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Novel 2-Carbonylbenzo[*b*]thiophene 1,1-Dioxide Derivatives as Potent Inhibitors of STAT3 Signaling Pathway

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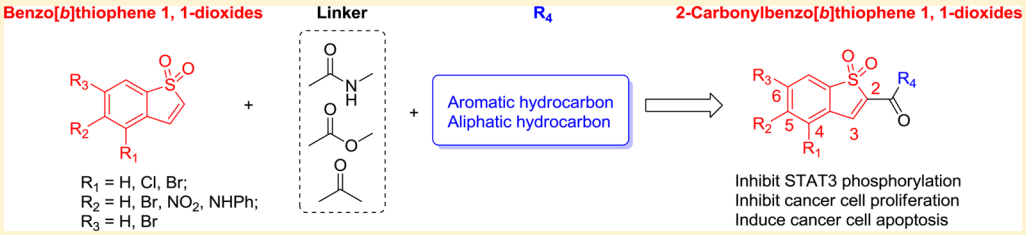
Supporting Information



ABSTRACT: Signal transducer and activator of transcription 3 (STAT3) is considered to be an attractive therapeutic target for cancer therapy. In this study, a series of 2-carbonylbenzo[*b*]thiophene 1,1-dioxide derivatives (**CBT**) were designed to inhibit the STAT3 SH2 domain phosphorylation site Try 705. We demonstrated that incorporation of basic flexible groups through amide bond linkage to benzo[*b*]thiophene 1,1-dioxide (**BTP**) achieved compounds with higher antiproliferative potency than **BTP** itself. The most potent compound **6o**, as indicated from luciferase reporter gene assay, inhibited the STAT3 pathway by decreasing the phosphorylation level of STAT3 Tyr705, while the phosphorylation level of other upstream tyrosine kinases in this pathway was not significantly inhibited. Compound **6o** was also shown to trigger ROS generation and accumulation, thus consequently attributed partially to the observed cell apoptosis. This study provided important structural information for the development of inhibitors targeting the STAT3 pathway.

KEYWORDS: *STAT3, inhibitor, 2-carbonylbenzo[b]thiophene 1,1-dioxide derivatives, antiproliferative activity, apoptosis, reactive oxygen species*

Signal transducer and activator of transcription 3 (STAT3), one of seven different STAT isoforms (STAT1−6), regulates many critical functions in human normal and malignant tissues, such as cell proliferation, differentiation, apoptosis, immune response, and angiogenesis.[1−5] Upon binding to membrane-bound receptors, the STATs are brought into close proximity for the phosphorylation of the critical tyrosine residue (Tyr 705), the phosphorylated STAT3 undergoes dimerization through a reciprocal pTyr-SH2 domain interaction to form transcriptionally active STAT:STAT dimer, which translocates into the nucleus and binds to specific DNA-response elements to promote STAT3 target gene transcription.[6] Aberrant activation of STAT3 is reported in many human solid and hematological tumors, including ovarian, breast, colon, pancreatic, lung, brain, renal, and prostate cancers.[7−9] On account of its crucial role in malignant transformation and tumorigenesis, STAT3 has emerged as a promising tumor therapeutic target.[10−15]

The first reported small molecule STAT3 inhibitor is **STA21** (Figure 1), which is discovered to inhibit STAT3-dependent luciferase activity in breast and ovarian cancer cell lines.[16] Derived from **STA21**, compound **LLL12** (Figure 1) also shows inhibitory effect on STAT3 phosphorylation, STAT3-DNA binding, and STAT3-dependent transcriptional activity.[17−19] In addition, the substituted 1,4-naphthoquinone-based compound **Ly5** and **PD15** have been revealed to inhibit STAT3 pTyr705 phosphorylation.[20] Another structurally different skeleton like **Stattic** (Figure 1) is discovered as a STAT3 SH2 domain inhibitor, which selectively suppresses STAT3 protein activation, dimerization, and nuclear translocation in numerous cancer cell lines.[21] Structurally similar to **Stattic**, compound **HJCo146** was also discovered to be a STAT3 inhibitor.[22] Other reported small molecules targeting the STAT3 signaling

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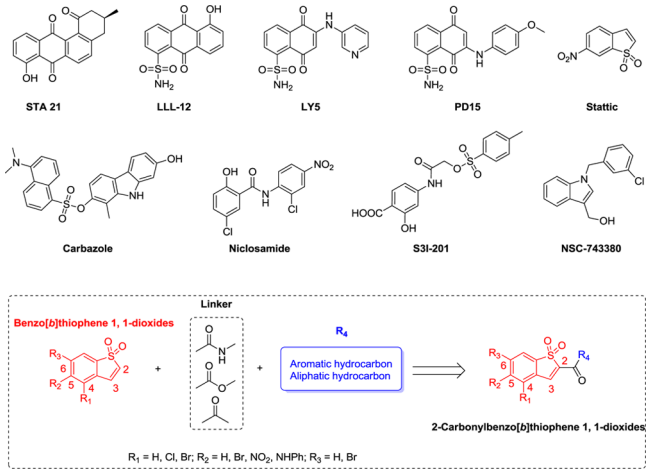


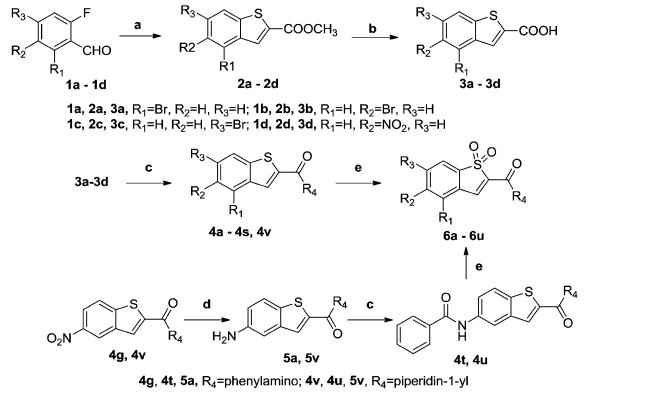
Figure 1. Representative STAT3 small molecule inhibitors and the design of compounds.

pathway include: **Carbazole**,[23] **Niclosamide**,[24,25] **S3I-201**,[26,27] **NSC-743380**,[28,29] and so on. Most of the reported STAT3 inhibitors are demonstrated to target the pTyr-SH2 domain site Tyr705. Despite these reported efforts, none of these STAT3 inhibitors have been developed into a clinical trial, most possibly due to the lack of ideal physicochemical properties of the studied molecules.[11]

To develop selective and potent STAT3 inhibitors, the benzo[*b*]thiophene 1,1-dioxide, as in **Stattic**, was employed as a leading scaffold. Previous studies have shown that this compound binds to the cleft of the STAT3 SH2 domain (PDB code 1BG1),[21] which primarily consists of three sits: the polar and basic pTyr705 site, the adjacent hydrophobic side pocket, and the Leu706 site.[30,31] By molecular docking analysis (Figure S1), it was envisioned that attachment of aromatic/aliphatic moiety at 2-position through different chemical tethers (linker), such as amide, carbonyl, and ester (Figure 1), would enforce the **BTP**-protein interaction and consequently improve the compound biological potency and binding selectivity. For these reasons, a series of 2-carbonylbenzo[*b*]thiophene 1,1-dioxide derivatives (**CBT**) were designed and synthesized, and their antiproliferative activity as well as the inhibitory effect toward the STAT3 pathway were studied. In addition, the biological effect of **Stattic** was shown to be associated with the formation of reactive oxygen species (ROS).[32,33] Therefore, the **CBT** were evaluated on their ability to induce ROS generation. Collectively, this study led to the identification of **CBT**-based potent inhibitors targeting the STAT3 pathway.

Treatment of the substituted 2-fluorobenzaldehyde (**1a−1d**) with methyl ethyl 2-mercaptoacetate and potassium carbonate in *N*,*N*-dimethylformamide(DMF) at 60 °C resulted in the formation of methyl benzo[*b*]thiophene-2-carboxylate (**2a−2d**), and those were conveniently hydrolyzed to provide the corresponding acids **3a−3d** (Scheme 1). Using 1-ethyl-3-(3-dimethyllaminopropyl)carbodiiehydrochlide (EDC) as coupling reagent, the acids (**3a−3d** or benzoic acid) were reacted with either aromatic amines or aliphatic amines to afford intermediates benzo[*b*]thiophene-2-carboxanilide (**4a−4k**, **4t**, **4u**). Compound *p*-tolyl 4-bromobenzo[*b*]thiophene-2-carboxylate (**4l**) was prepared by coupling of 4-bromobenzo[*b*]-thiophene-2-carboxylic acid (**3a**) with *p*-cresol in the presence of DCC and DMAP. Intermediates **4m−4s** and **4v** were prepared from **3b** and **3d** with the corresponding secondary

Scheme 1. Synthetic Routes for 2-Carbonylbenzo[*b*]thiophene 1,1-Dioxide Derivatives[a]



[a]Reagents and conditions: (a) $SHCH_2COOCH_3$, K_2CO_3, DMF, 60 °C; (b) KOH, MeOH, 60 °C; (c) (i) for (**4a−4k**, **4t−4u**) RNH_2, EDC, pyridine, RT; (ii) for (**4l**) *p*-cresol, DCC, DMAP, DCM, RT; (iii) for (**4m−4s**, **4v**) secondary amines, HOBt, EDC, TEA, DCM, RT, overnight; (d) Pd/C, H_2, MeOH; (e) *m*-CPBA, DCM, 45 °C.

amines using EDC and 1-hydroxybenzotriazole (HOBt) as coupling reagents. Treatment of **4g** and **4v** with Pd/C gave compounds **5a** and **5v**, which were coupled with benzoic acid to give **4t** and **4u**. Finally, all benzo[*b*]thiophenes were oxidized to 2-carbonylbenzo[*b*]thiophene 1,1-dioxides using *m*-chloroperbenzoic acid (*m*-CPBA) as the oxidant.

We first evaluated the antiproliferative activity of these compounds against selected cancer cell lines, which are reported with overexpression of STAT3 protein: breast cancer cell lines include MCF-7 (ER-positive), MDA-MB-231 (ER-negative and triple-negative), and MDA-MB-435S (ER-negative and triple-negative), and nonbreast cancer cell lines include the prostate cancer cell line DU145, the pancreatic cancer cell line PANC-1, and the nonsmall cell lung cancer cell line A549. The established MTT assay was used to determine the compound IC_{50} values with **Stattic** as positive control. As shown in Table 1, except **6l**, all prepared compounds exhibited significant antiproliferative activity against most tested cancer cells, with IC_{50}s in single digit micromole range. For compound **6l**, the *p*-cresol has an ester linkage with 4-bromobenzo[*b*]thiophene-2-carboxylic acid and showed the weakest inhibitory activity. All other compounds with the amide linkage ensured their good antiproliferative activity. Apparently, a variety of aliphatic amines at the position of R_4 were well tolerated (**6i**, **6j**, and **6m−6s**). These compounds exhibited higher activity against all selected cancer cells. This result was consistent with our molecular docking analysis. By comparison, aromatic amines at R_4 resulted in derivatives with relatively low potency (**6a−6g** and **6t**). In addition, large size substituents at R_2 or R_4 exerted unfavorable influence on the potency of compounds (**6g**, **6k**, **6t**, and **6u**).

To demonstrate whether or not these compounds would show the expected selectivity between normal cell vs cancer cell, the IC_{50}s of compounds **6a**, **6j**, and **6o** against normal human liver cell HL-7702 and liver cancer cell line SMMC-

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Table 1. Effect of Newly Synthesized Analogues on the Proliferation of Human Cancer Cell Lines

		IC_{50}[b] (μM) ± SEM					
		breast cancer cells			nonbreast cancer cells		
compd	cLogP[a]	MDA-MB-231	MDA-MB-435S	MCF-7	DU145	PANC-1	A549
6a	3.01	1.74 ± 0.53	1.39 ± 0.19	1.73 ± 0.15	2.64 ± 0.15	6.67 ± 0.27	4.09 ± 0.32
6b	3.19	2.46 ± 0.95	n.d.[c]	n.d.	3.03 ± 0.05	n.d.	n.d.
6c	3.83	3.78 ± 0.89	n.d.	n.d.	6.34 ± 0.21	n.d.	n.d.
6d	3.85	2.21 ± 0.77	n.d.	n.d.	3.39 ± 0.60	n.d.	n.d.
6e	3.03	1.72 ± 0.39	n.d.	n.d.	2.77 ± 0.30	n.d.	n.d.
6f	3.00	1.84 ± 0.47	n.d.	n.d.	2.66 ± 1.08	n.d.	n.d.
6g	1.90	3.04 ± 0.60	n.d.	n.d.	5.19 ± 1.12	n.d.	n.d.
6h	2.49	2.31 ± 0.07	n.d.	n.d.	3.86 ± 0.68	n.d.	n.d.
6i	2.49	1.34 ± 0.36	0.82 ± 0.08	1.27 ± 0.16	1.35 ± 0.01	3.34 ± 0.15	2.85 ± 0.71
6j	2.28	1.09 ± 0.21	0.93 ± 0.04	1.24 ± 0.10	2.17 ± 0.23	5.05 ± 1.68	2.97 ± 0.26
6k	3.11	5.73 ± 0.68	n.d.	n.d.	5.73 ± 0.50	n.d.	n.d.
6l	3.69	>15	n.d.	n.d.	>15	n.d.	n.d.
6m	2.45	1.67 ± 0.03	1.54 ± 0.19	0.89 ± 0.11	1.46 ± 0.09	1.94 ± 0.08	1.58 ± 0.07
6n	1.71	1.27 ± 0.41	0.94 ± 0.12	1.38 ± 0.29	1.54 ± 0.14	3.47 ± 0.24	3.47 ± 1.25
6o	2.05	0.70 ± 0.34	0.79 ± 0.09	0.91 ± 0.07	1.03 ± 0.29	2.81 ± 0.46	1.70 ± 0.29
6p	2.48	0.95 ± 0.21	0.98 ± 0.11	0.99 ± 0.05	0.98 ± 0.11	2.14 ± 0.16	1.3 ± 0.08
6q	2.67	1.07 ± 0.42	1.37 ± 0.17	1.26 ± 0.16	1.41 ± 0.26	2.98 ± 0.15	1.94 ± 0.16
6r	1.44	1.04 ± 0.49	0.80 ± 0.14	1.05 ± 0.16	1.91 ± 0.26	3.95 ± 1.34	2.31 ± 0.45
6s	2.85	0.88 ± 0.12	0.95 ± 0.04	1.13 ± 0.09	1.26 ± 0.14	4.38 ± 0.78	2.08 ± 0.05
6t	3.30	3.21 ± 0.50	n.d.	n.d.	6.21 ± 0.79	n.d.	n.d.
6u	2.64	1.68 ± 0.90	n.d.	n.d.	2.07 ± 0.42	n.d.	n.d.
Stattic	0.90	1.56 ± 0.56	1.87 ± 0.11	2.16 ± 0.29	2.50 ± 0.31	2.90 ± 0.56	2.50 ± 0.37

[a]cLogP: http://146.107.217.178/lab/alogps/start.html. [b]The inhibitory effects of individual compounds on the proliferation of cancer cell lines were determined by the MTT assay. The data are the mean ± SD from at least three independent experiments. [c]n.d.: not determined (for compounds with IC_{50} values higher than **Stattic** against MDA-MB-321 and DU145 cells, their antiproliferative activity was not determined in other cancer cells).

7721 were determined. As presented in Figure 2A, their IC_{50} values were 2.4−6.0 μM against liver cancer cell line SMMC-7721. By comparison, the IC_{50}s against normal human liver cell line HL-7702 were 15−25 μM, suggesting that these compounds exhibited high toxicity and good selectivity for cancer cells.

Based on the IC_{50} values in Table 1, compound **6o** was selected to investigate the mechanism of inhibition by using STAT3-dependent dual luciferase reporter assay in HeLa cells, which expresses a high level of constitutive STAT3. As shown in Figure 2B, **6o** inhibited STAT3 activity in a concentration-dependent manner. Compound **6o** at 2 μM decreased 14% of the STAT3-induced luciferase activity; when the concentration of **6o** was doubled to 4 μM, the luciferase activity was decreased by 48%, which was higher than the positive STAT3 inhibitor **Stattic**. At the same concentration, **Stattic** was decreased by 36%. These results indicated that **6o** displayed a remarkable inhibitory effect on STAT3-induced luciferase activity in HeLa cells.

The effect of **6o** on the intracellular STAT3 activation was further investigated using Western blot analysis. The MDA-MB-231 breast cancer cells were treated with **6o** for 12 h, the total cell lysates were prepared and the Y705 phosphorylated STAT3 proteins were detected using specific antibody. As shown in Figure 2C−E, phosphorylation of Tyr705 was inhibited by **6o** in a dose-dependent manner, while the total level of STAT3 protein was not affected at these concentrations. Moreover, **6o** displayed higher inhibitory effect on STAT3 phosphorylation than **Stattic** in all the tested cells (Figure 2C−E). By contrast, **6o** did not show marked inhibition on the phosphorylation of other kinases including Src, Erk1/2, and mTOR (Figure 3A), which are all involved in

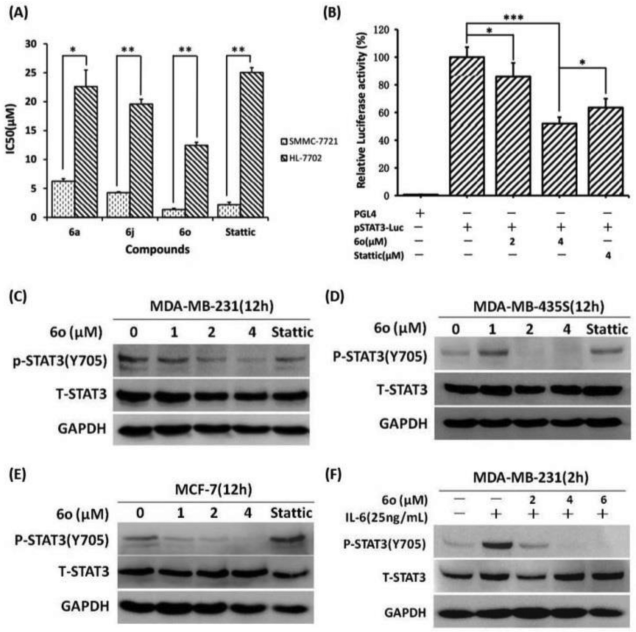

Figure 2. (A) Compounds **6a**, **6j**, and **6o** showed lower toxicity against normal human liver cells HL-7702 than liver cancer cells. (B) Compound **6o** inhibited the STAT3-mediated luciferase activity in transfected HeLa cells. (A,B) Data were expressed as mean ± SD from experiments performed in triplicate. ((*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$ compared with vehicle-treated control). (C−E) Compound **6o** affected the STAT3-Y705 phosphorylation in MDA-MB-231, MDA-MB-435S, and MCF-7 cells. (F) **6o** inhibited IL-6-induced STAT3 phosphorylation in the MDA-MB-231 cell.

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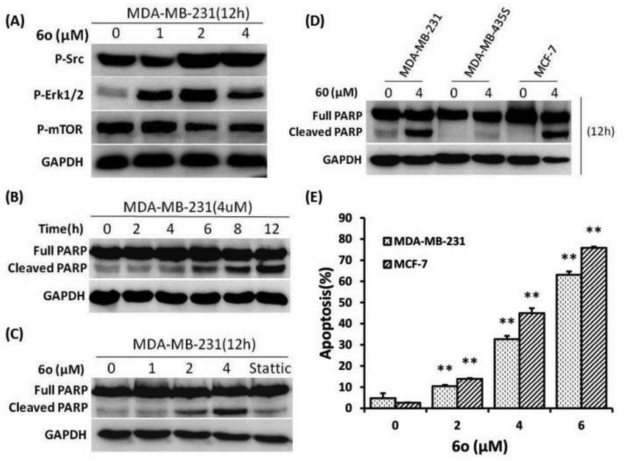

Figure 3. (A) Compound **6o** had no obvious effect on upstream tyrosine kinases. (B) Compound **6o** increased the cleaved PARP levels in MDA-MB-231 in a dose-dependent manner. (C) Compound **6o** increased the cleaved PARP levels in MDA-MB-231 in a time-dependent manner. (D) Compound **6o** up-regulated the cleavage of PARP in three breast cancer cells. (E) Apoptotic ratio of different concentrations of **6o** in MDA-MB-231 and MCF-7 cells. The values are presented as means ± SD from at least three independent experiments ((*) $p < 0$ 0.05, (**) $p < 0.01$, (***) $p < 0.001$ compared with vehicle-treated control).

the STAT3 signaling pathway. These results thus indicated that **6o** could effectively inhibit the STAT3 phosphorylation in a manner probably independent of these associated kinases (Figure S2).

To further evaluate the inhibition of **6o** on STAT3 activity, MDA-MB-231 and MCF-7 cells were starved overnight, followed by treatment with **6o** for 2 h before IL-6 stimulation. As shown in Figure 2F, IL-6 markedly increased the phosphorylation level of STAT3, which was decreased by **6o** in a concentration-dependent manner. Compound **6o** at 4 μM almost completely abolished STAT3 phosphorylation induced by IL-6. These results revealed that **6o** effectively inhibited IL-6-induced STAT3 phosphorylation (Figure S3).

To investigate whether **6o** could induce apoptosis, the poly(ADP-ribose) polymerase (PARP) was used as a characteristic indication to demonstrate the early apoptotic event in the intact cells. After treatment of MDA-231 cells with **6o**, cleavage of PARP protein was determined by Western blot analysis. Notably, **6o** elevated the cleaved PARP levels in a time- and concentration-dependent manner (Figure 3B,C). In addition, cleavage of PARP was also observed in both MDA-MB-435S and MCF-7 cells with **6o** treatment (Figure 3D).

The percentages of apoptotic cells were further determined by flow cytometry. As shown in Figure 3E, the percentage of the apoptotic cells increased in a concentration-dependent manner after treatment with compound **6o**. In the MDA-MB-231 cells, these values were 10.5%, 32.7%, and 63.1% (early and late apoptosis) at the indicated concentration, while only 4.8% was observed in the control cells. Similarly, the induced apoptosis rates in MCF-7 at 0, 2, 4, and 6 μM were 2.6%, 13.9%, 45.0%, and 75.8%, respectively. These results suggested that compound **6o** could efficiently induce cancer cell apoptosis, which partially explained the antiproliferative effect of **6o** on human cancer cells (Figure S4).

It was reported that benzo[*b*]thiophenesulphonamide 1,1-dioxide derivatives could induce reactive oxygen species (ROS)-mediated apoptotic process in tumor cells.[32] To investigate whether or not our newly synthesized derivatives could also induce ROS production, MDA-MB-231 and MCF-7 cells were pretreated with **6o**, and then cells were collected and stained by DCFH-DA and subjected to flow cytometry analysis. As shown in Figure 4A, **6o** triggered a dose-dependent ROS

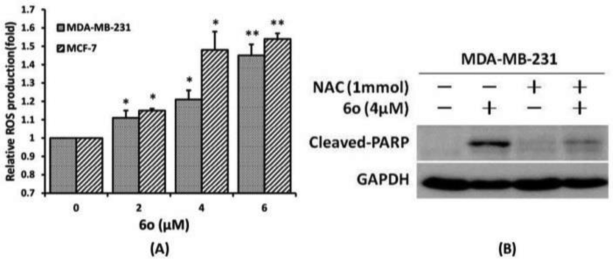

Figure 4. (A) Measurement of ROS production in MDA-MB-231 and MCF-7 cells by using a ROS-sensitive fluorometric probe. (B) Cleaved-PARP was determined by Western blot analysis ((*) $p < 0$ 0.05, (**) $p < 0.01$, (***) $p < 0.001$ compared with vehicle-treated control).

generation in both MDA-MB-231 and MCF-7 cancer cells, which would partially contribute to the observed PARP protein cleavage and cell apoptosis.

Next, Western blot analysis was conducted to detect whether the **6o**-induced cleavage of PARP could be abrogated by addition of a ROS scavenger. The MDA-MB-231 cancer cells were pretreated with an antioxidant, *N*-acetylcysteine (NAC). Then, compound **6o** was added, and PARP was collected and determined. As depicted by Figure 4B, the antioxidant NAC could significantly decrease **6o**-induced cleavage of PARP. Besides, **6o** could decrease the intracellular GSH content in the MDA-MB-231, which confirmed **6o** induced apoptosis partially by interacting with GSH (Figure S5). These results verified our hypothesis that compound **6o** could induce the generation and accumulation of ROS, which was possibly involved in the cell apoptosis by interacting with GSH.

In summary, a series of 2-carbonylbenzo[*b*]thiophene 1,1-dioxide derivatives (**CBT**) were developed to inhibit the phosphorylation of the STAT3. Through incorporation of basic flexible groups to the benzo[*b*]thiophene 1,1-dioxide (**BTP**) core structure, higher potency compounds were discovered. We confirmed that the compounds inhibited the phosphorylation of STAT3 and cell proliferation and induced the apoptotic process of cancer cells with constitutively active STAT3. The present study provided more structural reference for the development of a useful tool in elucidating the inhibition of STAT signaling pathway. Further *in vivo* antitumor activity studies on these compounds are undergoing and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00228.

Supplemental data, spectroscopic data with methods of synthesis of all compounds, and methods of biological evaluation (PDF)

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

DCM, dichloromethane; DCC, dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine; TEA, triethylamine; RT, room temperature; SD, standard deviation; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide; DCFH-DA, 2′,7′-dichlorofluorescin diacetate

REFERENCES

(1) Bromberg, J. F.; Wrzeszczynska, M. H.; Devgan, G.; Zhao, Y.; Pestell, R. G.; Albanese, C.; Darnell, J. E. Stat3 as an oncogene. *Cell* **1999**, *98*, 295−303.

(2) Bromberg, J.; Darnell, J. E. The role of STATs in transcriptional control and their impact on cellular function. *Oncogene* **2000**, *19*, 2468−2473.

(3) Turkson, J.; Jove, R. STAT proteins: novel molecular targets for cancer drug discovery. *Oncogene* **2000**, *19*, 6613−6626.

(4) Hirano, T.; Ishihara, K.; Hibi, M. Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. *Oncogene* **2000**, *19*, 2548−2556.

(5) Bromberg, J. Stat proteins and oncogenesis. *J. Clin. Invest.* **2002**, *109*, 1139−1142.

(6) Johnston, P. A.; Grandis, J. R. STAT3 signaling: anticancer strategies and challenges. *Mol. Interventions* **2011**, *11*, 18.

(7) Yu, H.; Jove, R. The STATs of cancer-new molecular targets come of age. *Nat. Rev. Cancer* **2004**, *4*, 97−105.

(8) Wei, L. H.; Kuo, M. L.; Chen, C. A.; Chou, C. H.; Lai, K. B.; Lee, C. N.; Hsieh, C. Y. Interleukin-6 promotes cervical tumor growth by VEGF-dependent angiogenesis via a STAT3 pathway. *Oncogene* **2003**, *22*, 1517−1527.

(9) Ahluwalia, A.; Busse, B. A.; Thiruvengadam, S. S.; Tarnawski, A. S. Importins are critical for colorectal cancer (CRC) growth and are novel biomarkers of CRC. Underlying mechanisms include:increased nuclear transport of P-CREB and p-STAT3, VEGF gene promoter activation and aberrant VEGF expression. *Gastroenterology.* **2011**, *140*, S184.

(10) Page, B. D.; Ball, D. P.; Gunning, P. T. Signal transducer and activator of transcription 3 inhibitors: A patent review. *Expert Opin. Ther. Pat.* **2011**, *21*, 65−83.

(11) Debnath, B.; Xu, S.; Neamati, N. Small molecule inhibitors of signal transducer and activator of transcription 3 (Stat3) protein. *J. Med. Chem.* **2012**, *55*, 6645−6668.

(12) Deng, J.; Grande, F.; Neamati, N. Small molecule inhibitors of Stat3 signaling pathway. *Curr. Cancer Drug Targets* **2007**, *7*, 91−107.

(13) Al Zaid Siddiquee, K.; Turkson, J. STAT3 as a target for inducing apoptosis in solid and hematological tumors. *Cell Res.* **2008**, *18*, 254−267.

(14) Darnell, J. E. Transcription factors as targets for cancer therapy. *Nat. Rev. Cancer* **2002**, *2*, 740−749.

(15) Darnell, J. E. Validating Stat3 in cancer therapy. *Nat. Med.* **2005**, *11*, 595−596.

(16) Song, H.; Wang, R.; Wang, S.; Lin, J. A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 4700−4705.

(17) Lin, L.; Hutzen, B.; Li, P. K.; Ball, S.; Zuo, M.; DeAngelis, S.; Foust, E.; Sobo, M.; Friedman, L.; Bhasin, D.; Cen, L.; Li, C.; Lin, J. A novel small molecule, LLL12, inhibits STAT3 phosphorylation and activities and exhibits potent growth-suppressive activity in human cancer cells. *Neoplasia.* **2010**, *12*, 39−50.

(18) Ball, S.; Li, C.; Li, P. K.; Lin, J. The small molecule, LLL12, inhibits STAT3 phosphorylation and induces apoptosis in medulloblastoma and glioblastoma cells. *PLoS One* **2011**, *6*, e18820.

(19) Liu, A.; Liu, Y.; Li, P. K.; Li, C.; Lin, J. LLL12 inhibits endogenous and exogenous interleukin-6-induced STAT3 phosphorylation in human pancreatic cancer cells. *Anticancer Res.* **2011**, *31*, 2029−2035.

(20) Bhasin, D.; Chettiar, S. N.; Etter, J. P.; Mok, M.; Li, P. Anticancer activity and SAR studies of substituted 1, 4-naphthoquinones. *Bioorg. Med. Chem.* **2013**, *21*, 4662−4669.

(21) Schust, J.; Sperl, B.; Hollis, A.; Mayer, T. U.; Berg, T. **Stattic**: a small-molecule inhibitor of STAT3 activation and dimerization. *Chem. Biol.* **2006**, *13*, 1235−1242.

(22) Chen, H.; Yang, Z.; Ding, C.; Xiong, A.; Wild, C.; Wang, L.; Ye, N.; Cai, G.; Flores, R. M.; Ding, Y.; Shen, Q.; Zhou, J. Discovery of potent anticancer agent HJC0416, an orally bioavailablesmall molecule inhibitor of signal transducer and activator oftranscription 3 (STAT3). *Eur. J. Med. Chem.* **2014**, *82*, 195−203.

(23) Hou, S.; Yi, Y. W.; Kang, H. J.; Zhang, L.; Kim, H.; Kong, Y.; Liu, Y.; Wang, K.; Kong, H.; Grindrod, S.; Bae, I.; Brown, L. M. Novel Carbazole Inhibits Phospho-STAT3 through Induction of Protein−Tyrosine Phosphatase PTPN6. *J. Med. Chem.* **2014**, *57*, 6342−6353.

(24) Ren, X.; Duan, L.; He, Q.; Zhang, Z.; Zhou, Y.; Wu, D.; Pan, J.; Pei, D.; Ding, K. Identification of niclosamide as a new small-molecule inhibitor of the STAT3 signaling pathway. *ACS Med. Chem. Lett.* **2010**, *1*, 454−459.

(25) Chen, H.; Yang, Z.; Ding, C.; Chu, L.; Zhang, Y.; Terry, K.; Liu, H.; Shen, Q.; Zhou, J. Discovery of O-Alkylamino-Tethered Niclosamide Derivatives as Potent and Orally Bioavailable Anticancer Agents. *ACS Med. Chem. Lett.* **2013**, *4*, 180−185.

(26) Siddiquee, K.; Zhang, S.; Guida, W. C.; Blaskovich, M. A.; Greedy, B.; Lawrence, H. R.; Yip, M. L.; Jove, R.; McLaughlin, M. M.; Lawrence, N. J.; Sebti, S. M.; Turkson, J. Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 7391−7396.

(27) Haftchenary, S.; Luchman, H. A.; Jouk, A. O.; Veloso, A. J.; Page, B. D. G.; Cheng, X. R.; Dawson, S. S.; Grinshtein, N.; Shahani, V. M.; Kerman, K.; Kaplan, D. R.; Griffin, C.; Aman, A. M.; Al-awar, R.; Weiss, S.; Gunning, P. T. Potent Targeting of the STAT3 Protein in Brain Cancer Stem Cells: A Promising Route for Treating Glioblastoma. *ACS Med. Chem. Lett.* **2013**, *4*, 1102−1107.

(28) Liu, X.; Guo, W.; Wu, S.; Wang, L.; Wang, J.; Dai, B.; Kim, E. S.; Heymach, J. V.; Wang, M.; Girard, L.; Minna, J.; Roth, J. A.; Swisher, S. G.; Fang, B. Antitumor activity of a novel STAT3 inhibitor and redox modulator in non-small cell lung cancer cells. *Biochem. Pharmacol.* **2012**, *83*, 1456−1464.

(29) Guo, W.; Wu, S.; Wang, L.; Wei, X.; Liu, X.; Wang, J.; Lu, Z.; Hollingshead, M.; Fang, B. Antitumor activity of a novel oncrasin analogue is mediated by JNK activation and STAT3 inhibition. *PLoS One* **2011**, *6*, e28487.

(30) Yu, W.; Xiao, H.; Lin, J.; Li, C. Discovery of novel STAT3 small molecule inhibitors via in silico site-directed fragment-based drug design. *J. Med. Chem.* **2013**, *56*, 4402−4412.

(31) Li, H.; Liu, A.; Zhao, Z.; Xu, Y.; Lin, J.; Jou, D.; Li, C. Fragment-based drug design and drug repositioning using multiple ligand simultaneous docking (MLSD): identifying celecoxib and template compounds as novel inhibitors of signal transducer and activator of transcription 3 (STAT3). *J. Med. Chem.* **2011**, *54*, 5592−5596.

(32) Boengler, K.; Ungefug, E.; Heusch, G.; Schulz, R. The STAT3 inhibitor **Stattic** impairs cardiomyocyte mitochondrial function through increased reactive oxygen species formation. *Curr. Pharm. Des.* **2013**, *19*, 6890−6895.

(33) Alonso, M. M.; Asumendi, A.; Villar, J.; Gil, M. J.; Martınez-Merino, V.; Enc10, I. J.; Migliaccio, M. New benzo(b)-thiophenesulphonamide 1, 1-dioxide derivatives induce a reactive oxygen species-mediated process of apoptosis in tumour cells. *Oncogene* **2003**, *22*, 3759−3769.

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