Bioorganic & Medicinal Chemistry Letters 20 (2010) 1148–1152

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and structure–activity relationships of novel furazan-3,4-diamide analogs as potent anti-cancer agents

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ARTICLE INFO

Article history:
Received 8 October 2009
Revised 1 December 2009
Accepted 3 December 2009
Available online 6 December 2009

Keywords:
Anti-cancer agents
Drug discovery
Synthesis
Structure–activity relationship

ABSTRACT

This study describes the synthesis and structure–activity relationships of a series of furazan-3,4-diamide analogs. 1,2,5-Oxadiazole ring and electron-withdrawing substituent on the phenyl ring are proposed to be the important elements which contribute to a significant extent maximal potency of anti-proliferation effect.

The search of biologically promising new chemical entity (NCE)[1–6] against deadly cancer disease remains great attention in drug discovery. Major sources of bioactive NCE are identified from or inspired by natural products,[7,8] marine metabolites[9,10] and random screening of chemical library.[11–13] Furoxan **1** and benzofuroxan analogs **2** (Fig. 1) were recently found to be potent as the anti-cancer, anti-microbial, anti-aggregating, anti-ulcer, and immunosuppressive agents.[14–18] Similarly, biological studies revealed that oxadiazolopyrazines **3**, furazans **4**, and diaminofurazans **5** (Fig. 1) exhibited significant anti-bacterial properties and also showed the active use in the treatment of cancer, atherosclerosis, angiogenesis, neurodegenerative diseases, and inflammatory diseases.[19–24] Given that the potential of compounds **1–5** is confirmed as the therapeutic candidates, the 1,2,5-oxadiazole (furazan) moiety might represent a satisfactory pharmacophore to design anti-cancer agents. To test our initial hypothesis, we synthesized a set of furazan-3,4-diamide analogs **6** and evaluated the tumor cell growth inhibitory activity in two human cancer cell lines.

Initial synthesis of aliphatic and aromatic furazan-3,4-diamides, **7–11**, **33**, and **12–22** (Fig. 2), started with 3,4-diaminofurazan **34** and the corresponding acyl chloride (Scheme 1). Lewis acid ($BF_3{\cdot}Et_2O$)-mediated coupling reaction in dioxane at reflux gave the desired aliphatic furazan-3,4-diamides **7–11**/**33** and aromatic furazan-3,4-diamides **12–22** in high yields,[25] respectively. This method displays a significant improvement over the original synthesis of compound **7**, which required 2–6 equiv of 2,2,2-trichloroacetyl chloride in the presence of Et_3N and gave the product in low yield at room temperature.

The HPLC-purified furazan-3,4-diamides **7–22** were dissolved in DMSO and tested for growth-inhibitory potency in U-87 MG human glioblastoma and SW480 human colon adenocarcinoma cell

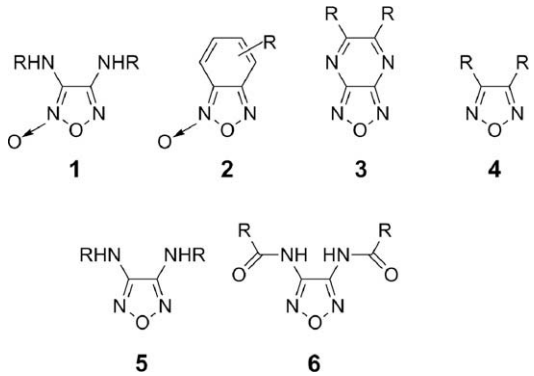

Figure 1. Six 1,2,5-oxadiazole derivatives.

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doi:10.1016/j.bmcl.2009.12.017

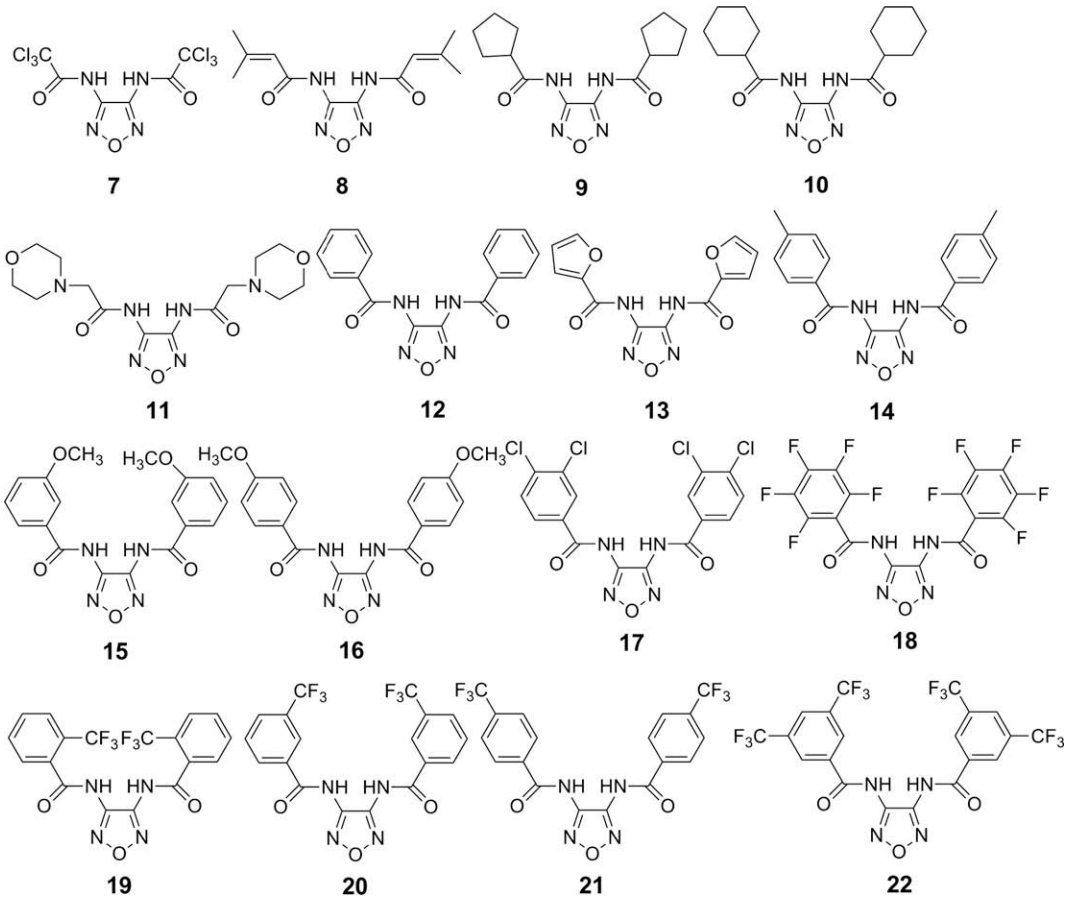
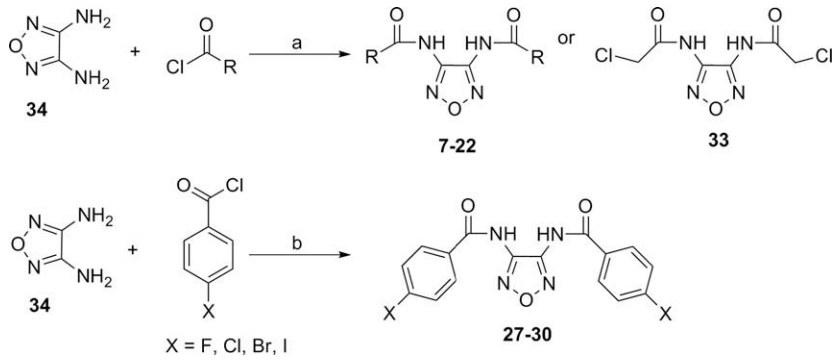


Figure 2. Rationale for the design of aliphatic and aromatic furazan-3,4-diamides (**7**–**11** and **12**–**22**).



Scheme 1. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, dioxane, reflux, 10 h, 55–90%; (b) MW, 5–10 min, 70–85%.

lines. The assay results, summarized in Table 1, show that unlike compounds **7**–**11** which exhibited weak activities, compound **33** having two chloroacetyl moieties possessed a significantly lower IC_{50} in both cell lines (IC_{50} = 17.1 and 7.4 μM) as compared to other aliphatic furazan-3,4-diamides (IC_{50} >200 μM). Efforts to analyze compound **33**-induced cellular pathway required for effective inhibition of cell growth are in progress.

In comparison with aliphatic furazan-3,4-diamides **7**–**11**, eleven aromatic furazan-3,4-diamides **12**–**22** showed similar or greater growth-inhibitory potency against U-87 MG and SW480 cells. Compound **12** with two phenyl substituents moderately inhibited cell growth with IC_{50} values of 121.6 and 137.0 μM, respectively, whereas, compound **13** bearing two furanyl groups displayed weak activity, suggesting that the phenyl moiety is an acceptable pharmacophore. Compound **21** having two 4-(trifluoromethyl)phenyl substituents was the most active compound with IC_{50} values of 14.6 and 11.2 μM against two cancer cell lines (Table 1). Similar to compound **21**, compounds **17**, **18**, and **22**, which bear the electron-withdrawing groups in the phenyl ring, gave promising results in inhibition of cell growth with IC_{50} values between 10 and 39 μM. Compounds **19** and **20** having the trifluoromethyl group at the C-2 and C-3 position on the phenyl ring possessed higher IC_{50} values in both cell lines and lower solubility as compared to the parent compound **21**. In comparison with **21**, three electron-

Table 1
In vitro antitumor activity for compounds **7**–**33**

Compds	IC_{50}[a] (μM)	
	U-87 MG	SW480
7	>200	>200
8	>200	>200
9	>200	>200
10	>20[b]	>20[b]
11	>200	>200
12	121.6 ± 4.2	137.0 ± 2.3
13	>200	>200
14	>40[b]	>40[b]
15	70.7 ± 15.3	71.0 ± 7.5
16	>40[b]	>40[b]
17	24.0 ± 3.8	39.7 ± 3.2
18	25.5 ± 1.2	17.2 ± 1.1
19	>100	89.3 ± 6.4
20	>10[b]	>10[b]
21	14.6 ± 2.3	11.2 ± 1.3
22	17.7 ± 0.8	10.8 ± 1.0
23	>8[b]	>8[b]
24	>8[b]	>8[b]
25	>200	>200
26	>200	>200
27	6.0 ± 1.0	10 (50%)[c]
28	14 (70%)[c]	8 (50%)[c]
29	14 (77%)[c]	4 (50%)[c]
30	1 (70%)[c]	2 (50%)[c]
31	18.2 ± 1.0	18.5 ± 1.1
32	20.6 ± 1.0	22.9 ± 2.0
33	17.1 ± 2.7	7.4 ± 1.1

[a] Amount of compound necessary to inhibit the growth of cancer cells by 50% in 48 h. Values are means of three experiments (IC_{50}, mean ± SEM, $n = 3$).
[b] Due to poor solubility, the value is expressed as the maximum concentration used in study.
[c] Compound became inactive at indicated concentration or greater. The percent (%) inhibition, in parentheses, is expressed as the maximum percent (%) inhibition of cells growth.

donating compounds **14**–**16** displayed weaker growth-inhibitory activities against two human cancer cell lines. Thus, these results indicate that the electron-withdrawing functionalities (Cl, F or CF_3) identified by screening were clearly required for the improved antitumor activity.

As shown in Table 1, two 4-(trifluoromethyl)phenyl substituents in the aromatic furazan-3,4-diamide series is critical for strong activity. To elucidate an initial structure–activity profile, our first aim was the design and synthesis of the suitable core structures of aromatic 3,4-diamides **23**–**25** (Fig. 3), for which we chose thiophene, benzene and 1*H*-pyrazol-5-ol to replace the 1,2,5-oxadiazole group. Employing the method of Lewis acid-mediated coupling reaction in Scheme 1, compounds **23**–**25** were synthesized by reacting 4-(trifluoromethyl)benzoyl chloride with thiophene-3,4-diamine, benzene-1,2-diamine, and 3,4-diamino-1*H*-pyrazol-5-ol, respectively, under reflux condition. Unluckily, this exercise did not improve the growth-inhibitory activity and diminished solubility in cellular solution was also found with compounds **23** and **24**. Compound **25** with a hydrophilic 3,4-diamino-1H-pyrazol-5-ol resulted in at least 14–18-fold less potent than **21**, indicating that the 1,2,5-oxadiazole core structure is essential for potency. Our results suggest that the core structure of aromatic 3,4-diamides would be preferred for the 1,2,5-oxadiazole ring.

To enhance the potency, we synthesized numerous analogs **27**–**30** (Fig. 4) with alterations of the trifluoromethyl substituent.[26] In addition, we next evaluate the effect of carbonyl group of amide bond in compound **21** for growth-inhibitory activity. The preparation starting with 3,4-diaminofurazan **34**, and a 4-(trifluoromethyl)benzoyl group was introduced by pyridine-mediated acylation to obtain a 4-aminofurazan-3-amides **26** in high yield (with trace **21**, Scheme 2). Amide **26** was subjected to reduction with lithium aluminum hydride followed by selective acylation on primary amine and purification through column chromatography, affording the asymmetrical 4-aminofurazan-3-amides **31**. For the reduction of the amide moiety of **31**, an excess of lithium aluminum hydride was used to give symmetrical furazan-3,4-diamine **32**.

As expected, compound **26** displayed a significant loss of growth-inhibitory activity compared to **21** (Table 1). The results suggest that the introduction of two 4-(trifluoromethyl)benzoyl groups into the 3,4-diaminofurazan **34** scaffold is clearly beneficial for activity. The replacement of the 4-trifluoromethyl group of **21** with other electron-withdrawing substituents (F, Cl, Br, and I) led to the improvement of activity. For example, the 4-fluorobenzoyl derivative **27** exhibited an IC_{50} of 6.0 μM against U-87 MG cells (Table 1, Fig. 5), representing a 2.4-fold improvement over **21** and 20-fold improvement over **12**. The concentrations of compounds **28**–**30** required to inhibit U-87 MG cells growth by 50% were lower (Table 1) as compared to compound **21**. Similar results were observed for **27**–**30** against SW480 cell line. Unfortunately, these compounds reached a plateau of effective potency and could not completely inhibit cancer cell growth under high dose condition or dose-dependent manner. For example, compound **30** having two 4-iodobenzoyl moieties possessed a significant anti-proliferation effect to inhibit 50% U-87 MG cells growth at the level of nanomolar range; whereas it became inactive at concentration of 1 μM or greater (inhibiting U-87 MG cells growth at the maximum of 70%, Table 1). Similar results were found for **27**–**30** against SW480 cell line. These observations gave us a clue that the hydrophobic trifluoromethyl group holds promise for effective potency.

To examine whether the carbonyl groups of **21** could affect the growth-inhibitory activity, we tested the anti-proliferation effect of compounds **31** and **32**. Slight diminished activity was observed with **31** and **32**, in which a carbonyl or two carbonyl groups were reduced into methylene moiety. For instance, compounds **31** and **32** are 1.2–1.4- and 1.7–2.0-fold less potent than **21** against U-87 MG and SW480 cells, respectively.

Altogether, the structure–activity relationship (SAR) study (Fig. 6) revealed that two aromatic amide substituents of analogs

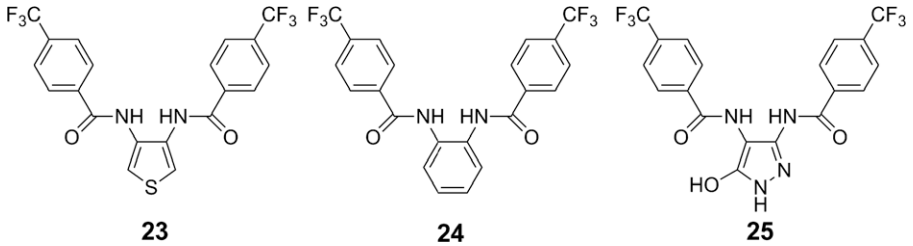


Figure 3. The core structures of aromatic 3,4-diamides **23**–**25** used in this study.

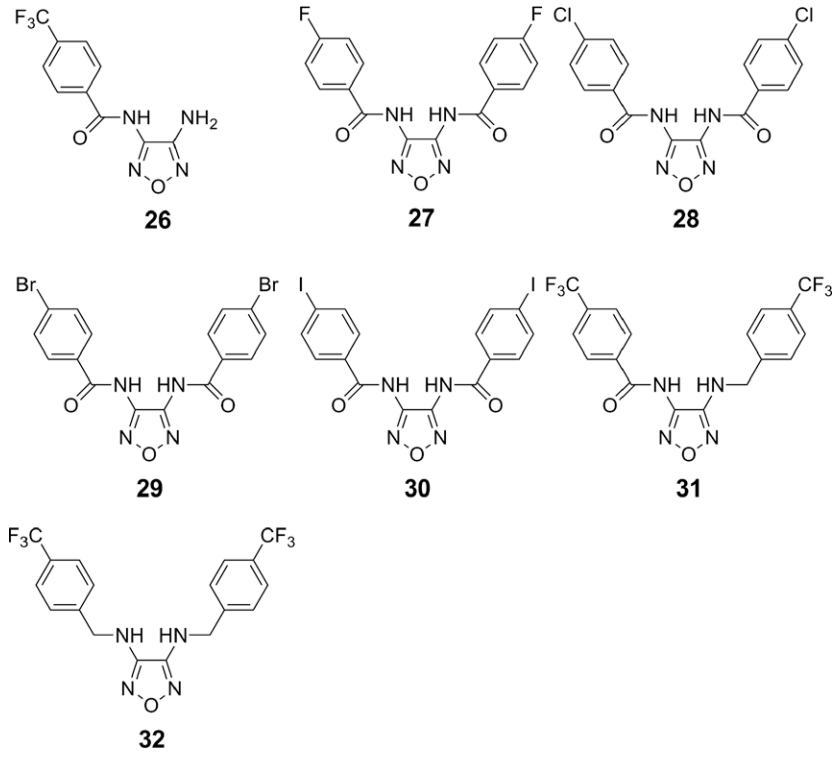
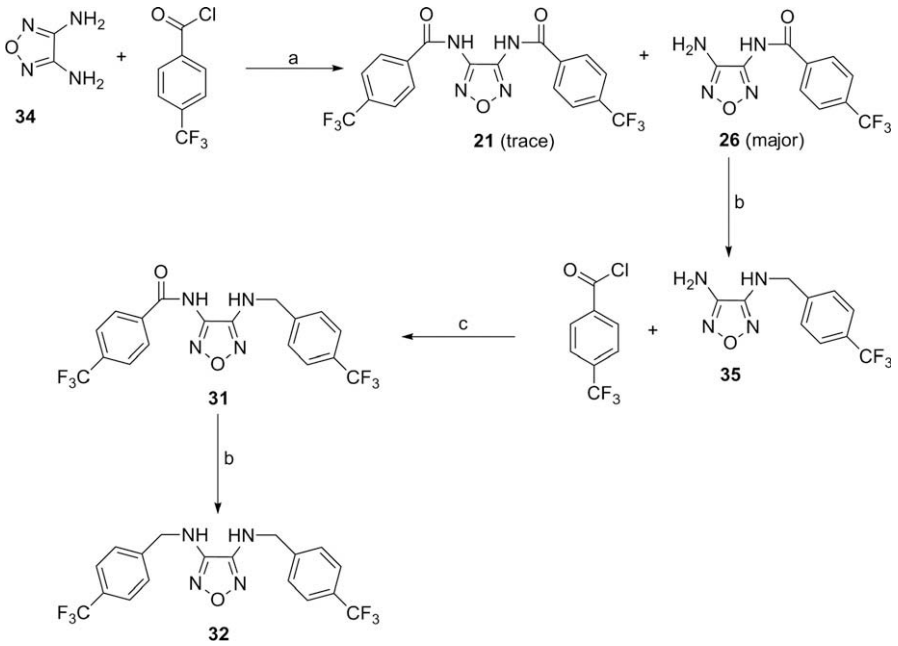


Figure 4. Symmetrical and asymmetrical 3,4-diaminofurazan derivatives **26**–**32** used in this study.



Scheme 2. Reagents and conditions: (a) pyridine, DCM, 0 °C to rt, 5 h, 70%; (b) LAH, THF, 0 °C to rt, 2 h, 70%; (c) MW, 4 min, 72%.

6 play an important role for inhibition of cancer cell growth. Electron-withdrawing substituents (CF_3 and F) in the *para*-position of phenyl ring are favorable for achieving anti-proliferation effect. A 1,2,5-Oxadiazole ring core structure is essential for the effective activity, whereas the existence of carbonyl groups is not critical for potency (Fig. 6).

In summary, we have identified bioactive NCEs from a set of furazan-3,4-diamide analogs by random screening and SAR approaches. This work not only established an effective route to synthesize diverse analogs of aliphatic and aromatic furazan-3,4-diamides but also provided a general strategy for discovering potent anti-cancer agents.

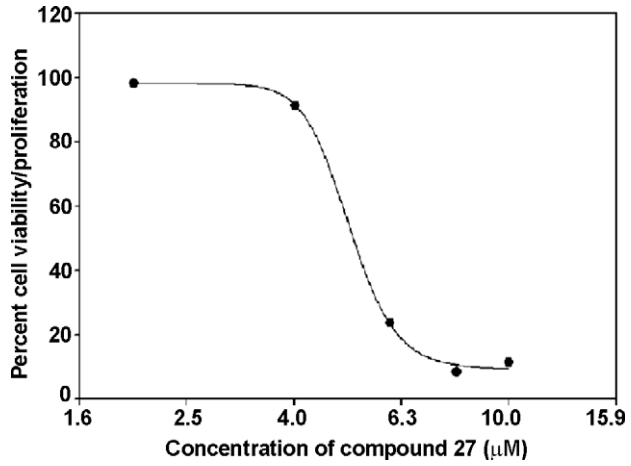


Figure 5. Dose-dependent anti-proliferative effect of compound **27** on U-87 MG cells.

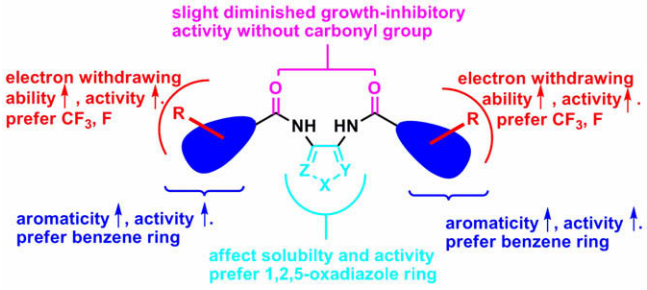


Figure 6. Schematic representation of structure–activity relationships based on diverse furazan-3,4-diamide analogs.

Acknowledgments

We are grateful to Ping-Yu Lin and Dr. Mei-Chun Tseng for assistance with mass spectrometry. We thank the National Science Council(NSC 95-2113-M-001-018-MY3) and Academia Sinica (program project AS-95-TP-AB1) for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.017.

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25. *A representative experiment for acid catalyzed reaction*: Benzoyl chloride (281 mg, 2 mmol) was mixed with dioxane (3 mL) and followed by addition of 3,4-diaminofurazan **34** (100 mg, 1 mmol) in dioxane (2 mL) and $BF_3{\cdot}Et_2O$ 6.78 mg (10 mol %). The reaction mixture was refluxed for 10 h (monitored by TLC). The crude residue was diluted with DCM (15 mL) and washed with water (2×10 mL). Organic layer was dried over anhydrous $MgSO_4$, filtered, evaporated, and the crude product was purified by column chromatography (SiO_2, 20% EtOAc/hexane) to afford compound **12** (262 mg, 85%).
26. *A representative experiment for MW condition*: In 5 mL sample vial, 3,4-diaminofurazan **34** (100 mg, 1 mmol) and 4-fluorobenzoyl chloride (317 mg, 2 mmol) were mixed. The sample vial was closed with Teflon cap and allowed to react at 100 °C in microwave for 5 min (monitored by TLC). The crude residue was directly purified by column chromatography (SiO_2, 20% EtOAc/hexane) to afford compound **27** (275 mg, 80%).