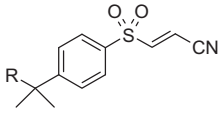


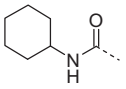
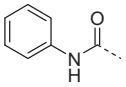
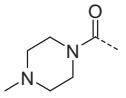
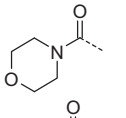
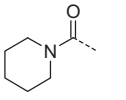
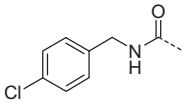
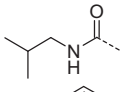
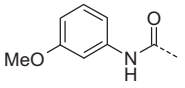
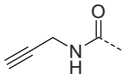
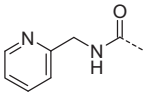
Scheme 1. (a) (i) PhLi; (ii) *n*-BuLi; (iii) SO_2; (iv) 2-chloroacrylonitrile; AcOH; (v) Et_3N; (b) amine, EDCI, HOBT, MeCN or DCM.



Scheme 2. (a) DMF, 80 °C, 3–15% yield; (b) 4-*t*-BuPhSH, Et_3N, $CHCl_3$, 97%.

Table 1
Biological data for compounds **1**, **4**, **5a–j**



Compd	R	Efficacy[a] of compounds by co-culture assay			
		Su86	BxPC3	A2780	SKOV3
1	Me	0.13 ± 0.01	0.07 ± 0.03	0.04 ± 0.00	0.32 ± 0.02
4	CO_2H	0.89 ± 0.03	0.92 ± 0.03	1.08 ± 0.04	1.07 ± 0.03
5a		0.43 ± 0.02	0.22 ± 0.03	0.20 ± 0.01	0.60 ± 0.04
5b		0.52 ± 0.02	0.22 ± 0.02	0.17 ± 0.00	0.55 ± 0.04
5c		0.95 ± 0.03	0.98 ± 0.05	0.95 ± 0.01	1.09 ± 0.03
5d		0.94 ± 0.09	0.85 ± 0.11	0.66 ± 0.04	1.01 ± 0.07
5e		0.51 ± 0.00	0.25 ± 0.00	0.08 ± 0.01	0.51 ± 0.06
5f		0.43 ± 0.05	0.27 ± 0.02	0.15 ± 0.04	0.63 ± 0.02
5g		0.29 ± 0.05	0.24 ± 0.02	0.05 ± 0.00	0.47 ± 0.08
5h		0.65 ± 0.05	0.62 ± 0.05	0.55 ± 0.01	0.98 ± 0.04
5i		0.65 ± 0.04	0.64 ± 0.14	0.63 ± 0.01	0.93 ± 0.06
5j		0.29 ± 0.05	0.24 ± 0.02	0.05 ± 0.00	0.47 ± 0.08

[a] The final concentration of all compounds was defined at 10 μM. Luminescence of cancer cells = living cancer cell number. Efficacy = luminescence of cancer cells treated with analog/luminescence of cells treated with DMSO; the data are presented as mean ± SEM, $n = 3$.

Table 2
Biological data for compounds **7a–e**, **8**

Compd	R	Efficacy[a] of tested compounds by co-culture assay			
		Su86	BxPC3	A2780	SKOV3
1	CN	0.13 ± 0.01	0.07 ± 0.03	0.04 ± 0.00	0.32 ± 0.02
7a	H	0.80 ± 0.03	0.84 ± 0.11	1.16 ± 0.02	1.05 ± 0.06
7b	Me	0.99 ± 0.04	0.99 ± 0.05	1.15 ± 0.01	1.08 ± 0.02
7c	Ph	0.97 ± 0.02	1.01 ± 0.10	1.13 ± 0.02	1.17 ± 0.05
7d	$CONH_2$	0.59 ± 0.03	0.44 ± 0.08	0.77 ± 0.01	0.50 ± 0.03
7e	CO_2Et	0.52 ± 0.01	0.20 ± 0.01	0.39 ± 0.04	0.67 ± 0.02
8	CN (sulfide)	1.03 ± 0.04	1.08 ± 0.05	1.06 ± 0.08	0.92 ± 0.04

[a] The final concentration of all compounds was defined at 10 μM. Luminescence of cancer cells = living cancer cell number. Efficacy = luminescence of cancer cells treated with analog/luminescence of cells treated with DMSO; the data are presented as mean ± SEM, $n = 3$.

luciferase plasmid, which allowed the cancer cells to be detected by bioluminescence in the presence of substrate (D-luciferin). The analogs were added to the cancer cell suspensions and then the mixture overlaid on the mesothelial cell monolayer. The cancer cells that were able to adhere to the mesothelial cells and survive were detected by bioluminescence which correlates with the number of surviving and adherent cancer cells present.

The synthesis of the amide analogs began with bromophenyl isobutyric acid **3**,[11] which was treated sequentially with phenyl- and *n*-butyl lithium and the resultant lithiated salt was quenched with sulfur dioxide (Scheme 1). The sulfinic acid was then coupled with 2-chloroacrylonitrile and after workup, triethylamine-induced elimination[12] of HCl generated the *E*-sulfonyl acrylonitrile acid **4**. The target amides **5a–j** were then prepared following standard peptide amide coupling with EDCI and HOBT in low to acceptable yields (17%–82%).[13]

Several amide analogs of **1** were prepared to assess functional group tolerability. The free acid **4** was inactive in the cellular screening assays, but once coupled to an amine, the resultant amides showed comparable activity to **1** (Table 1). Secondary amides **5a**–**b** showed good activity when screened at 10 μM across the four cell lines. Tertiary amides **5c** and **5d** showed a dramatic decrease in activity, but when piperidine amide **5e** was assessed, activity returned, highlighting the preference for non-polar functionality. Additional secondary amides **5f–i** showed moderate to good potency in the four cell lines, while pyridine-containing analog **5j** maintained potency.

To further investigate the possible mechanism of action, a series of analogs with alkene modifications were prepared (Scheme 2)[14,15] and screened (Table 2). All of the replacements of the nitrile fragment present in **1** showed significant reduction in antitumor activity by co-culture assay in all four cell lines assessed. An additional modification, vinyl sulfide **8**,[16] the reduced analog of **1**, was screened as well and found to be weakly active. The lack of activity highlights the need for an electron-deficient Michael acceptor to effect apoptosis of cancer cells during adhesion to mesothelial cells in the co-culture assay. Sulfonyl acrylonitriles are known to readily undergo Michael addition/elimination reactions in the literature,[17,16] and it is postulated that this is the mechanism by which the active analogs are affecting the cancer cells.

Concentration–response studies of the active analogs **5b**, **5f**, **5g** and **5h** were performed in the co-culture assay. All of these analogs showed inhibition in the micromolar range, similar to the activity of **1** (Table 3). Additionally, treatment of SKOV3.luc cells with **1**, **5b** or **5f–h** at a concentration of 7.5 μM during their adhesion to mesothelial cell monolayers resulted in the formation of detached apoptotic cells for all five compounds, relative to vehicle.[2,18]

In order to validate the in vitro co-culture assay results, an in vivo murine peritoneal carcinomatosis model was employed. Analogs were chosen based on their activity in the co-culture assays in order to determine if they performed similarly in an in vivo model. As previously published,[1] athymic 5-week-old female nu/nu mice were randomized to IP (intraperitoneal) injection with 5 mg/kg of drug or an equal volume of vehicle (4:1 PEG400:EtOH) 4 h before IP injection of 1.5 million suspended A2780-luc ovarian cancer cells. The mice continued to receive vehicle or drug every three days for 14 days total. The peritoneal tumor implants were examined weekly using a bioluminescence detection system and monitored out to 14 days. The results are shown in Figure 2.

Significant inhibition of intraperitoneal implantation was observed in the groups treated with BAY 11-7085 and analog **5g** compared with the control mice which received vehicle only. Analog **5b** inhibited peritoneal carcinomatosis compared with the controls,

Table 3
Dose response data for active compounds

Compd	IC_{50} (μM)[a] by co-culture assay			
	Su86	BxPC3	A2780	SKOV3
1	5.9 ± 0.2	3.7 ± 0.1	2.6 ± 0.1	7.6 ± 0.5
5b	7.5 ± 0.4	4.1 ± 0.0	2.7 ± 0.1	9.9 ± 0.2
5f	8.7 ± 0.1	4.1 ± 0.1	3.4 ± 0.0	11.4 ± 0.2
5g	7.6 ± 0.1	4.2 ± 0.2	3.3 ± 0.1	12.9 ± 0.1
5h	7.3 ± 0.1	4.1 ± 0.1	2.4 ± 0.1	10.9 ± 0.2

[a] IC_{50} values are reported as the average of three separate determinations.

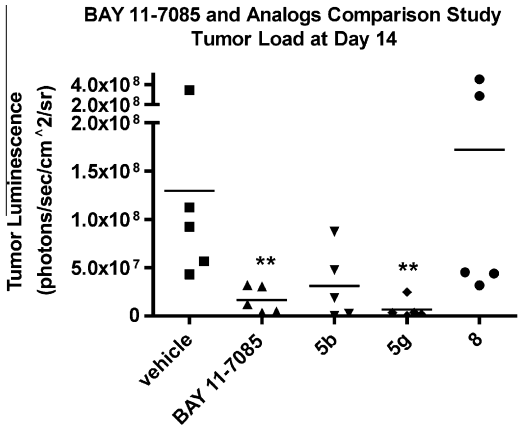


Figure 2. BAY 11-7085 and active analogs inhibit peritoneal carcinomatosis. The mice were pre-treated intraperitoneally (IP) with drug or vehicle. Four hours later, 1.5×10^6 A2780-luciferase ovarian cancer cells were injected IP into the mice. The mice subsequently underwent treatment (5 mg/kg, IP) every three days for 14 days. The intraperitoneal tumor implants were detected and quantified using a bioluminescence imager following IP D-luciferin administration. The graph shows abdominal bioluminescence output for the mice at day 14. The horizontal bars represent the medians.**, $p < 0.01$ compared with control mice that were treated with vehicle alone.

but the effect was not statistically significant, which demonstrates differences between the in vitro co-culture assay and the in vivo model. Analog **8**, which failed to demonstrate efficacy in the co-culture assay, did not demonstrate significant inhibition of peritoneal carcinomatosis in the mice as well (Fig. 2), verifying the importance of the sulfonyl group on the anti-cancer activity of these analogs. Some degree of weight loss was initially observed in the mice treated with the active analogs but this corrected over the 14-day treatment period.

In summary, seventeen analogs of **1** with *t*-butyl or alkene modifications were synthesized and their antitumor activities evaluated in vitro with in vivo analysis of three select analogs. Analog **5g** showed similar activity to BAY 11-7085 in co-culture assay and greater inhibition of peritoneal carcinomatosis in mice suggesting that specific modifications of the *t*-butyl group could improve clinical activity of these drugs.

Through the synthesis of analogs of **1**, we have defined a group of compounds that are able to induce apoptosis of pancreatic and ovarian cancer cells during their adhesion to the peritoneum. Compound **1** inhibits c-FLIP$_L$, which restores apoptotic signaling by death receptors. The death receptors expressed on the cancer cells are activated in a juxtacrine fashion by death receptor ligands on the mesothelial cells. If the tolerability and intraperitoneal exposure of these compounds is acceptable, they may be useful when administered during the resection of intraabdominal cancers, a time when tumors are disrupted and cancer cells shed into the peritoneal cavity.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.085.

References and notes

1. Scaife, C. L.; Kuang, J.; Wills, J. C.; Trowbridge, D. B.; Gray, P.; Manning, B. M.; Eichwald, E. J.; Daynes, R. A.; Kuwada, S. K. *Cancer Res.* **2002**, *62*, 6870.
2. Shen, Y.; Herde, R.; Doxey, B. W.; Xu, C.; Gray, P. D.; Kuwada, S. K. *Int. J. Cancer* **2011**. doi:10.1002/ijc.26119.
3. Hase, K.; Ueno, H.; Kuranaga, N.; Utsunomiya, K.; Kanabe, S.; Mochizuki, H. *Dis. Colon Rectum* **1998**, *41*, 1249.
4. Yu, W.; Whang, I.; Suh, I.; Averbach, A.; Chang, D.; Sugarbaker, P. H. *Ann. Surg.* **1998**, *228*, 347.
5. Kim, S. H.; Milsom, J. W.; Gramlich, T. L.; Toddy, S. M.; Shore, G. I.; Okuda, J.; Fazio, V. W. *Dis. Colon Rectum* **1998**, *41*, 971.
6. Elnemr, A.; Ohta, T.; Yachie, A.; Kayahara, M.; Kitagawa, H.; Fujimura, T.; Ninomiya, I.; Fushida, S.; Nishimura, G. I.; Shimizu, K.; Miwa, K. *Int. J. Oncol.* **2001**, *18*, 311.
7. Burns, T. F.; El-Deiry, W. S. *J. Biol. Chem.* **2001**, *276*, 37879.
8. Zhang, L.; Fang, B. *Cancer Gene Ther.* **2005**, *12*, 228.
9. Li, H.; Cai, X.; Fan, X.; Moquin, B.; Stoicov, C.; Houghton, J. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2008**, *294*, G263.
10. Kataoka, T.; Budd, R. C.; Holler, N.; Thome, M.; Martinon, F.; Irmler, M.; Burns, K.; Hahne, M.; Kennedy, N.; Kovacsovics, M.; Tschopp, J. *Curr. Biol.* **2000**, *10*, 640.
11. Heck, R.; Winstein, S. *J. Am. Chem. Soc.* **1957**, *79*, 3432.
12. Bradley, P. J.; Grayson, D. H. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1794.
13. For procedures and characterization data, see the Supplementary data.
14. Sulfone **7a** is commercially available from ChemBridge or may be prepared as shown in Scheme 2.
15. Guan, Z.-H.; Zuo, W.; Zhao, L.-B.; Ren, Z.-H.; Liang, Y.-M. *Synthesis* **2007**, 1465.
16. Prepared from **1** and 4-*tert*-butylthiophenol by the method of Grayson, D. H.; O'Donnell, S. H. *ARKIVOC* **2003**, 4.
17. Benedetti, F.; Fabrissin, S.; Pricl, S.; Risaliti, A. *Gazz. Chim. Ital.* **1987**, *117*, 391.
18. For Western Blot analysis, see Supplementary Figure S1.