Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Synthesis and antitumor activity of ATB-429 derivatives containing a nitric oxide-releasing moiety

Chunlan Wang [a,†], Guimin Xia [a,†], Xiujun Liu [a,*], Rui Zhang [a], Yun Chai [a], Jun Zhang [a,b], Xiaoning Li [a], Yang Yang [a], Juxian Wang [a], Mingliang Liu [a,*]

[a] Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China
[b] Zhejiang Starry Pharmaceutical Co. Ltd, Xianju 317300, China

ARTICLE INFO

*Article history:*
Received 17 January 2016
Revised 25 February 2016
Accepted 4 March 2016
Available online xxxx

*Keywords:*
ATB-429 derivatives
Nitric oxide
Synthesis
Antitumor activity

ABSTRACT

A series of novel ATB-429 (an anti-inflammatory candidate) derivatives containing a nitric oxide (NO)-releasing moiety were designed, synthesized and evaluated for their in vitro activity against six human cancer cell lines. Our results reveal that phenylsulfonylfuroxan-based derivatives have considerable antitumor activity, and compounds **7**–**9** ($IC_{50}$s: 0.256–3.024 μM) against HT-29 and PANC-1, **8a**,**b** ($IC_{50}$s: 2.677–3.051 μM) against MCF-7 and **8a** ($IC_{50}$: 1.270 μM) against DU145 are more active than Vandetanib ($IC_{50}$s: 1.925–4.107 μM).



Nonsteroidal anti-inflammatory drugs (NSAIDs), in general, and aspirin, in particular, are recognized as the prototypical chemopreventive agents against many forms of cancers[1] and may serve as possible adjunct in cancer treatment.[2] However, NSAID-induced upper gastrointestinal (GI) side effects remain a major problem that affects a broad segment of the population, due to frequent prescription and over the counter dispensing.[3] And this has stimulated the search for alternative anti-inflammatory drugs that are safe for long-term use. Hydrogen sulfide ($H_2S$), nitric oxide (NO) and carbon monoxide (CO) form parts of a group of biologically active gases that are termed gasotransmitters or gasomediators.[4] It was reported that NO-releasing NSAIDs are indeed safer to the GI mucosa than the parent drugs.[5] Similarly, $H_2S$-releasing NSAIDs have been shown to be more effective than the parent drugs in reducing inflammation, while producing significantly less damage in the GI tract.[6,7] For example, ATB-429 (Fig. 1), a $H_2S$-releasing derivative of mesalamine (5-aminosalicylicacid, a first-line therapy for inflammatory bowel disease), exhibits a marked increase in anti-inflammatory activity and potency in a murine model of colitis and exerts antinociceptive effects in a model of post inflammatory hypersensitivity model of colitis.[6,8] On the other hand, many of NO-releasing aspirins and $H_2S$-releasing aspirins have proved to possess antitumor activity.[9,10] More recently, Kashfi et al. described a series of new NOSH (NO-, $H_2S$-releasing) aspirins (such as NOSH-1, Fig. 1) which are extremely effective in inhibiting the growth of different human cancer cell lines.[11] More interesting, different active compounds containing a novel phenylsulfonylfuroxan-based NO releasing moiety (such as hydrids **1** and **2**, Fig. 1) display potent antitumor activity both in vivo and in vitro.[12,13]

Receptor tyrosine kinases (RTKs) represent a large family of membrane-bound enzymes that play key roles in tumor growth, survival, and metastasis.[14] Inhibition of RTK pathways has become an important approach for the discovery of new anticancer drugs.[15] Many RTK inhibitors have been introduced to the market during the last decade, such as Sorafenib and Linifanib (Fig. 1) having a diaryl urea motif.[16–18]

Inspired by the above research results, we decided to make structural modifications on ATB-429 by introduction of a NO-releasing moiety and replacement of the amino group with an aryl urea unit in this study. Our primary object was to identify potent and novel antitumor compounds so as to lay a foundation for the further study.

Detailed synthetic pathways to the intermediate **5** and ATB-429 derivatives **7**–**10**, **13** and **14a**–**f** are shown in Schemes 1 and 2, respectively. Treatment of 5-(4-methoxyphenyl)-3*H*-1,2-dithiole-3-thione (ADT-$OCH_3$, **1**) with pyridine hydrochloride gave ADT-OH (**2**).[19] 5-Amino-2-hydroxybenzoic acid **3** was protected by reaction

* Corresponding authors.
*E-mail addresses:* liuxiujun2000@163.com (X. Liu), lmllyx@126.com (M. Liu).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmcl.2016.03.012
0960-894X/

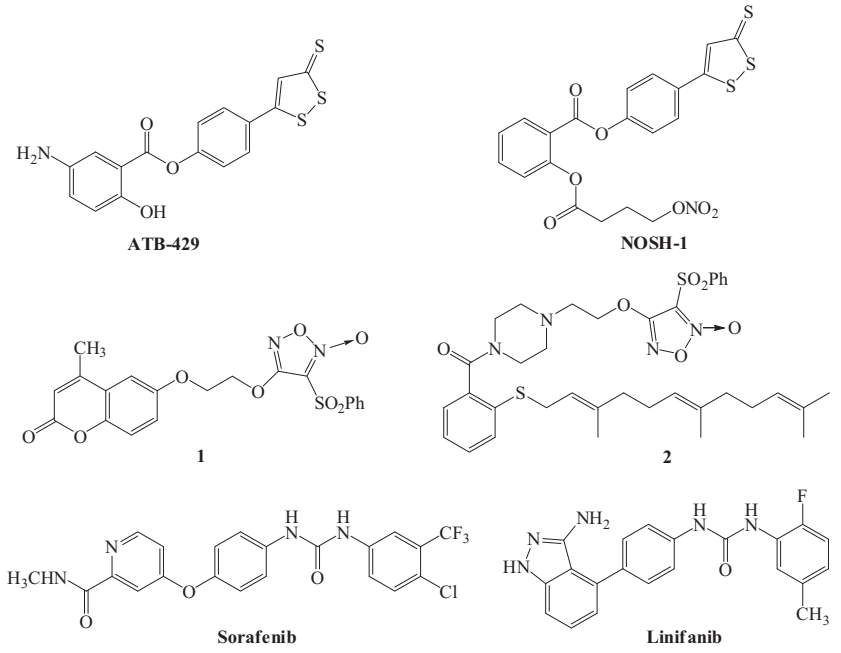
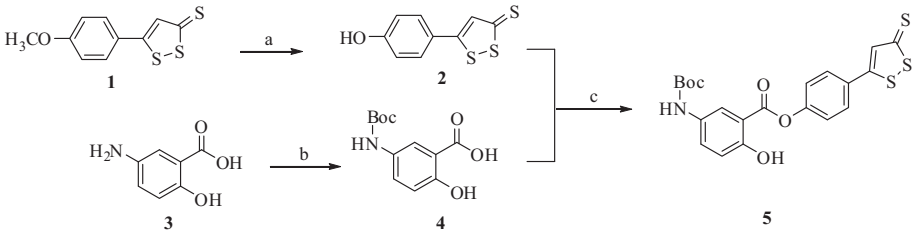



**Figure 1.** Structures of selected compounds.




**Scheme 1.** Synthesis of Boc-protected ATB-429 (**5**). Reagents and conditions: (a) $C_5H_5N$·HCl, 215 °C, 2 h, 80%; (b) $Boc_2O$, NaOH, dioxane/$H_2O$, rt, 4 h, 93%; (c) DCC/DMAP, $CH_2Cl_2$, 0 °C-rt, 8 h, 62%.

with di-tert-butyl dicarbonate ($Boc_2O$) produced compound **4**.[20] Esterification of ADT-OH and the acid **4** in the presence of dicyclohexylcarbodiimide (DCC)/4-dimethylaminopyridine (DMAP) yielded Boc-protected ATB-429 (**5**) (Scheme 1).

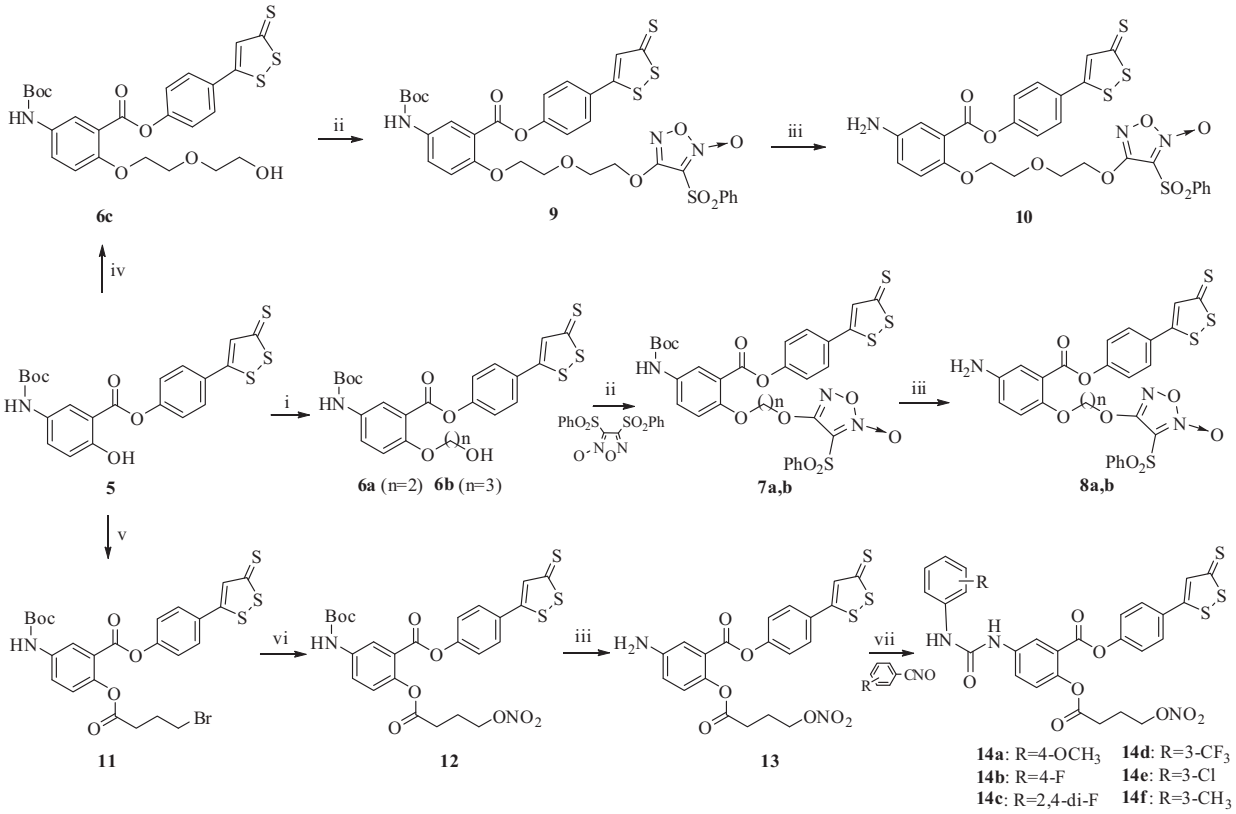
The Boc-protected ATB-429 (**5**) was treated with 2-bromoethan-1-ol, 3-bromopropan-1-ol or 2-(2-chloroethoxy)ethan-1-ol to provide the intermediates **6a–c**. Condensation of **6a**,**b** with 3,4-diphenylsulfonyl-1,2,5-oxadiazole 2-oxide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene(DBU) yielded compounds **7a**, **b**. The Boc-protecting group of **7a**,**b** was removed by pumping hydrogen chloride gas in $CH_2Cl_2$ to afford ATB-429 derivatives **8a**, **b**. The target compound **10** was obtained from **6c** in a similar manner as for the preparation of **8a**,**b** (Scheme 2).[23]

Esterification of the phenol **5** and 4-bromobutyric acid in the presence of DCC/DMAP yielded compound **11**. The bromo moiety in the bromide **11** was then substituted with nitrate using $AgNO_3$ to give compound **12**, on which the Boc-protecting group was removed by pumping hydrogen chloride gas in $CH_2Cl_2$ to afford amine **13**. Ureas **14a–f** were obtained by condensation of **13** with commercially available various phenyl isocyanates (Scheme 2).

For preliminary screening of antitumor candidates, all the newly synthesized ATB-429 derivatives **7–10**, **13** and **14a–f** were first investigated for cytotoxic activity in vitro against HT-29 (colon adenocarcinoma), PANC-1 (pancreatic carcinoma), MCF-7 (breast cancer), A549 (lung adenocarcinoma), DU-145 (prostate cancer) and HL60 (human leukemia), and the compounds having >100% inhibition against HT-29 or A549, and >90% inhibition against the other four cells at the concentration of 30 μM were subjected to $IC_{50}$s (50% inhibition concentrations) determination. Six target compounds **7–10** containing a phenylsulfonylfuroxan-based NO releasing moiety meeting this criterion (Table 1) were further evaluated for their in vitro antitumor activity in the above six cell lines by standard MTT assay.[21] The $IC_{50}$ values were compared with those of Vandetanib, a multi-targeted receptor TK inhibitor[22] (Table 2).

The selected ATB-429 derivatives **7–10** have potent in vitro antitumor activity against the tested cancer cell lines. Among them, compounds **7–9** ($IC_{50}$s: 0.256–3.024 μM) against HT-29 and PANC-1, **8a**,**b** ($IC_{50}$s: 2.677–3.051 μM) against MCF-7 and **8a** ($IC_{50}$: 1.270 μM) against DU145 are more active than Vandetanib ($IC_{50}$s: 1.925, 4.107, 3.536 and 1.974 μM against the four cell lines, respectively) (Table 2).

According to the biological evaluation results showed in Tables 1 and 2, antitumor activity of the ATB-429 derivatives **7–10**, **13** and **14a–f** depends mainly on the NO-releasing moiety. In general, the contribution of the phenylsulfonylfuroxan moiety to the antitumor activity is significantly greater than the corresponding nitrate one (—$ONO_2$) (**8a**,**b**, **10** vs **13**) (Table 1). Compound **13** and its derivatives **14a–f** having an aryl urea motif, contrary to expectation, have virtually no activity at 30 μM against the tested cell lines (Table 1). On the other hand, the antitumor activity of phenylsulfonylfuroxan-based ATB-429 derivatives is closely related to the linkers.




**Scheme 2.** Synthesis of ATB-429 derivatives **7**–**10**, **13** and **14a**–**f**. Reagents and conditions: (i) $Br(CH_2)_nOH$, $K_2CO_3$, DMF, reflux, 3 h, 44–50%; (ii) DBU, $CH_2Cl_2$, 0 °C-rt, 12 h, 35–38%; (iii) HCl (g), $CH_2Cl_2$, rt, 1 h, 50–90%; (iv) $Cl(CH_2)O(CH_2)_2OH$, $K_2CO_3$, DMF, reflux, 5 h, 42%; (v) $Br(CH_2)_3CO_2H$, DCC/DMAP, $CH_2Cl_2$, 0 °C-rt, 8 h, 69%; (vi) $AgNO_3$, $CH_3CN$, 70 °C, 3 h, 38%; (vii) $Et_3N$, $C_6H_5CH_3$, reflux, 4 h, 30–40%.

**Table 1**
In vitro activity of compounds **7**–**10**, **13** and **14a**–**f** against six cell lines (at 30 μM)

| Cell lines | % inhibition | | | | | |
|---|---|---|---|---|---|---|
| Compd | HT-29 | PANC1 | MCF-7 | A549 | DU145 | HL60 |
| **7a** | 102.87 | 97.12 | 83.12 | 88.45 | 61.60 | 84.62 |
| **7b** | 102.63 | 97.40 | 25.19 | −23.80 | 67.91 | 83.98 |
| **8a** | 101.92 | 96.64 | 90.13 | 100.42 | 93.81 | 92.33 |
| **8b** | 102.63 | 97.45 | 92.12 | 100.47 | 94.81 | 87.32 |
| **9** | 102.95 | 97.88 | 63.09 | 18.73 | 54.74 | 84.45 |
| **10** | 100.64 | 97.12 | 90.03 | 62.75 | 63.20 | 84.39 |
| **13** | −13.27 | −10.51 | −5.55 | −18.38 | −7.67 | 8.73 |
| **14a** | 12.11 | 2.19 | 15.97 | 3.19 | −6.39 | 8.30 |
| **14b** | 6.92 | −12.95 | 19.06 | −27.18 | 15.30 | 8.68 |
| **14c** | −6.41 | −14.80 | −6.87 | −28.03 | −7.47 | 0.43 |
| **14d** | 13.15 | −5.71 | −6.78 | −22.11 | −14.25 | 14.06 |
| **14e** | 21.53 | −17.26 | 6.10 | −23.29 | 5.49 | 10.90 |
| **14f** | 9.56 | −14.25 | 13.89 | −19.77 | −19.32 | 7.34 |

For example, compounds with an alkyl (ethyl or propyl) linker show much better activity for HT-29, PANC-1 and MCF-7 than the analogs containing an ethoxyethyl one ($IC_{50}$s: 0.559–3.051 μM for **8a**,**b** vs 5.437–11.894 μM for **10**) (Table 2), although all of the three have similar rates of growth inhibitions at 30 μM (Table 1). Moreover, the sizes of the alkyl linkers seem to have no significantly impact on the activity (**8a** vs **8b**) (Tables 1 and 2). Finally, some ATB-429 derivatives with a Boc moiety on the amino group at the C-5 position, by comparison, were found to be more active against some cell lines (**7a** vs **8a**, **9** vs **10**), which suggests that a carbamate moiety is permitted at the C-5 position of these phenylsulfonylfuroxan-basedATB-429 derivatives and would be more beneficial to the increase of the antitumor activity that could lay a solid foundation for the further study.

**Table 2**
In vitro activity of selected compounds **7**–**10** against six cell lines

| Cell lines | $IC_{50}$ (μM) | | | | | |
|---|---|---|---|---|---|---|
| Compd | HT-29 | PANC1 | MCF-7 | A549 | DU145 | HL60 |
| **7a** | 0.256 | 1.448 | – | – | – | – |
| **7b** | 0.926 | 3.024 | – | – | – | – |
| **8a** | 0.769 | 1.858 | 2.677 | 4.171 | 1.270 | 1.877 |
| **8b** | 0.559 | 0.971 | 3.051 | 3.275 | 2.236 | – |
| **9** | 0.536 | 2.797 | – | – | – | – |
| **10** | 5.437 | 8.002 | 11.894 | – | – | – |
| Vandetanib | 1.925 | 4.107 | 3.536 | 2.630 | 1.974 | 1.492 |

In summary, a series of novel ATB-429 derivatives containing a NO-releasing moiety were designed, synthesized and evaluated for their antitumor activity. Our results reveal that phenylsulfonyl-furoxan-based derivatives have potent antitumor activity, and compounds **7**–**9** ($IC_{50}$s: 0.256–3.024 μM) against HT-29 and PANC-1, **8a**,**b** ($IC_{50}$s: 2.677–3.051 μM) against MCF-7 and **8a** ($IC_{50}$: 1.270 μM) against DU145 are more active than Vandetanib ($IC_{50}$s: 1.925–4.107 μM).

## Acknowledgments

This work was supported by the National S&T Major Special Project on Major New Drug Innovations (2014ZX09507009-003, 2015ZX09102007) and NSFC 81373267-003, 21502237.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.03.

012. These data include MOL files and InChiKeys of the most important compounds described in this article.

## References and notes

1. Kashfi, K. *Adv. Pharmacol.* **2009**, *57*, 31.
2. Hinz, B.; Brune, K. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 367.
3. Wallace, J. L. *Baillieres Best Pract. Res. Clin. Gastroenterol.* **2000**, *14*, 147.
4. Li, L.; Hsu, A.; Moore, P. K. *Pharmacol. Ther.* **2009**, *123*, 386.
5. Fiorucci, S.; Santucci, L.; Gresele, P.; Faccino, R. M.; Del Soldato, P.; Morelli, A. *Gastroenterology* **2003**, *124*, 600.
6. Fiorucci, S.; Orlandi, S.; Mencarelli, A.; Caliendo, G.; Santagada, V.; Distrutti, E.; Santucci, L.; Cirino, G.; Wallace, J. L. *Br. J. Pharmacol.* **2007**, *150*, 996.
7. Wallace, J. L.; Caliendo, G.; Santagada, V.; Cirino, G. *Br. J. Pharmacol.* **2010**, *159*, 1236.
8. Distrutti, E.; Sediari, L.; Mencarelli, A.; Renga, B.; Orlandi, S.; Russo, G.; Caliendo, G.; Santagada, V.; Cirino, G.; Wallace, J. L.; Fiorucci, S. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 447.
9. Chattopadhyay, M.; Kodela, R.; Nath, N.; Barsegian, A.; Boring, D.; Kashfi, K. *Biochem. Pharmacol.* **2012**, *83*, 723.
10. Hulsman, N.; Medema, J. P.; Bos, C.; Jongejan, A.; Leurs, R.; Smit, M. J.; de Esch, I. J.; Richel, D.; Wijtmans, M. *J. Med. Chem.* **2007**, *50*, 2424.
11. Kodela, R.; Chattopadhyay, M.; Kashfi, K. *ACS Med. Chem. Lett.* **2012**, *3*, 257.
12. Han, C.; Huang, Z.; Zheng, C.; Wan, L.; Zhang, L.; Peng, S.; Ding, K.; Ji, H.; Tian, J.; Zhang, Y. *J. Med. Chem.* **2013**, *56*, 4738.
13. Liu, M. M.; Chen, X. Y.; Huang, Y. Q.; Feng, P.; Guo, Y. L.; Yang, G.; Chen, Y. *J. Med. Chem.* **2014**, *57*, 9343.
14. Khanwelkar, R. R.; Chen, G. S.; Wang, H. C.; Yu, C. W.; Huang, C. H.; Lee, O.; Chen, C. H.; Hwang, C. S.; Ko, C. H.; Chou, N. T.; Lin, M. W.; Wang, L. M.; Chen, Y. C.; Hseu, T. H.; Chang, C. N.; Hsu, H. C.; Lin, H. C.; Shih, Y. C.; Chou, S. H.; Tseng, H. W.; Liu, C. P.; Tu, C. M.; Hu, T. L.; Tsai, Y. J.; Chern, J. W. *Bioorg. Med. Chem.* **2010**, *18*, 4674.
15. Gschwind, A.; Fisher, O. M.; Ullrich, A. *Nat. Rev. Cancer.* **2004**, *4*, 361.
16. Ng, R.; Chen, E. X. *Curr. Clin. Pharmacol.* **2006**, *1*, 223.
17. Dai, Y.; Hartandi, K.; Ji, Z.; Ahmed, A. A.; Albert, D. H.; Bauch, J. L.; Bouska, J. J.; Bousquet, P. F.; Cunha, G. A.; Glaser, K. B.; Harris, C. M.; Hickman, D.; Guo, J.; Li, J.; Marcotte, P. A.; Marsh, K. C.; Moskey, M. D.; Martin, R. L.; Olson, A. M.; Osterling, D. J.; Pease, L. J.; Soni, N. B.; Stewart, K. D.; Stoll, V. S.; Tapang, P.; Reuter, D. R.; Davidsen, S. K.; Michaelides, M. R. *J. Med. Chem.* **2007**, *50*, 1584.
18. Albert, D. H.; Tapang, P.; Magoc, T. J.; Pease, L. J.; Reuter, D. R.; Wei, R. Q.; Li, J.; Guo, J.; Bousquet, P. F.; Ghoreishi-Haack, N. S.; Wang, B.; Bukofzer, G. T.; Wang, Y. C.; Stavropoulos, J. A.; Hartandi, K.; Niquette, A. L.; Soni, N.; Johnson, E. F.; McCall, J. O.; Bouska, J. J.; Luo, Y.; Donawho, C. K.; Dai, Y.; Marcotte, P. A.; Glaser, K. B.; Michaelides, M. R.; Davidsen, S. K. *Mol. Cancer Ther.* **2006**, *5*, 995.
19. Chen, P.; Luo, Y.; Hai, L.; Qian, S.; Wu, Y. *Eur. J. Med. Chem.* **2010**, *45*, 3005.
20. Wang, C.; Shi, W.; Cai, L.; Lu, L.; Wang, Q.; Zhang, T.; Li, J.; Zhang, Z.; Wang, K.; Xu, L.; Jiang, X.; Jiang, S.; Liu, K. *J. Med. Chem.* **2013**, *56*, 2527.
21. Green, L. M.; Reade, J. L.; Ware, C. F. *J. Immunol. Methods* **1984**, *70*, 257.
22. Commander, H.; Whiteside, G.; Perry, C. *Drugs* **2011**, *71*, 1355.
23. A mixture of **2**[19] (2.3 g, 10 mmol), **4**[20] (3.1 g, 12 mmol), DCC (2.5 g, 12 mmol) and DMAP (0.61 g, 5 mmol) in $CH_2Cl_2$ (150 mL) was stirred for 8 h at room temperature under nitrogen atmosphere and then filtered. The filtrate was washed with saturated brine (100 mL) and concentrated under reduced pressure. The residue was recrystallized from MeOH to get compound **5** (2.9 g, 62.8%) as a yellow solid, mp 162–164 °C.
A mixture of **5** (462 mg, 1 mmol), 2-bromoethanol/3-bromopropanol/2-(2-chloroethoxy)ethanol (1.2 mmol), $K_2CO_3$ (276 mg, 2 mmol), NaI (14.9 mg, 0.1 mmol) in anhydrous DMF (8 mL) was stirred for 3 h under refluxing, and poured into ice water and then extracted with EtOAc (5 mL × 3). The combined extracts were dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to afford crude compounds **6a–c**.
A mixture of the above crude compounds **6a–c**, 3,4-diphenylsulfonyl-1,2,5-oxadiazole 2-Oxide (1.2 mmol), DBU (2 mmol) and $CH_2Cl_2$ (8 mL) was stirred for 12 h at room temperature, washed with water (10 mL × 3) and then concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) eluting with$CH_2Cl_2$/MeOH (200:1, v/v) to get **7a**,**b** and **9** (20–25%, for two steps) as orange solids.
To a solution of **7a**,**b** or **9** (1 mmol) in $CH_2Cl_2$ (10 mL) was pumped dried hydrochloride gas for 1 h at room temperature. The precipitate was collected by suction, washed with $CH_2Cl_2$, and dried under vacuum to give **8a**,**b** or **10** (50–80%) as orange solids.
A mixture of **5** (3.0 g, 6 mmol), 4-bromobutyric acid (1.3 g, 8 mmol), DCC (1.6 g, 8 mmol) and DMAP (0.4 g, 3 mmol) in $CH_2Cl_2$ (150 mL) was stirred for 8 h at room temperature under nitrogen atmosphere and then filtered. The filtrate was washed with saturated brine (100 mL) and concentrated under reduced pressure. The residue was recrystallized from a ether to afford **11** (2.7 g, 68.7%) as a yellow solid.
A mixture of **11** (1.4 g, 2 mmol), $AgNO_3$ (1.0 g, 6 mmol) in anhydrous $CH_3CN$ was stirred for 3 h under refluxing in the dark, cooled to room temperature and then filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (silica gel) eluting with EtOAc/petroleum ether (1:5, v/v) to afford **12** (0.5 g, 38%) as an red oil. Compound **13** was obtained from **12** in a similar manner as for the preparation of **8a**,**b** (1.4 g, 90%) as an orange solid.
A solution of **13** (530 mg, 1 mmol), isocyanates (2 mmol) and $Et_3N$ (0.5 mL, 3 mmol) in toluene (8 mL) was stirred for 4 h under refluxing and then filtered. The solid was purified by column chromatography (silica gel) eluting with $CH_2Cl_2$/MeOH (100:1, v/v) to get target compounds **14a–f** (30–40%) as orange solids.
Compound **7a** (24.5%, from **5**), mp 155–156 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.74 (1H, s, –NH), 8.20 (1H, d, $J$ = 2.5 Hz, Ar-H), 7.97 (1H, dd, $J$ = 8.5, 2.5 Hz, Ar-H), 7.81–7.72 (5H, m, Ar-H), 7.69 (1H, s, =CH), 7.62–7.59 (2H, m, Ar-H), 7.24 (1H, d, $J$ = 8.5 Hz, Ar-H), 6.84–6.80 (2H, m, Ar-H), 4.48 (2H, t, $J$ = 5.0 Hz, —$OCH_2$), 4.37 (2H, t, $J$ = 5.0 Hz, —$OCH_2$), 1.49 (9H, s, —$C(CH_3)_3$). MS-ESI ($m/z$): 730.2 (M+H)$^+$. Compound **7b** (20%, from **5**), mp 170–172 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.72 (1H, s, —NH), 8.16 (d, $J$ = 2.5 Hz, 1H), 7.99–7.95 (2H, m, Ar-H), 7.87 (1H, dd, $J$ = 8.5, 2.5 Hz, Ar-H), 7.82–7.78 (2H, m, Ar-H), 7.73–7.71 (3H, m, Ar-H), 7.69 (1H, s, =CH), 7.20 (1H, d, $J$ = 8.5 Hz, Ar-H), 6.89 (2H, m, Ar-H), 4.23 (2H, t, $J$ = 6.0 Hz, —$OCH_2$), 4.19 (2H, t, $J$ = 6.0 Hz, —$CH_2OH$), 2.05–1.94 (2H, m, —$CH_2$—), 1.49 (9H, s, —$C(CH_3)_3$). MS-ESI ($m/z$): 744.2 (M+H)$^+$. Compound **9** (25%, from **5**), mp 175–177 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.71 (1H, s, —NH), 8.17 (1H, d, $J$ = 2.0 Hz, Ar-H), 8.00–7.97 (2H, m, Ar-H), 7.88–7.86 (2H, m, Ar-H), 7.85 (1H, dd, $J$ = 8.0, 2.0 Hz, Ar-H), 7.74 (1H, s, =CH), 7.74–7.68 (3H, m, Ar-H), 7.20 (1H, d, $J$ = 8.0 Hz, Ar-H), 6.95–6.91 (2H, m, Ar-H), 4.43 (2H, t, $J$ = 5.0 Hz, —$CH_2$—), 4.25 (2H, t, $J$ = 5.0 Hz, —$CH_2$—), 3.70 (2H, t, $J$ = 5.0 Hz, —$CH_2$—), 3.59 (2H, t, $J$ = 5.0 Hz, —$CH_2$—), 1.48 (9H, s, —$C(CH_3)_3$). MS-ESI ($m/z$): 774.1 (M+H)$^+$.
Compound **8a** (75.5%), mp 95–96 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.98–7.95 (2H, m, Ar-H), 7.83–7.80 (1H, m, Ar-H), 7.72–7.70 (2H, m, Ar-H), 7.67 (1H, s, =CH), 7.64–7.61 (2H, m, Ar-H), 7.21 (1H, d, $J$ = 2.5 Hz, Ar-H), 6.98 (1H, d, $J$ = 8.5 Hz, Ar-H), 6.91 (1H, dd, $J$ = 8.5, 2.5 Hz, Ar-H), 6.79–6.77 (2H, m, Ar-H), 5.53 (2H, s, —$NH_2$), 4.43 (2H, dd, $J$ = 5.0, 3.0 Hz, —$OCH_2$), 4.31 (2H, dd, $J$ = 5.0, 3.0 Hz, —$OCH_2$). $^{13}$C NMR (500 MHz, DMSO-$d_6$) $\delta$ 214.65, 173.04, 164.52, 162.67, 158.15, 146.68, 142.10, 136.97, 135.95, 134.10, 129.78, 128.61, 128.17, 124.21, 124.07, 123.40, 119.35, 115.90, 115.44, 109.99, 68.89, 61.84. MS-ESI ($m/z$): 630.0 (M+H)$^+$. HRMS-ESI ($m/z$): Calcd. for $C_{26}H_{19}N_3O_8S_4$ (M+H)$^+$: 630.0055; Found: 630.0100. Compound **8b** (80%), mp 103–105 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.99–7.97 (2H, m, Ar-H), 7.90–7.87 (1H, m, Ar-H), 7.79–7.77 (2H, m, Ar-H), 7.74–7.71 (2H, m, Ar-H), 7.68 (1H, s, =CH), 7.17 (1H, d, $J$ = 2.0 Hz, Ar-H), 6.94 (1H, d, $J$ = 8.0 Hz, Ar-H), 6.87 (1H, dd, $J$ = 8.0, 2.0 Hz, Ar-H), 6.86–6.83 (2H, m, Ar-H), 5.49 (2H, s, —$NH_2$), 4.19 (2H, t, $J$ = 6.0 Hz, —$OCH_2$), 4.15 (2H, t, $J$ = 6.0 Hz, —$OCH_2$), 1.95 (2H, m, —$CH_2$—). $^{13}$C NMR (500 MHz, DMSO-$d_6$) $\delta$ 214.81, 173.35, 164.95, 162.87, 158.53, 146.80, 142.16, 137.15, 136.16, 134.34, 130.04, 129.00, 128.30, 124.39, 124.25, 123.83, 119.32, 116.12, 115.50, 110.29, 67.77, 60.76, 27.26. MS-ESI ($m/z$): 644.1 (M+H)$^+$. HRMS-ESI ($m/z$): Calcd. for $C_{27}H_{21}N_3O_8S_4$ (M+H)$^+$: 644.0211; Found: 644.0256. Compound **10** (50%), mp 118–120 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.98–7.96 (2H, m, Ar-H), 7.85–7.83 (1H, m, Ar-H), 7.71 (1H, s, =CH), 7.70–7.67 (2H, m, Ar-H), 7.12 (1H, d, $J$ = 2.0 Hz, Ar-H), 6.93 (1H, d, $J$ = 8.0 Hz, Ar-H), 6.88–6.86 (2H, m, Ar-H), 6.84 (1H, dd, $J$ = 8.0, 2.0 Hz, Ar-H), 5.45 (2H, s, —$NH_2$), 4.41 (2H, t, $J$ = 5.0 Hz, —$CH_2$—), 4.19 (2H, t, $J$ = 5.0 Hz, —$CH_2$—), 3.66 (2H, t, $J$ = 5.0 Hz, —$CH_2$—), 3.54 (2H, t, $J$ = 5.0 Hz, —$CH_2$—). $^{13}$C NMR (500 MHz, DMSO-$d_6$) $\delta$ 214.85, 173.55, 164.77, 162.97, 158.82, 146.72, 142.28, 137.18, 136.10, 134.37, 129.97, 129.01, 128.29, 124.33, 124.24, 123.90, 119.21, 116.29, 115.35, 110.47, 70.76, 68.31, 67.71, 63.95. MS-ESI ($m/z$): 674.1 (M+H)$^+$. Compound **11**, mp 199–201 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.73 (1H, s, —NH), 8.38 (1H, d, $J$ = 2.5 Hz, Ar-H), 8.06–8.03 (2H, m, Ar-H), 7.86 (1H, s, =CH), 7.76 (1H, dd, $J$ = 8.8, 2.5 Hz, Ar-H), 7.44–7.40 (2H, m, Ar-H), 7.27 (1H, d, $J$ = 8.8 Hz, Ar-H), 3.58 (2H, t, $J$ = 6.5 Hz, —$CH_2$Br), 2.71 (2H, t, $J$ = 7.5 Hz, —$COCH_2$), 2.16–2.10 (2H, m, —$CH_2$—), 1.50 (9H, s, —$C(CH_3)_3$). MS-ESI ($m/z$): 612.1 (M+H)$^+$. Compound **12**, $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.73 (1H, s, —NH), 8.38 (1H, d, $J$ = 2.5 Hz, Ar-H), 8.06–8.01 (2H, m, Ar-H), 7.86 (1H, s, =CH), 7.76 (1H, dd, $J$ = 8.8, 2.5 Hz, Ar-H), 7.44–7.38 (2H, m, Ar-H), 7.26 (1H, d, $J$ = 8.8 Hz, Ar-H), 4.56 (2H, t, $J$ = 6.5 Hz, —$ONO_2CH_2$), 2.70 (2H, t, $J$ = 7.0 Hz, —$COCH_2$), 1.98–2.03 (2H, m, —$CH_2$—), 1.50 (9H, s, —$C(CH_3)_3$). MS-ESI ($m/z$): 593.1 (M+H)$^+$. Compound **13**, mp 160–162 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.02–8.00 (2H, m, Ar-H), 7.85 (1H, s, =CH), 7.41–7.37 (2H, m, Ar-H), 7.36 (1H, d, $J$ = 2.7 Hz, Ar-H), 6.98 (1H, d, $J$ = 8.7 Hz, Ar-H), 6.91 (1H, dd, $J$ = 8.7, 2.8 Hz, Ar-H), 5.50 (2H, s, —$NH_2$), 4.55 (2H, t, $J$ = 6.5 Hz, —$ONO_2CH_2$), 2.64 (2H, t, $J$ = 7.0 Hz, —$COCH_2$), 2.01–1.96 (2H, m, —$CH_2$—). MS-ESI ($m/z$): 493.0 (M+H)$^+$. Compound **14a** (38.5%), mp 187–188 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.01 (1H, s, —NH), 8.58 (1H, s, —NH), 8.35 (1H, d, $J$ = 2.7 Hz, Ar-H), 8.05–8.02 (2H, m, Ar-H), 7.87 (1H, s, =CH), 7.77 (1H, dd, $J$ = 8.8, 2.7 Hz, Ar-H), 7.45–7.42 (2H, m, Ar-H), 7.38–7.35 (2H, m, Ar-H), 7.27 (1H, d, $J$ = 8.8 Hz, Ar-H), 6.90–6.86 (2H, m, Ar-H), 4.56 (2H, t, $J$ = 6.5 Hz, —$ONO_2CH_2$), 3.72 (3H, s, —$OCH_3$), 2.70 (2H, t, $J$ = 7.0 Hz, —$COCH_2$), 2.02–1.97 (2H, m, —$CH_2$—). $^{13}$C NMR (500 MHz, DMSO-$d_6$) $\delta$ 215.74, 172.87, 171.56, 162.44, 154.87, 153.32, 152.95, 144.66, 138.40, 136.12, 132.53, 129.42, 129.07, 124.71, 123.37, 121.75, 120.83, 120.60, 114.16, 72.77, 55.36, 29.82, 21.62. MS-ESI ($m/z$): 642.0 (M+H)$^+$. HRMS-ESI ($m/z$): Calcd. for $C_{28}H_{23}N_3O_9S_3$ (M+H)$^+$: 642.0596; Found: 642.0686. Compound **14b** (35.5%), mp 189–191 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.10 (1H, s, —NH), 8.83 (1H, s, —NH), 8.36 (1H, d, $J$ = 2.4 Hz, Ar-H), 8.03–8.05 (2H, m, Ar-H), 7.87 (1H, s, =CH), 7.78 (1H, dd, $J$ = 8.8, 2.5 Hz, Ar-H), 7.49 (2H, m, Ar-H), 7.42–7.44 (2H, m, Ar-H), 7.28 (1H, d, $J$ = 8.8 Hz, Ar-H), 7.14 (2H, t, $J$ = 8.8 Hz, Ar-H), 4.56 (2H, t, $J$ = 6.5 Hz, —$ONO_2$-$CH_2$), 2.70 (2H, t, $J$ = 7.0 Hz, —$COCH_2$), 2.08–1.96 (2H, m, —$CH_2$—). $^{13}$C NMR (500 MHz, DMSO-$d_6$) $\delta$ 215.99, 173.13, 171.82, 162.68, 158.93, 157.03, 153.58, 153.15, 145.11, 138.45, 136.39, 136.17, 129.69, 129.34, 125.09, 123.63, 122.06, 121.29, 120.83, 115.77, 73.04, 30.10, 21.89. MS-ESI ($m/z$): 630.0 (M+H)$^+$. HRMS-ESI ($m/z$): Calcd. for $C_{27}H_{20}FN_3O_8S_3$ (M+H)$^+$: 630.0397; Found: 630.0498. Compound **14c** (32.5%), mp 181–183 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.41 (1H, s, —NH), 8.58 (1H, s, —NH), 8.36 (1H, d, $J$ = 2.6 Hz, Ar-H), 8.03 (3H, m, Ar-H), 7.87 (1H, s, =CH), 7.77 (1H, dd, $J$ = 8.8, 2.6 Hz, Ar-H),

7.44 (2H, m, Ar-H), 7.29 (2H d, $J$ = 8.8 Hz, Ar-H), 7.06 (1H, t, $J$ = 9.0 Hz, Ar-H), 4.56 (2H, t, $J$ = 6.5 Hz, —$ONO_2CH_2$), 2.71 (2H, t, $J$ = 7.0 Hz, —$COCH_2$), 2.03–1.98 (2H, m, —$CH_2$—). MS-ESI ($m/z$): 648.0 $(M+H)^+$. Compound **14d** (34.5%), mp 170–172 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.24 (1H, s, —NH), 9.18 (1H, s, —NH), 8.37 (1H, d, $J$ = 2.7 Hz, Ar-H), 8.05–8.01 (3H, m, Ar-H), 7.87 (1H, s, =CH), 7.82 (1H, dd, $J$ = 8.8, 2.7 Hz, Ar-H), 7.63 (1H, d, $J$ = 8.9 Hz, Ar-H), 7.53 (1H, t, $J$ = 8.0 Hz, Ar-H), 7.45–7.42 (2H, m, Ar-H), 7.34 (1H, d, $J$ = 7.7 Hz, Ar-H), 7.30 (1H, d, $J$ = 8.8 Hz, Ar-H), 4.56 (2H, t, $J$ = 6.5 Hz, —$ONO_2CH_2$), 2.71 (2H, t, $J$ = 7.0 Hz, —$COCH_2$), 2.03–1.98 (2H, m, —$CH_2$—). MS-ESI ($m/z$): 680.0 $(M+H)^+$. Compound **14e** (31.5%), mp 168–170 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.23 (1H, s, —NH), 9.06 (1H, s, —NH), 8.37 (1H, d, $J$ = 2.7 Hz, Ar-H), 8.04 (2H, d, $J$ = 8.7 Hz, Ar-H), 7.87 (1H, s, =CH), 7.79 (1H, dd, $J$ = 8.8, 2.7 Hz, Ar-H), 7.72 (1H, s, Ar-H), 7.46–7.42 (2H, m, Ar-H), 7.28–7.32 (3H, m, Ar-H), 7.05–7.02 (1H, m, Ar-H), 4.56 (2H, t, $J$ = 6.5 Hz, —$ONO_2CH_2$), 2.71 (2H, t, $J$ = 7.0 Hz, —$COCH_2$), 2.03–1.98 (2H, m, —$CH_2$—). MS-ESI ($m/z$): 646.0 $(M+H)^+$. Compound **14f** (31.5%), mp 178–180 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.08 (1H, s, —NH), 8.70 (1H, s, —NH), 8.37 (1H, d, $J$ = 2.7 Hz, Ar-H), 8.05–8.02 (2H, m, Ar-H), 7.87 (1H, s, =CH), 7.77 (1H, dd, $J$ = 8.8, 2.7 Hz, Ar-H), 7.46–7.42 (2H, m, Ar-H), 7.33 (1H, s, Ar-H), 7.28 (1H, d, $J$ = 8.8 Hz, Ar-H), 7.24 (1H, d, $J$ = 7.7 Hz, Ar-H), 7.17 (1H, t, $J$ = 7.8 Hz, Ar-H), 6.81 (1H, d, $J$ = 7.4 Hz, Ar-H), 4.56 (2H, t, $J$ = 6.5 Hz, —$ONO_2CH_2$), 2.70 (2H, t, $J$ = 7.0 Hz, —$COCH_2$), 2.28 (3H, s, —$CH_3$), 2.04–1.97 (2H, m, —$CH_2$—). MS-ESI ($m/z$): 626.1 $(M+H)^+$.

Please cite this article in press as: Wang, C.; et al. *Bioorg. Med. Chem. Lett.* (2016), http://dx.doi.org/10.1016/j.bmcl.2016.03.012