ACS Medicinal Chemistry Letters

Novel nitric oxide donors of phenylsulfonylfuroxan and 3-benzyl coumarin derivatives as potent antitumor agents

Yalan Guo, Yujie Wang, Haihong Li, Ke Wang, Qi Wan, Jia Li, Yubo Zhou, and Ying Chen

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.8b00125 • Publication Date (Web): 20 Apr 2018

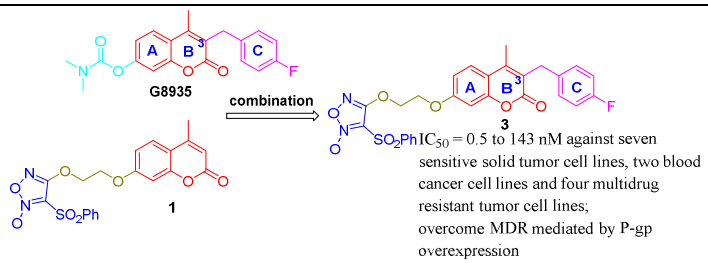
Novel nitric oxide donors of phenylsulfonylfuroxan and 3-benzyl coumarin derivatives as potent antitumor agents

Yalan Guo,[†] Yujie Wang,[‡] Haihong Li,[†] Ke Wang,[†] Qi Wan,[†] Jia Li,[‡] Yubo Zhou,[‡*] Ying Chen[†*]

†Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai, 201203, China

‡Chinese National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

KEYWORDS: Phenylsulfonylfuroxan, 3-benzyl coumarin, Anti-cancer, Multi-drug resistance, P-gp overexpression



ABSTRACT: In this work, five new hybrids of phenylsulfonylfuroxan merging 3-benzyl coumarin and their seco-B-ring derivatives **2-6** were designed and synthesized. Among them, compound **3** showed the most potent antiproliferation activities with IC_{50} values range from 0.5 nM to 143 nM against nine drug-sensitive and four drug-resistant cancer cell lines. Preliminary pharmacologic studies showed that these compounds displayed lower toxicities than that of lead compound **1**. Compound **3** obviously induced the early apoptosis and hardly affected the cell cycle of A2780, which was significantly different from compound **1**. Especially, it gave 559 and 294 folds selectivity antiproliferation activity in P-gp overexpressed drug-resistant cancer cell lines MCF-7/ADR and KB-V compared to their drug-sensitive ones MCF-7 and KB, implying that compounds **2-6** might have an extra mechanism of anti-MDR-cancer with P-gp overexpression.

Nitric oxide (NO), which was reported by Furchgott and Zawadzki as an endothelium-derived relaxing factor (EDRF) in 1980,[1-2] plays important roles in diverse physiological and pathophysiological processes.[3-8] Additionally, NO can down-regulate PI3K/Akt pathway and up-regulate MEK/ERK pathway.[9-11] Hideo Baba et al. reported that the combined administration of NO donor and MEK inhibitor can synergistically inhibit the viability of cancer cells through downregulating both PI3K/ Akt and MEK/ERK pathways.[12] (3,4-Bis(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (phenylsulfonylfuroxan) as an important NO donor, was widely used in the design of anticancer agents.[13-17] We previously reported that phenylsulfonylfuroxan and coumarin hybrid **1** showed remarkably antitumor activity through multi-target mechanism containing disruption of MEK pathway. However, its MEK inhibitory activity was relatively weak.[18] Considering 4-fluorobenzyl at 3-position of coumarin skeleton in G8935, which was a MEK inhibitor[19-20], occupied a new binding pocket in the MEK docking model,[21] we also introduced several benzyl groups covering 4-fluorobenzyl to the same position of lead compound **1** and obtained five new derivatives (**2-6**, **Scheme 1**). The structure optimization aimed at developing stronger synergistic antiproliferation activity with both NO donor and MEK inhibitory activitiy compared to lead compound **1**. Besides, concerning coumarin core integrity for sustaining anti-cancer activity, two seco-B-ring derivatives were also synthesized.

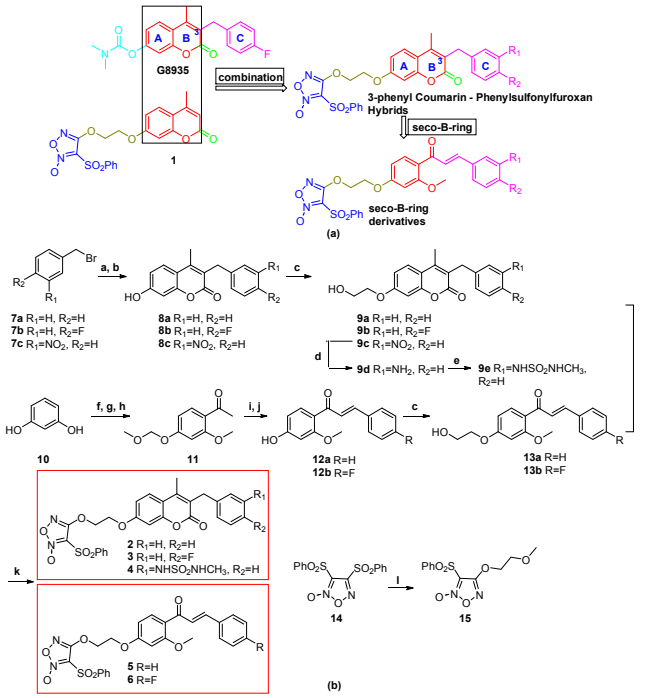
As showed in **Scheme 1**, compounds **8a-c**, synthesized according to a previously described procedure,[22-23] were treated with 2-chloro-1-ethanol to provide intermediates **9a-c**. Compound **9c** was reduced by stannous chloride dehydrate to form **9d** and sulfonylated with *N*-methyl-2-oxooxazolidine-3-sulfonamide[24] to obtain **9e**. Compound **11** was prepared from resorcinol *via* Friedel-Crafts reaction, 4-hydroxyl protection and 2-hydroxyl methylation. After aldol condensation of **11** with benzaldehyde or 4-fluorobenzaldehyde, deprotection of the 4-hydroxyl gave **12a**, **b**. Seco-B-ring compounds **13a**, **b** were synthesized by the same procedure used to obtain **9a-c**. Finally, **9a-b**, **e** and **13a**, **b** were merged with phenylsulfonylfuroxan to provide compounds **2-6**. Meanwhile, compound **15** containing phenylsulfonylfuroxan-linker fragment was also

synthesized as a reference for bio-evaluation analyses. Structures of **2-6** were confirmed by ^{1}H NMR, ^{13}C NMR and MS spectra. In ^{1}H NMR, the chemical shift of two alkenyl hydrogens of α,β-unsaturated ketone in compound **6** were 7.65 and 7.43 ppm, respectively, and the coupling constantis 15.8 Hz, indicating a *trans*-conformation of the ethylenic double bond structure.

Then **2-6** were screened for their bioactivities against nine drug-sensitive solid cancer cell lines, two hematological tumors, four drug-resistant cancer and four nontumorigenesis cell lines with lead compound **1** and NO donor **15** as references, and cisplatin, doxorubicin, gemcitabine, vincristine, SAHA, lenalidomide and CC-885[25] were chosen as positive controls. Except for MCF-7 and KB (**Table 1**), compounds **2-6** all showed higher antiproliferation activities (0.5 to 35.8 nM) than that of **1** and **15**. Particularly, compound **3** with 4-fluorobenzyl at 3-position of coumarin core was the most potent compound (0.8 to 8.3 nM). In A549, OVCA429 and MDA-MB-231, **2-6** all displayed significant activities in single digit nanomolar levels of IC_{50} values. While in HeLa, SKOV3, OVCA433 and A2780, **2** and **3** bearing 3-benzyl coumarin skeleton showed slightly better activities than that of their seco-B-ring derivatives (**5** and **6**). Introduction *N*-methylsulfomicamino group into the 3-position of phenyl group (**4**) resulted in a slightly decreasing activities relative to compounds **3** and **6** bearing 4-fluorophenyl group. Additionally, they had strong bioactivities with a range from 5.1 to 156.8 nM of IC_{50} values against two hematological tumor cell lines MV-4-11, MM-1S and four drug-resistant cancer cell lines A2780/CDDP, MDA-MB-231/Gem, MCF-7/ADR and KB-V (**Table 2**, **3**). Notably, the selective ratios of IC_{50} values about **3** were 559 and 294 (**Table 3**, 2.9 and 3.2 μM in MCF-7 and KB *vs* 5.1 and 11.0 nM in MCF-7/ADR and KB-V).Whereas, compounds **2-6** were almost at the same activity levels against A2780/CDDP *vs* A2780 and MDA-MB-231/Gem *vs* MDA-MB-231. The distinct selectivities presumed that new NO donors **2-6** probably had an extra pathway to inhibit certain MDR cancer. Moreover, **2-6** expressed far lower toxicities than compound **1** in HUVEC, T29, WI-38 and MCF-10A (**Table 4**, 0.19 to 20μM), which indicated they have a noteworthy selectivity between tumor and nontumorigenesis cell lines.

Assay of NO release showed that **2-6** produced much less NO compared to control **1** (See **Figure S1**), and the antiproliferation activities of compound **3** was declined with the increasing concentration of scavenger c-PTIO in both A2780 and A2780/CDDP (**Figure 1**). However, compounds **2-6** showed much weaker MEK inhibiting potency than that of **1**, which elucidated activities of **2-6** might be mainly related to the release of NO. Furthermore, compounds **3** and **6** could remarkably induce cell apoptosis, but hardly affect cell cycle. Interestingly, **3** and **6** seemed to mainly induce early apoptosis, while reference **1** mainly late apoptosis (see **Figure S3**). Recently, some compounds including Dp44mT were reported to specifically inhibit the proliferation of drug-resistant cancer with P-gp overexpression.[26-31] This promoted us to detect P-gp expression of the drug-resistant and their drug-sensitive cancer cell lines mentioned above. As expected (see **Figure S4**), P-gp expression was outstanding in KB-V and MCF-7/ADR, to which compounds **2-6** showed significant selective antiproliferation potency compared to KB and MCF-7; while P-gp overexpression wasn't observed in A2780/CDDP, MDA-MB-231/GEM, A2780 and MDA-MB-231, which having no relevant selective antiproliferation activities for **2-6**. Further work is under way to find the possible pharmacologic mechanism of these compounds against P-gp overexpression MDR cancer.

Scheme 1. Design (a) and synthesis (b) of 3-benzyl coumarin and phenylsulfonylfuroxan hybrids and their seco-B-ring derivatives[a].



[a]Reagents and conditions: (a) ethyl 3-oxobutanoate (1.0 equiv), NaH (1.2 equiv), dry THF, 60 °C, 2 h; (b) resorcinol (1.0 equiv), 70% H_2SO_4, rt, 2 h, 35% ~ 95% for two steps (a and b); (c) 2-chloro-1-ethanol (1.0 equiv), K_2CO_3 (3.0 equiv), KI (0.1 equiv), DMF, reflux, 2 h ~ 10 h, 76% ~ 100%; (d) stannous chloride dehydrate (4.0 equiv), DMF, rt, 6 h, 99%; (e) *N*-methyl-2-oxooxazolidine-3-sulfonamide (2.0 equiv), NEt_3 (3.0 equiv), MeCN, 80 °C, 8 h, 99%; (f) $ZnCl_2$ (1.5 equiv), HOAc, reflux, 70 min, 51%; (g) chloromethyl methyl ether (2.0 equiv), K_2CO_3 (2.5 equiv), actone, rt, overnight, 84%; (h) CH_3I (1.2 equiv), K_2CO_3 (3.0 equiv), DMF, 80 °C, 30 min, 82%; (i) benzaldehyde or 4-fluorobenzaldehyde (1.05 equiv), 60% KOH aqueous solution (2 mL/mM compound **11**), EtOH, 2 ~ 5 h, rt; (j) conc. HCl : EtOH = 1:25 (v/v), reflux, 30 min, 80% ~ 85% for two steps (i and j); (k) **14** (1.3 equiv), DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) (2.0 equiv), anhydrous DCM, rt, 3.5 h ~ 12 h, 51% ~ 85%; (l) 2-methoxyethan-1-ol (1.1 equiv), DBU (2.0 equiv); anhydrous DCM, rt, overnight, 90%.

In conclusion, novel NO donors **2-6** not only displayed significant antiproliferation activities in nine drug-sensitive tumor cell lines, but also showed strong activities against four drug-resistant tumor cell lines with nanomolar even sub-nanomolar level IC_{50} values. The less NO-releasing levels and lower toxicity on nontumorigenesis cell lines compared to lead compound **1** suggested that they had a better selectivity against malignant cells *in vitro*. Notably, some of their preliminary pharmacologic study results were different from lead compound **1**. Especially, compound **3** exhibited obvious selectivity ratios of anti-cancer potency in drug-resistant and their drug-sensitive cell lines MCF-7/ADR vs MCF-7 and KB-V vs KB with 559 and 294 folds, respectively. Western Blot analysis discovered the overexpression of P-gp in MCF-7/ADR and KB-V, while did not overexpress in MCF-7 and KB. The resu-

Table 1. Antiproliferation activities for 2-6 against nine solid cancer cell lines[a]

Compd.	IC_{50} (nM)								
	HeLa	SKOV3	A549	OVCA429	OVCA433	A2780	MDA-MB-231	MCF-7	KB
15	8826	3909	4778	5173	4389	1635	1233	3503	3691
1	62.2	47.3	45.8	41.1	130.8	20.9	85.9	445	127
2	22.9	17.3	2.0	1.7	1.1	12.7	6.0	2351	4542
3	2.8	8.3	3.7	3.9	3.3	6.6	0.8	2853	3234
4	15.4	19.2	7.9	6.1	16.3	10.2	1.1	3131	1143
5	17.8	35.8	3.3	4.4	10.1	8.7	0.5	1865	2182
6	6.9	25.0	3.5	4.0	6.6	14.5	1.4	1413	1665
Cisplatin	2741	1440	13220	7296	24630	2549	NA	NA	NA
Doxorubicin	NA	NA	NA	NA	NA	NA	NA	879	NA
Gemcitabine	NA	NA	NA	NA	NA	NA	36.4	NA	NA
Vincristine	NA	NA	NA	NA	NA	NA	NA	NA	0.65

[a] MTT assay. The data are the mean of triplicate determinations. NA: not available. HeLa: human cervical cancer cell lines; SKOV3, OVCA429, OVCA433 and A2780: human ovarian cancer cell lines; A549: human non-small cell lung cancer cell lines; MDA-MB-231 and MCF-7: human breast cancer cell lines; KB: human oral epidermoid cancer cell lines.

Table 2. Antiproliferation activities for 2-6 in hematological tumor cell lines[a]

IC_{50} (nM)	**1**	**2**	**3**	**4**	**5**	**6**	SAHA	Lenalidomide	CC-885
MV-4-11	28.3	24.5	27.2	66.3	29.3	29.6	NA	> 20000	0.3
MM-1S	21.0	12.0	143.0	24.0	28.0	25.0	2844	NA	NA

[a] MTS assay. The data are the mean of triplicate determinations. NA: not available. MV-4-11: human myeloid leukemia cell lines; MM-1S: human myeloma cell lines.

Table 3. Antiproliferation activities for 2-6 against drug-resistant tumor cells[a]

Compd.	IC_{50} (nM)				Selectivity ratio			
	A2780/CDDP	MDA-MB-231/Gem	MCF-7/ADR	KB-V	$IC_{50(A2780)}$/ $IC_{50(A2780/CDDP)}$	$IC_{50(MDA-MB-231)}$/ $IC_{50(MDA-MB-231/Gem)}$	$IC_{50(MCF-7)}$/ $IC_{50(MCF-7/ADR)}$	$IC_{50(KB)}$/ $IC_{50(KB-V)}$
15	4820	4747	7845	1959	0.3	0.3	0.5	1.9
1	85.7	85.9	156.8	24.9	0.2	1.0	2.8	5.1
2	94.0	25.2	16.3	16.0	0.1	0.2	144.2	283.9
3	22.8	6.9	5.1	11.0	0.3	0.1	559.4	294.0
4	47.2	50.4	50.1	65.4	0.2	0.02	62.5	17.5
5	51.7	10.5	18.0	13.9	0.2	0.05	103.6	157.0
6	34.1	93.0	47.9	10.1	0.4	0.02	29.5	164.9
Cisplatin	201370 (r.r. = 79)	NA	NA	NA	NA	NA	NA	NA
Doxorubicin	NA	NA	>100000 (r.r. > 113)	NA	NA	NA	NA	NA
Gemcitabine	NA	>50000 (r.r. > 1373)	NA	NA	NA	NA	NA	NA
Vincristine	NA	NA	NA	417.0(r.r. = 646)	NA	NA	NA	NA

[a] MTT assay. The data are the mean of triplicate determinations. r.r. : resistant ratio, r.r. = $IC_{50\ (drug\text{-}resistant\ cell\ lines)}$ / $IC_{50\ (drug\text{-}sensitive\ cell\ lines)}$, NA: not available. A2780/CDDP: cisplatin resistant human ovarian cancer cell lines; MDA-MB-231/Gem: gemcitabine

resistant human breast cancer cell lines; MCF-7/ADR: doxorubicin resistant human breast cancer cell lines; KB-V: vincristine resistant human oral epidermoid cancer cell lines

Table 4. Antiproliferation activities for 2-6 in nontumorigenesis cells

IC_{50} (μM)	**1**	**2**	**3**	**4**	**5**	**6**	cisplatin	Le-nalimide	CC-885
HUVEC[a]	0.13	0.44	0.27	0.19	0.41	0.36	1.10	NA	NA
T29[a]	1.8	> 5	> 5	> 5	2.5	3.5	1.8	NA	NA
WI-38[b]	0.09	> 20	> 20	1.36	0.44	0.56	NA	> 20	> 20
MCF-10A[b]	0.66	> 20	> 20	NA	NA	NA	NA	> 20	0.005

[a] MTT assay; [b] MTS assay. The data are the mean of triplicate determinations. NA: not available. HUVEC: human umbilical vein endothelial cell lines; T29: human immortalized but non-tumorigenic ovarian epithelial cell lines; WI-38: human lung fibroblasts; MCF-10A: human non-tumorigenic breast epithelial cell lines

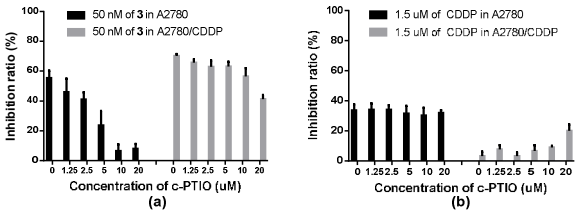


Figure 1. Antiproliferation activities of compound **3** at the concentration of 50 nM together with different concentrations of c-PTIO. Results were indicated as the Mean ± SEM of two independent experiments.

-lts suggested that there was a close relationship between P-gp overexpression and antiproliferation selectivity. Research of the detailed pharmacologic mechanism will further proceed for the development of desirable anti-cancer agents to overcome MDR mediated by P-gp overexpression.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:
anti-proliferative assay, inhibition activities of colony, nitrite measurement, cell apoptosis and western-blot analysis, HRMS, ^{1}H &^{13}C NMR and HSQC spectra of compounds (PDF).

AUTHOR INFORMATION

Corresponding Author

Email: yingchen71@fudan.edu.cn

Author Contributions

Y. L. Guo† and *Y. J. Wang*‡ contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

Funding from "Major New Drugs Innovation and Development" of National Science and Technology Major Projects (2018ZX09711002-007-005); The authors wish to thank Zhimin Shao and Gong Yang for providing cancer cell lines.

ABBREVIATIONS

P-gp, P-glycoprotein; MDR, multi-drug resistant; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; MEK, Mitogen-activated protein kinase kinase; ERK, extracellular regulated protein kinases; NMR, nuclear magnetic resonance; HSQC, heteronuclear single quantum correlation; THF, tetrahydrofuran; DMF, *N,N*-dimethylformamide; DCM, dichloromethane; SAHA, suberoylanilide hydroxamic acid; DMSO, dimethyl sulfoxide; PI, propidium iodide; HRMS, high resolution mass spectra

REFERENCES

1. Furchgott R. F.; Zwazki J. V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **1980**, *288*, 373-376.

2. Mocellin, S.; Bronte, V.; Nitti, D. Nitric oxide, a double edged sword in cancer biology: searching for therapeutic opportunities. *Med. Res. Rev.* **2007**, *27*, 317-352.

3. Bonavida, B.; Baritaki, S.; Huerta-Yepez, S.; Vega, M. I.; Chatterjee, D.; Yeung, K. Novel therapeutic applications of nitric oxide donors in cancer: roles in chemo- and immunosensitization to apoptosis and inhibition of metastases. *Nitric Oxide* **2008**, *19*, 152-157.

4. Riganti, C.; Miraglia, E.; Viarisio, D.; Costamagna, C.; Pescarmona, G.; Ghigo, D.; Bosia, A. Nitric oxide reverts the resistance to doxorubicin in human colon cancer cells by inhibiting the drug efflux. *Cancer Res.* **2005**, *65*, 516-525.

5. Kiechle, F. L.; Zhang X. Apoptosis: biochemical aspects and clinical implications. *Clinica Chimica Acta* **2002**, *326*, 27-45.

6. Sullivan, R.; Graham, C. H. Chemosensitization of cancer by nitric oxide. *Curr. Pharm. Des.* **2008**, *14*, 1113-1123.

7. Dong, R.; Wang, X.; Wang, H.; Liu, Z.; Liu, J.; Saavedra, J. E. Effects of JS-K, a novel anti-cancer nitric oxide prodrug, on gene expression in human hepatoma Hep3B cells. *Biomed. Pharmacother.* **2017,** *88*, 367-373.

8. Zhao, H.; Ning, S.; Scicinski, J.; Oronsky, B.; Knox, S. J.; Peehl, D. M. Epigenetic effects of RRx-001: a possible unifying mechanism of anticancer activity. *Oncotarget* **2015**, *6*, 43172-43181.

9. Sugita, H.; Kaneki, M.; Furuhashi, S.; Hirota, M.; Takamori, H.; Baba, H. Nitric oxide inhibits the proliferation and invasion of pancreatic cancer cells through degradation of insulin receptor substrate-1 protein. *Mol. Cancer Res.* **2010**, *8*, 1152-1163.

10. Shields, J. M.; Pruitt, K.; McFall, A.; Shaub, A.; Der, C. J. Understanding Ras: 'it ain't over 'til it's over'. *Trends in cell biolo.* **2000,** *10*, 147-154.

11. Lim, K. H.; Ancrile, B. B.; Kashatus, D. F.; Counter, C. M. Tumour maintenance is mediated by eNOS. *Nature* **2008**, *452*, 646-649.

12. Furuhashi, S.; Sugita, H.; Takamori, H.; Horino, K.; Nakahara, O.; Okabe, H.; Miyake, K.; Tanaka, H.; Beppu, T.; Baba, H. NO donor and MEK inhibitor synergistically inhibit proliferation and invasion of cancer cells. *Int. J. Oncol.* **2012**, *40*, 807-815.

13. Huang, Y.; Liu, M.; Meng, L.; Feng, P.; Guo, Y.; Ying, M.; Zhu, X.; Chen, Y. Synthesis and antitumor evaluation of novel hybrids of phenylsulfonylfuroxan and epiandrosterone/dehydroepiandrosterone derivatives. *Steroids* **2015**, *101*, 7-14.

14. Ai, Y.; Kang, F.; Huang, Z.; Xue, X.; Lai, Y.; Peng, S.; Tian, J.; Zhang, Y. Synthesis of CDDO-amino acid-nitric oxide donor trihybrids as potential antitumor agents against both drug-sensitive and drug-resistant colon cancer. *J. Med. Chem.* **2015**, *58*, 2452-2464.

15. Ling, Y.; Ye, X.; Zhang, Z.; Zhang, Y.; Lai, Y.; Ji, H.; Peng, S.; Tian, J. Novel nitric oxide-releasing derivatives of farnesylthiosalicylic acid: synthesis and evaluation of antihepatocellular carcinoma activity. *J. Med. Chem.* **2011**, *54*, 3251-3259.

16. Gu, X.; Huang, Z.; Ren, Z.; Tang, X.; Xue, R.; Luo, X.; Peng, S.; Peng, H.; Lu, B.; Tian, J.; Zhang, Y. Potent inhibition of nitric oxide-releasing bifendate derivatives against drug-resistant K562/A02 cells in vitro and in vivo. *J. Med. Chem.* **2017**, *60*, 928-940.

17. Chegaev, K.; Riganti, C.; Lazzarato, L.; Rolando, B.; Guglielmo, S.; Campia, I.; Fruttero, R.; Bosia, A.; Gasco, A. Nitric oxide donor doxorubicins accumulate into Doxorubicin-resistant human colon cancer cells inducing cytotoxicity. *ACS Med. Chem. Lett.* **2011**, *2*, 494-497.

18. Liu, M.; Chen, X.; Huang, Y.; Feng, P.; Guo, Y.; Yang, G.; Chen, Y. Hybrids of phenylsulfonylfuroxan and coumarin as potent antitumor agents. *J. Med. Chem.* **2014**, *57*, 9343-9356.

19. Ohren, J. F.; Chen, H.; Pavlovsky, A.; Whitehead, C.; Zhang, E.; Kuffa, P.; Yan, C.; McConnell, P.; Spessard, C.; Banotai, C.; Mueller, W. T.; Delaney, A.; Omer, C.; Sebolt-Leopold, J.; Dudley, D. T.; Leung, I. K.; Flamme, C.; Warmus, J.; Kaufman, M.; Barrett, S.; Tecle, H.; Hasemann, C. A. Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition. *Nat. Struct. Mol. Biol.* **2004**, *11*, 1192-1197.

20. Wallace, E. M.; Lyssikatos, J.; Blake, J. F.; Seo, J.; Yang, H.; Yeh, T. C.; Perrier, M.; Jarski, H.; Marsh, V.; Poch, G.; Livingston, M. G.; Otten, J.; Hingorani, G.; Woessner, R.; Lee, P.; Winkler, J.; Koch, K. Potent and selective mitogen-activated protein kinase kinase (MEK) 1,2 inhibitors. 1. 4-(4-bromo-2-fluorophenylamino)-1-methylpyridin-2(1H)-ones. *J. Med. Chem.* **2006**, *49*, 441-444.

21. Han, S.; Zhou, V.; Pan, S.; Liu, Y.; Hornsby, M.; McMullan, D.; Klock, H. E.; Haugen, J.; Lesley, S. A.; Gray, N.; Caldwell, J.; Gu, X. J. Identification of coumarin derivatives as a novel class of allosteric MEK1 inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5467-5473.

22. Aoki, T.; Hyohdoh, I.; Furuichi, N.; Ozawa, S.; Watanabe, F.; Matsushita, M.; Sakaitani, M.; Morikami, K.; Takanashi, K.; Harada, N.; Tomii, Y.; Shiraki, K.; Furumoto, K.; Tabo, M.; Yoshinari, K.; Ori, K.; Aoki, Y.; Shimma, N.; Iikura, H. Optimizing the physicochemical properties of Raf/MEK inhibitors by nitrogen scanning. *ACS Med. Chem. Lett.* **2014**, *5*, 309-314.

23. Cheng, J. F.; Chen, M.; Wallace, D.; Tith, S.; Arrhenius, T.; Kashiwagi, H.; Ono, Y.; Ishikawa, A.; Sato, H.; Kozono, T.; Sato, H.; Nadzan, A. M. Discovery and structure-activity relationship of coumarin derivatives as TNF-alpha inhibitors. *Bioorg. Med. Chem. Lett.* **2004,** *14*, 2411-2415.

24. Aoki, T.; Hyohdoh, I.; Furuichi, N.; Ozawa, S.; Watanabe, F.; Matsushita, M.; Sakaitani, M.; Ori, K.; Takanashi, K.; Harada, N.; Tomii, Y.; Tabo, M.; Yoshinari, K.; Aoki, Y.; Shimma, N.; Iikura, H. The sulfamide moiety affords higher inhibitory activity and oral bioavailability to a series of coumarin dual selective RAF/MEK inhibitors. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6223-6227.

25. Matyskiela, M. E.; Lu, G.; Ito, T.; Pagarigan, B.; Lu, C.; Miller, K.; Fang, W.; Wang, N.; Nguyen, D.; Houston, J.; Carmel, G.; Tran, T.; Riley, M.; Nosaka, L. A.; Lander, G. C.; Gaidarova, S.; Xu, S.; Ruchelman, A. L.; Handa, H.; Carmichael, J.; Daniel, T. O.; Cathers, B. E.; Lopez-Girona, A.; Chamberlain, P. P. A novel cereblon modulator recruits GSPT1 to the CRL4(CRBN) ubiquitin ligase. *Nature* **2016**, 535, 252-257.

26. Szakacs, G.; Hall, M. D.; Gottesman, M. M.; Boumendjel, A.; Kachadourian, R.; Day, B. J.; Baubichon-Cortay, H.; Di Pietro, A. Targeting the achilles heel of multidrug-resistant cancer by exploiting the fitness cost of resistance. *Chem. Rev.* **2014**, *114*, 5753-5774.

27. Jansson, P. J.; Yamagishi, T.; Arvind, A.; Seebacher, N.; Gutierrez, E.; Stacy, A.; Maleki, S.; Sharp, D.; Sahni, S.; Richardson, D. R. Di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT) overcomes multidrug resistance by a novel mechanism involving the hijacking of lysosomal P-glycoprotein . *J. Biol. Chem.* **2015,** *290*, 9588-9603.

28. Gutierrez, E. M.; Seebacher, N. A.; Arzuman, L.; Kovacevic, Z.; Lane, D. J.; Richardson, V.; Merlot, A. M.; Lok, H.; Kalinowski, D. S.; Sahni, S.; Jansson, P. J.; Richardson, D. R. Lysosomal membrane stability plays a major role in the cytotoxic activity of the anti-proliferative agent, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT). *Biochim. Biophys. Acta* **2016**, *1863*, 1665-1681.

29. Al-Eisawi, Z.; Stefani, C.; Jansson, P. J.; Arvind, A.; Sharpe, P. C.; Basha, M. T.; Iskander, G. M.; Kumar, N.; Kovacevic, Z.; Lane, D. J.; Sahni, S.; Bernhardt, P. V.; Richardson, D. R.; Kalinowski, D. S. Novel mechanism of cytotoxicity for the selective selenosemicarbazone, 2-acetylpyridine 4,4-dimethyl-3-selenosemicarbazone (Ap44mSe): lysosomal membrane permeabilization. *J. Med. Chem.* **2016**, *59*, 294-312.

30. Stacy, A. E.; Palanimuthu, D.; Bernhardt, P. V.; Kalinowski, D. S.; Jansson, P. J.; Richardson, D. R. Structure-activity relationships of di-2-pyridylketone, 2-benzoylpyridine, and 2-acetylpyridine thiosemicarbazones for overcoming pgp-mediated drug resistance. *J. Med. Chem.* **2016**, *59*, 8601-8620.

31. Seebacher, N.; Lane, D. J. R.; Richardson, D. R.; Jansson, P. J. Turning the gun on cancer: utilizing lysosomal P-glycoprotein as a new strategy to overcome multi-drug resistance. *Free Radical Bio. Med.* **2016**, *96*, 432-445.

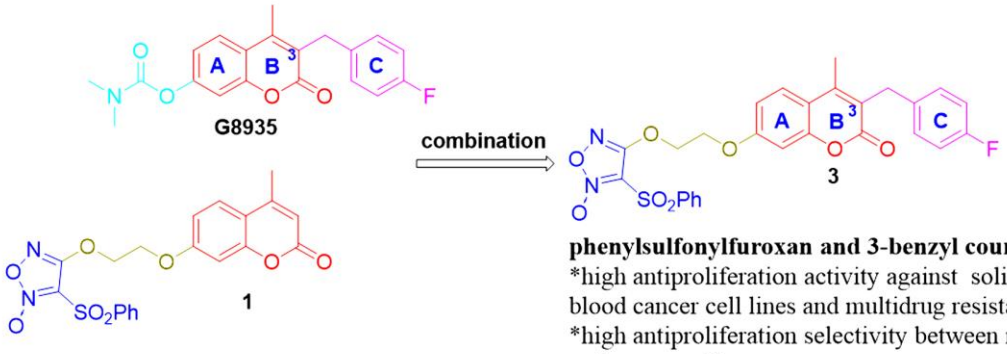
Novel nitric oxide donors of phenylsulfonylfuroxan and 3-benzyl coumarin derivatives as potent antitumor agents

Yalan Guo,† Yujie Wang,‡ Haihong Li,† Ke Wang,† Qi Wan,† Jia Li,‡ Yubo Zhou,‡* Ying Chen†*

†Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai, 201203, China

‡Chinese National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

KEYWORDS: Phenylsulfonylfuroxan, 3-benzyl coumarin, Anti-cancer, Multi-drug resistance, P-gp overexpression



phenylsulfonylfuroxan and 3-benzyl coumarin hybrid

*high antiproliferation activity against solid tumor cell lines, blood cancer cell lines and multidrug resistant tumor cell lines;

*high antiproliferation selectivity between nontumorigenesis cells and cancer cells;

*overcome MDR mediated by P-gp overexpression.