Bioorganic & Medicinal Chemistry Letters 24 (2014) 3948–3951

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel 1,5-benzodiazepine-2,4-dione derivatives as potential anticancer agents

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ARTICLE INFO

Article history:
Received 25 March 2014
Revised 30 May 2014
Accepted 13 June 2014
Available online 25 June 2014

Keywords:
1,5-Benzodiazepine-2,4-dione
Anthranilic diamide
Conformational restriction
Synthesis
Antitumor activity

ABSTRACT

A series of novel 1,5-benzodiazepine-2,4-dione derivatives with C-6 amide substituents were designed and synthesized using three-component reactions. The preliminary assays showed that most of them displayed moderate to good antitumor activities against human lung carcinoma (A549), human breast epithelial carcinoma (MCF-7), human colon carcinoma (HCT116), human cervical carcinoma (Hela) and Lewis lung carcinoma (2LL). Exhilaratingly, the activity level of **6m** rivaled that of 5-Fluorouracil (5-Fu) against MCF-7 cell lines, which might be used as novel lead scaffold for potential anticancer development.

Nowadays, cancer has been one of the worldwide serious diseases that represent a real crisis for threatening human health and life.[1–3] Apart from the use of surgical treatment and irradiation, chemotherapy still remains the backbone of treatment against cancer.[4,5] However, one of the major hurdles in cancer chemotherapy is attributed to the prevalence of the drug and multidrug resistance.[6–8] Therefore, considerable efforts have been made on the design and the discovery of new anticancer agents focusing on the neotype chemical entity for the successful treatment of cancer.[9–11]

Anthranilic diamide scaffolds are privileged structures in medicinal chemistry and also serve as versatile building blocks for the construction of therapeutic leads. They are usually embedded in many bioactive compounds (Fig. 1, A–D), which elicit a wide range of biological potencies including antitumor,[12,13] insecticidal,[14,15] and antiviral,[16] etc. This gives us a great impetus to the search for potential pharmacologically active compounds carrying anthranilic diamide unit. The compounds containing the amide group exist as a mixture of atropisomers in solution due to restricted rotations at the amide (CO–N) and aryl–carbonyl (Ar–CO) bonds.[17] The conformational influence of amides may result in the difference of activity and selectivity.[18,19]

In recent years, conformational restriction is a promising strategy in drug discovery and makes an ideal platform for structural modification and derivatization.[20–22] Rigidification of a functional group through formation of five- to seven-membered ring is the common approach for conformational restriction.[23–25] Our research group has been interested in the molecular design using conformational restriction strategy.[26,27] Thus, locking one amide group of anthranilic diamide unit arouses our interest. Also, it is well documented that several synthetic approaches can make it. For example, 1,5-benzodiazepine-2,4-dione scaffold[28] provides a feasible method for locking the amide group into phenyl ring condensed with a seven-membered heterocycle (Fig. 2).

Furthermore, 1,5-benzodiazepine-2,4-dione core is a highly efficient pharmacophore and presents in a number of bioactive

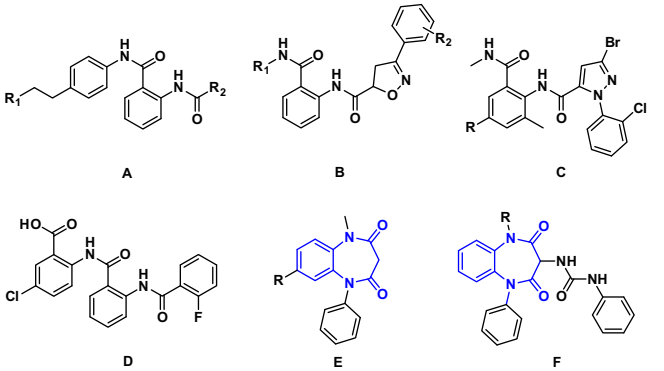

Figure 1. Representative derivatives with anthranilic diamide and 1,5-benzodiazepine-2,4-dione scaffolds.

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http://dx.doi.org/10.1016/j.bmcl.2014.06.041
0960-894X/

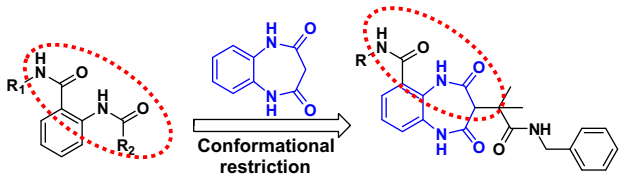


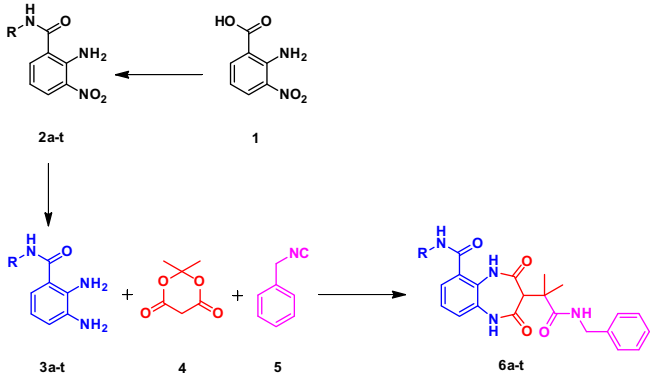
Figure 2. Design strategy of anthranilic diamide derivatives with conformational restriction.

compounds. They were developed as anxiolytic agents (Fig. 1, E)[29,30] and cholecystokinin-B receptor antagonists (Fig. 1, F).[31] Inspired by the biological diversity described above, we intended to investigate whether there would be some new beneficial properties if 1,5-benzodiazepine-2,4-dione moiety hybridized anthranilic diamide core structure.

Enlightened by all of the descriptions above, we utilized anthranilic diamide scaffold as a key prototype structure unit and introduced the 1,5-benzodiazepine-2,4-dione scaffold with dual effects of locking the amide group and hybridizing its versatility. Herein we described the molecular design, synthesis and initial findings on antitumor activity.

The synthetic route employed for the synthesis of the target compounds **6a**–**t** was outlined in Scheme 1. The commercially available 2-amino-3-nitrobenzoic acid **1** was selected as the starting material, which was transferred to the corresponding benzamides by the general condensation reaction. When the R was alkyl or benzyl amines, the treatment of 2-amino-3-nitrobenzoic acid with amines in the presence of EDCI and HOBt afforded the intermediates **2a**–**k** in 68–76% yields; when the R was anilines, **1** was firstly converted to the corresponding acid chloride and then coupled with anilines using DIPEA as acid acceptor to obtain the intermediates **2l**–**t** in 57–75% yields. The catalytic hydrogenation of intermediates **2a**–**t** with 15% Pd/C in MeOH afforded the key diamide derivatives **3a**–**t** in high yields, which were used for the next reaction without further purification. The benzyl isocyanide **5** was synthesized following the literature method.[32] In the final step, a one-pot three-component reaction using diamide derivatives **3a**–**t**, Meldrum's acid **4** and benzyl isocyanide **5** in 1,2-dichloroethane at reflux under Ar produced the target compounds **6a**–**t** in 25–45% yields after column chromatography or recrystallization. The chemical structures of the synthesized compounds **6a**–**t** were summarized in Table 1. Spectral data for all synthesized compounds were given in the Supplementary material.

The in vitro antitumor activities of the synthesized compounds **6a**–**t** against five cancer cell lines, including human lung carcinoma (A549), human breast epithelial carcinoma (MCF-7), human colon carcinoma (HCT116), human cervical carcinoma (Hela) and Lewis



Scheme 1. Protocol for synthesis of the target compounds.

Table 1
The chemical structure of compounds **6a**–**t**

No	Compds	Substituents R
1	**6a**	Isopropyl
2	**6b**	*t*-Butyl
3	**6c**	Cyclopropyl
4	**6d**	Benzyl
5	**6e**	3-(Trifluoromethyl)benzyl
6	**6f**	3-Chloro-4-methylbenzyl
7	**6g**	3-Chloro-4-fluorobenzyl
8	**6h**	4-Methylbenzyl
9	**6i**	2,5-Difluorobenzyl
10	**6j**	2,3,6-Trifluorobenzyl
11	**6k**	Pyridin-2-ylmethyl
12	**6l**	Phenyl
13	**6m**	3-(Trifluoromethyl)phenyl
14	**6n**	3-Chlorophenyl
15	**6o**	2-Fluorophenyl
16	**6p**	4-(Trifluoromethyl)phenyl
17	**6q**	2,3-Difluorophenyl
18	**6r**	2,4-Difluorophenyl
19	**6s**	5-chloro-2-Fluorophenyl
20	**6t**	2,3,4-Trifluorophenyl

lung carcinoma (2LL), were assayed by the standard MTT method[33] using 5-FU (5-Fluorouracil) as a positive control. The cancer cells were preincubated for 48 h. The results expressed as IC_{50} were summarized in Table 2.

All of the prepared compounds were screened against A549 cell lines, and most of them exhibited moderate to good antitumor activity. Firstly, the lower activity of compounds **6a**–**c** derived from alkyl groups than the corresponding benzylated and phenylated counterparts **6d**–**t** implied that the antitumor activity might be influenced by the size or electronic effect of the substituents. Compound **6m** showed the most potent activity with IC_{50} value of 27.8 μg/mL. Introducing electron-withdrawing groups, electron-donating groups, or halogen atoms at different positions did not contribute to great improvement of the activity. Moreover, compounds **6h** and **6p** were inactive, which indicated that the 4-position was unfavorable to the activity. And, replacement of phenyl group in **6d** with pyridine group to generate analogue **6k** resulted in a big drop of potency. Maybe the pyridine unit was an ineffective

Table 2
Growth inhibition[a] effect of the synthesized compounds **6a**–**t** on 5 tumor cell lines

Compds	A549 cells	MCF-7 cells	HCT116 cells	Hela cells	2LL cells
	IC_{50} (μg/mL)				
6a	>100	n.t.[b]	n.t.	n.t.	n.t.
6b	>100	n.t.	n.t.	n.t.	n.t.
6c	>100	n.t.	n.t.	n.t.	n.t.
6d	45.3 ± 0.9	91.2 ± 0.8	85.0 ± 0.2	42.0 ± 1.1	48.5 ± 0.8
6e	80.5 ± 0.7	36.1 ± 0.2	46.1 ± 0.4	8.1 ± 0.3	30.5 ± 1.0
6f	59.4 ± 0.5	87.0 ± 0.2	>100	16.7 ± 1.2	58.3 ± 0.9
6g	68.1 ± 0.8	68.2 ± 0.4	49.9 ± 0.1	21.1 ± 1.2	66.8 ± 1.0
6h	>100	n.t.	n.t.	n.t.	n.t.
6i	43.1 ± 1.0	74.8 ± 0.3	80.1 ± 0.2	25.1 ± 1.2	40.2 ± 1.1
6j	97.7 ± 2.0	n.t.	n.t.	n.t.	n.t.
6k	>100	n.t.	n.t.	n.t.	n.t.
6l	85.3 ± 2.3	21.3 ± 2.6	26.6 ± 1.7	41.9 ± 1.5	29.3 ± 2.3
6m	27.8 ± 0.8	10.0 ± 1.3	20.5 ± 2.4	42.0 ± 2.0	17.6 ± 0.9
6n	56.2 ± 1.3	11.2 ± 1.4	17.3 ± 1.9	24.0 ± 0.9	17.1 ± 0.6
6o	96.1 ± 3.2	n.t.	n.t.	n.t.	n.t.
6p	>100	n.t.	n.t.	n.t.	n.t.
6q	80.4 ± 0.7	21.0 ± 1.8	25.0 ± 2.8	37.8 ± 1.2	32.9 ± 2.2
6r	>100	n.t.	n.t.	n.t.	n.t.
6s	63.3 ± 2.0	20.4 ± 2.2	21.3 ± 2.6	35.4 ± 1.6	19.3 ± 1.1
6t	>100	n.t.	n.t.	n.t.	n.t.
5-Fu	4.1 ± 0.6	12.1 ± 0.3	4.8 ± 0.1	5.7 ± 0.3	2.9 ± 0.8

[a] As measured by the MTT assay after 48 h incubation.
[b] n.t.: not tested.