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Novel Type of Prodrug Activation through a Long-Range *O,N*-Acyl Transfer: A Case of Water-Soluble CREB Inhibitor

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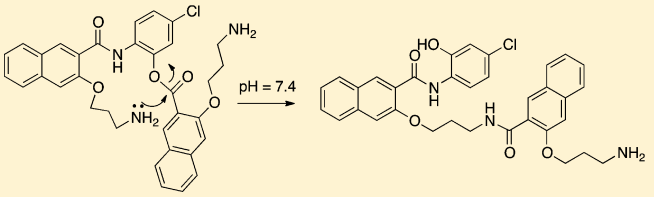
Supporting Information

ABSTRACT: CREB (cAMP response element binding protein) has been shown to play an important role in tumor initiation, progression, and metastasis. We discovered that naphthol AS-E, a cell-permeable CREB inhibitor, presented antiproliferative activity in a broad panel of cancer cell lines in vitro. However, it has limited aqueous solubility. In this report, we described a water-soluble inhibitor (compound **6**) of CREB-mediated gene transcription with *in vivo* anticancer activity. Unexpectedly, compound **6** was found to be a prodrug of compound **12** necessitating an unprecedented long-range *O,N*-acyl transfer. The rate of this transfer was pH- and temperature-dependent. To the best of our knowledge, this is the first time to show that a long-range *O,N*-acyl transfer could be exploited as a prodrug activation strategy to improve aqueous solubility. This type of prodrug may be applicable to other structures with spatially arranged hydroxyl amide to improve their aqueous solubility.



KEYWORDS: *CREB, prodrug, water-soluble inhibitor, anticancer, O,N-acyl transfer*

Among the various transcription factors that are involved in the pathogenesis of cancer is cyclic-AMP-response element binding protein (CREB).[1−3] The signaling pathways leading to phosphorylation and subsequent activation of CREB start with extracellular signals through a variety of intracellular oncogenic protein serine/threonine kinases.[1,3] The critical residue to be phosphorylated in CREB for its activation is Ser133.[4] Once phosphorylated, it binds to histone acetyltransferase and transcription coactivator, CREB-binding protein (CBP), and its closely related paralog p300.[5] Further recruitment of additional components in the transcription machinery to the gene promoter initiates CREB-dependent gene transcription.[3,5] However, three protein phosphatases with known tumor suppressor functions can dephosphorylate CREB to attenuate CREB-mediated gene transcription. These are protein phosphatase 1 (PP1),[6] protein phosphatase 2A (PP2A),[7] and phosphatase and tensin homologue (PTEN).[8] The mechanisms leading to CREB activation and inactivation suggest that CREB plays a critical role in tumorigenesis. Indeed, CREB has been found to be overexpressed and overactivated in a variety of cancers including breast,[9,10] prostate,[11] lung,[12] brain,[13] and acute leukemia.[14] Therefore, CREB has been proposed as a promising drug target for a variety of cancers.[1,15]

An essential step for activation of CREB-dependent gene transcription is to recruit CBP.[5] The CREB−CBP interaction is mediated by kinase-inducible domain (KID) from CREB and KIX (KID-interacting) domain from CBP.[16] Naphthol AS-E (**1**, Figure 1) was recently identified as an inhibitor of KIX−KID interaction and a cell-permeable inhibitor of CREB-mediated gene transcription.[17] Subsequent studies also demonstrated that compound **1** inhibited proliferation of cancer cells of different organs with a GI_{50}, which is the concentration required to inhibit the cancer cell proliferation by 50%, in the low micromolar range.[18] Importantly, normal cells tolerated **1** very well,[18] consistent with the idea of cancer cells' addiction to CREB.[1] One of the major limitations associated with compound **1** is its poor aqueous solubility. In this report, we describe our results in generating a water-soluble inhibitor of CREB-mediated gene transcription (compound **6**) with in vivo anticancer activity. Unexpectedly, compound **6** was found to be a prodrug to be activated by a long-range *O,N*-acyl transfer reaction under physiological conditions.

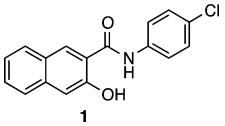


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Figure 1. Chemical structure of naphthol AS-E (**1**).

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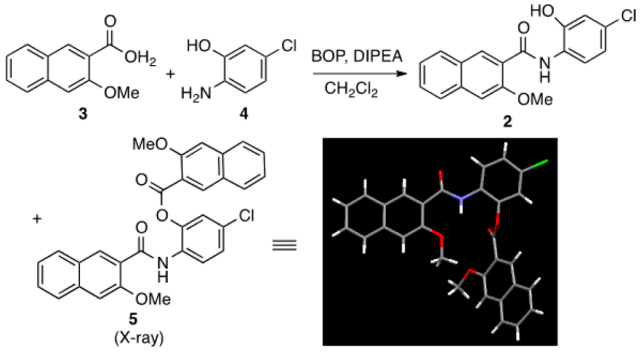
In order to improve the aqueous solubility of **1**, compound **2** was designed (Scheme 1), which contains a free phenol group

Scheme 1. Synthesis of Compounds 2 and 5



envisioned to be a facile point to attach polar groups to enhance its aqueous solubility. Therefore, commercially available acid **3** was coupled with aniline **4** in the presence of BOP/DIPEA. In addition to the desired compound **2** (39%), a bisacylated product **5** was also obtained from the reaction mixture in 55% yield. The structure of **5** was initially deduced from its ^{1}H NMR, ^{13}C NMR, MS data, and then confirmed by single crystal X-ray analysis (Scheme 1). Both **2** and **5** were evaluated for in vitro inhibition of KIX−KID interaction using a split *Renilla* luciferase complementation assay we recently described (Table 1).[17] This assay directly monitored KIX−KID interaction in vitro with a purified KIX fusion and KID fusion-containing cell lysates. Even though compound **2** was inactive in this assay (IC_{50} >50 μM), the bisacylated product **5** displayed potent activity in inhibiting KIX−KID interaction with an IC_{50} = 0.17 μM. This compound represents one of the most potent inhibitors of KIX−KID interaction reported to date[18−21] and is 17-fold more potent than the initial lead compound **1** (Table 1).

The potent in vitro activity of compound **5**, however, is plagued by its poor aqueous solubility (<0.1 mg/mL in H_2O). In addition, the ester group present in **5** was not expected to be stable toward serum esterases. Indeed, we found that the half-life ($t_{1/2}$) of compound **5** in regular tissue culture media (Dulbecco's Modified Eagle Medium, DMEM, pH = 7.70) containing 10% fetal bovine serum (FBS) was ∼20 h (data not shown). These two factors may explain why **5** was still a low micromolar inhibitor of CREB-mediated gene transcription in living HEK 293T cells (IC_{50} = 1.95 μM, Table 1) as evaluated by a CREB-*Renilla* luciferase reporter assay.[17] Similarly, the antiproliferative activity (GI_{50}) of **5** in cancer cell lines (A549, MCF-7, MDA-MB-231, and MDA-MB-468) was not improved compared to the original compound **1** (Table 1). In order to improve these two inherent limitations associated with **5**, a bis-aminoalkylated compound **6** was designed (Figure 2). The

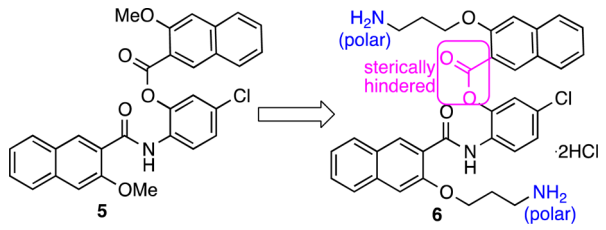
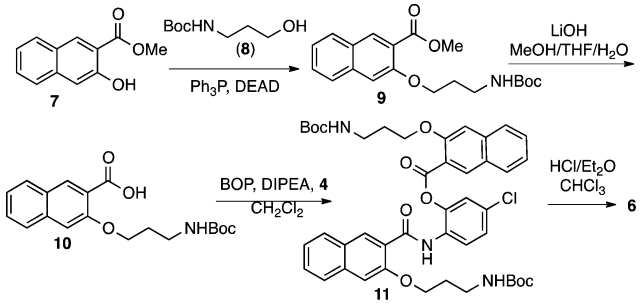


Figure 2. Design of a water-soluble compound **6**.

charged polar amino groups were added to improve its aqueous solubility. In addition, the aminopropyl groups are sterically more hindered than the methyl groups. Thus, it was predicted that compound **6** would be hydrolytically more stable than **5** because esterase-mediated hydrolysis of esters is known to be sensitive to sterics.[22]

The synthesis of **6** is presented in Scheme 2. Naphthol **7**, prepared from methylation of the corresponding acid,[20] was

Scheme 2. Synthesis of Compound 6



coupled with alcohol **8** under standard Mitsunobu condition (Ph_3P, DEAD) to give **9** uneventfully. Saponification of methyl ester **9** generated acid **10**, which was then used for double acylation with *ortho*-aminophenol **4** to yield **11**. Removal of the

Table 1. Biological Activities of Newly Synthesized Compounds

	IC_{50} (μM)[a]		GI_{50} (μM)[b]			
compd	KIX−KID inhibition[c]	CREB inhibition[d]	A549	MCF-7	MDA-MB-231	MDA-MB-468
1	2.90 ± 0.81	2.29 ± 0.31	2.90 ± 0.33	2.81 ± 0.35	2.35 ± 0.60	1.46 ± 0.30
2	>50	>50	>100	>100	>100	>100
5	0.17 ± 0.12	1.95 ± 1.14	8.78 ± 2.55	3.38 ± 0.49	23.51 ± 2.27	8.11 ± 5.47
6	4.40 ± 0.19	2.27 ± 0.28	0.59 ± 0.02	0.42 ± 0.15	1.30 ± 0.66	0.33 ± 0.004
12	19.72 ± 1.78	2.22 ± 0.38	0.29 ± 0.014	0.14 ± 0.03	0.37 ± 0.13	0.22 ± 0.07
14	>50	26.25 ± 13.64	2.73 ± 0.20	1.85 ± 1.23	2.19 ± 0.34	1.20 ± 0.20

[a]The IC_{50} is presented as mean ± SD of at least two independent experiments or >50 in the cases where IC_{50} did not reach at the highest tested concentration (50 μM). [b]GI_{50} is the concentration required to inhibit the cancer cell growth by 50% as evaluated by the MTT assay. The compounds were incubated with cells for 72 h. The GI_{50} is presented as mean ± SD of at least two independent experiments or >100 in the cases where GI_{50} did not reach at the highest tested concentration (100 μM). [c]KIX−KID interaction inhibition was evaluated by an in vitro *Renilla* luciferase complementation assay. [d]CREB inhibition refers to inhibition of CREB-mediated gene transcription in HEK 293T cells using a CREB reporter assay.

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Boc protecting groups in **10** under acidic condition delivered desired compound **6**.

Consistent with the design rationale, compound **6** was found to be highly water-soluble (>100 mg/mL in H_2O), which is a more than 1000-fold improvement over **5**. However, this compound was surprisingly found to be extremely unstable in regular tissue culture media with $t_{1/2}$ < 5 min (Figure 3). Unlike

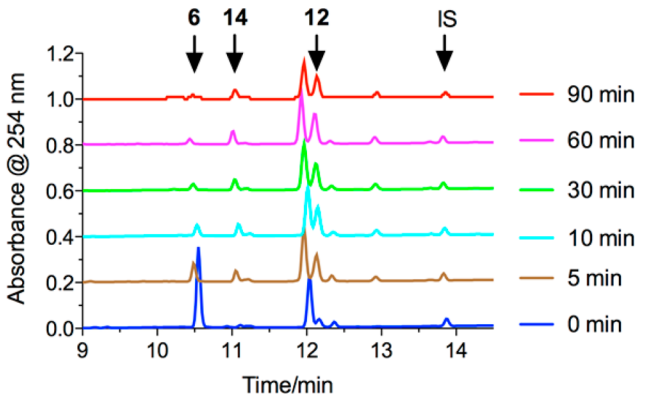


Figure 3. Compound **6** was converted into **12** in tissue culture media. Compound **6** was incubated in DMEM containing 10% FBS at 37 °C. At the indicated time point, an aliquot (10 μL) was withdrawn and mixed with acetonitrile (90 μL) to precipitate the proteins. Then 10 μL of the supernatant was subjected to RP-HPLC analysis monitored by UV absorbance at 254 nm. IS: internal standard. The other peaks eluted between 12.0 and 12.5 min are unidentified components from the tissue culture media.[23]

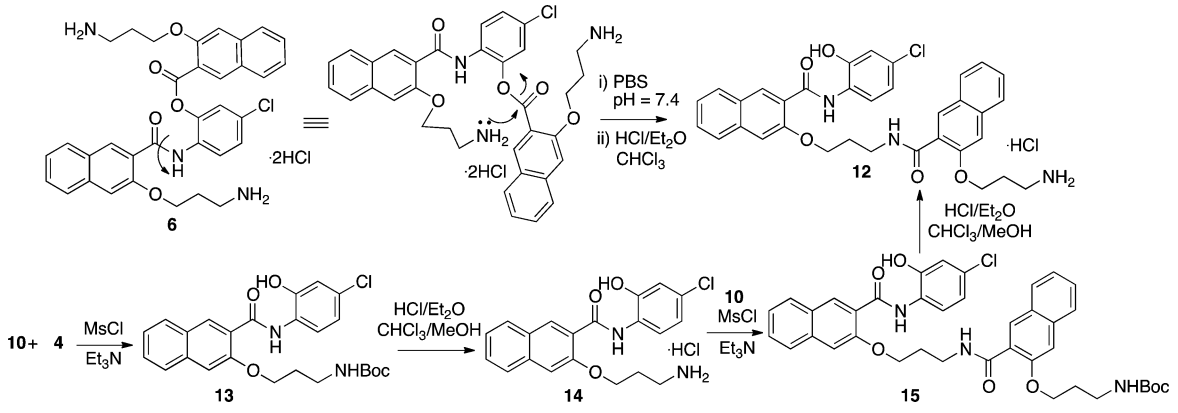
compound **5**, the instability of **6** was not due to its ester hydrolysis to produce phenol **14** (Scheme 3) because this hydrolysis product only constituted a minor species in the incubation mixture (Figure 3). Instead, the major species generated was the peak eluted at ~12.2 min in the HPLC chromatogram (Figure 3). This new species became apparently important because compound **6** was found to be a potent inhibitor of all four cancer cell lines tested even though its potencies of CREB inhibition in HEK 293T cells and in vitro KIX−KID interaction inhibition remained modest (Table 1). To facilitate identification of the new species, we investigated if this conversion was an enzyme-mediated process by switching the tissue culture media to phosphate buffered saline (PBS, pH = 7.40). Similar instability of compound **6** in PBS was also observed (Figure S1, Supporting Information), demonstrating that the proteins and enzymes in the tissue culture media were not required for this conversion. Therefore, compound **6** was treated with PBS, and the newly generated product was isolated in 50% yield.[24] Extensive NMR and MS analyses indicated that the new product was *O*,*N*-acyl transferred product **12** (Scheme 3). To further confirm its structural assignment, compound **12** was synthesized through an independent route (Scheme 3). Thus, acid **10** was coupled with *ortho*-aminophenol **4** at 1:1 ratio to give **13**, whose Boc was removed under acidic condition to give amine **14**. Coupling of **14** with another copy of acid **10** followed by acidic hydrolysis provided **12**. The two samples of **12** individually or a mixture thereof were identical in NMR and HPLC, confirming the original structural assignment of **12**.

To investigate if the conversion from **6** to **12** was an intramolecular or intermolecular process, the kinetics of this conversion was studied in detail in PBS at room temperature. At physiological pH of 7.40, the decay of **6** followed a first-order kinetics (Figure 4A), which was further confirmed by the linearity of the semilog plot (r^2 = 0.9892) (Figure S2, Supporting Information). These results support that **12** was formed from intramolecular aminolysis of **6**, which would require a 13-membered ring transition state. To understand why this unusual transition state was favored, compound **6** was subjected to molecular dynamics simulation in MacroModel. During the 1000 ps production run, the distance between C_a and N_b in **6** was monitored (Figure 5). It is evident from this simulation that these two reaction centers are very close to each other averaged at ~4 Å during the majority of the simulation time. This optimal distance may facilitate the intramolecular aminolysis reaction to generate **12**. Similar to other intramolecular aminolysis,[25] conversion from **6** to **12** was pH-dependent. Thus, the $t_{1/2}$ of **6** in PBS at room temperature was 27.3, 37.7, 114.6, and 447.7 min at pH of 7.40, 6.98, 6.48, and 6.01, respectively (Figures 4 and S2, Supporting Information).

The rapid intramolecular aminolysis of **6** at physiological pH suggested that **12** was the active species in all the cellular experiments with **6**, which is thus considered as a prodrug. Other prodrugs involving intramolecular aminolysis has been known for *O*-acyl-1,2-hydroxylamines such as the ester derivatives of propranolol,[26,27] paclitaxel,[25] and peptidomimetics.[28] However, to the best of our knowledge, this type of prodrug activation necessitating a long-range *O*,*N*-acyl transfer has not been known before in the literature.

To further test the prodrug hypothesis, the newly synthesized compound **12** was biologically evaluated, and the results are presented in Table 1. Compound **12** was about as potent as **6**

Scheme 3. Two Syntheses of Compound 12



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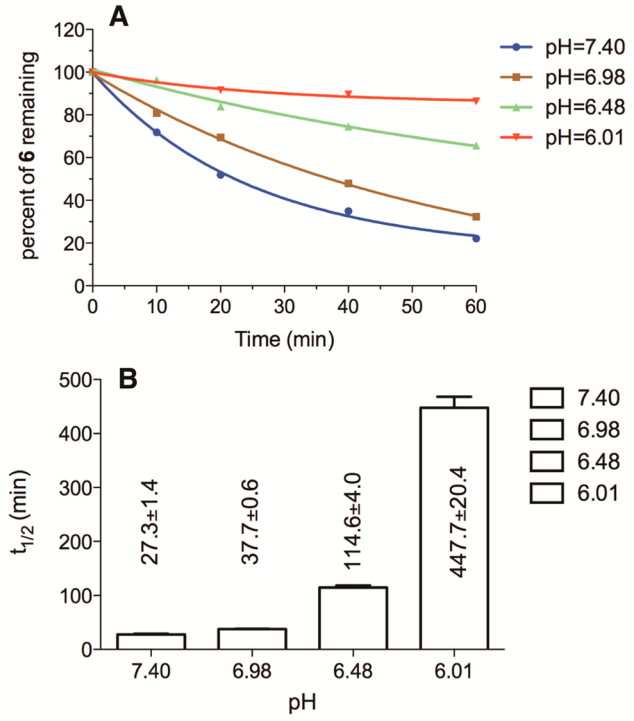

Figure 4. Kinetics of conversion from **6** to **12** in PBS at room temperature at different pH. (A) Conversion from **6** to **12** was a first-order, unimolecular process and pH-dependent in PBS solution. Compound **6** was incubated in PBS at different pH values at room temperature. At the indicated time points, an aliquot was taken and analyzed by RP-HPLC. The percentage of **6** remaining was quantified from each chromatogram. The lines represent the best fit curves based on first-order kinetics. (B) The $t_{1/2}$ of **6** in PBS at different pH values at room temperature. The data are presented as mean ± SD of two independent experiments.

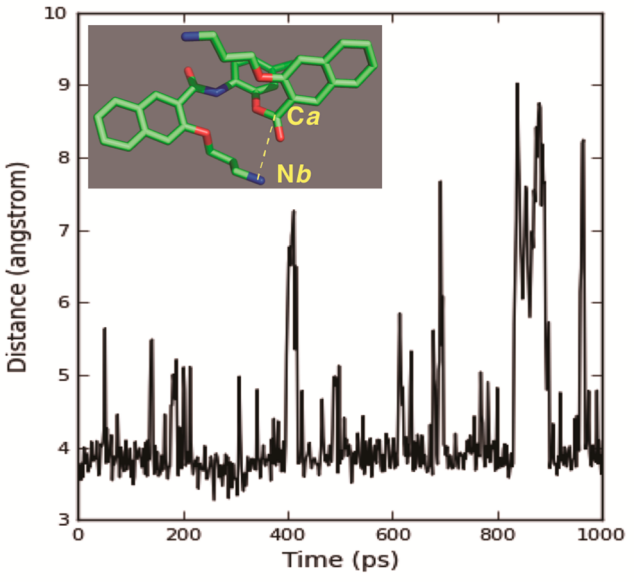

Figure 5. Distance profile of C_a and N_b in **6** during 1000 ps molecular dynamics simulation.

in inhibiting CREB's activity in HEK 293T cells in the CREB reporter assay. To further confirm their CREB inhibition, the expression level of endogenous CREB target gene *NR4A2*[29] was evaluated using quantitative reverse transcription polymerase chain reaction (qRT-PCR) (Figure 6). As an endogenous CREB target gene, forskolin (10 μM), robustly stimulated

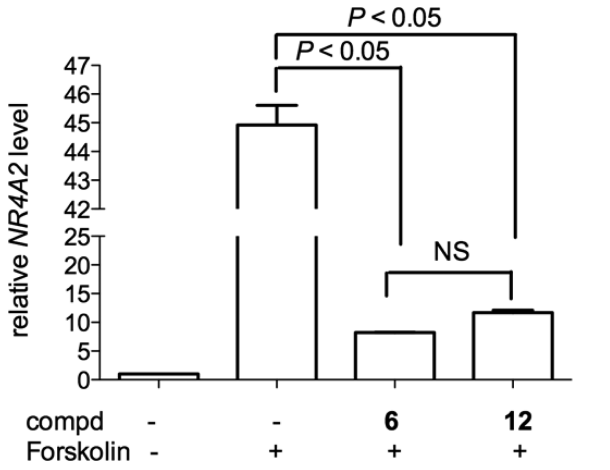

Figure 6. Compounds **6** and **12** suppressed expression of endogenous CREB target *NR4A2*. HEK 293T cells were treated with different drugs for 1 h, when forskolin was added for another 45 min. The relative mRNA level was quantified by qRT-PCR. The student t test was used to evaluate significance.

NR4A2 expression level to ~44-fold. Both compounds **6** (2.5 μM) and **12** (2.5 μM) suppressed the expression of *NR4A2* to ~10-fold (Figure 6).

In suppressing cancer cell growth, **12** was also similar to or only marginally more potent than **6** in A549, MCF-7, MDA-MB-231, and MDA-MB-468 cells. Under these cellular experimental conditions, the small amount of hydrolysis product **14** generated did not likely contribute to the observed activity for **6** because **14** was less potent in all the evaluated assays (Table 1). It is clear that the antiproliferative activity observed for **14** is not due to CREB inhibition or KIX−KID interaction inhibition.

In the in vitro KIX−KID interaction inhibition assay, compound **6** was substantially more potent than **12** (4.40 vs 19.72 μM, Table 1). We attributed this difference to the fact that this assay was conducted at 4 °C and conversion from **6** to **12** was temperature-dependent. We found that the $t_{1/2}$ of **6** in PBS (pH = 7.40) at 4 °C was about 24 h (data not shown). Therefore, the IC_{50} of KIX−KID interaction presented in Table 1 for **6** actually represented a mixture of both **6** and **12**. The IC_{50} discordance between CREB transcription (IC_{50} = 2.22 μM) and KIX−KID interaction (IC_{50} = 19.72 μM) for **12** suggests that **12** inhibited CREB's transcription activity through a mechanism independent of inhibition of KIX−KID interaction.

Although **12** was the active species of prodrug **6** under cellular experimental conditions, it showed considerably decreased aqueous solubility (~0.12 mg/mL in H_2O). On the other hand, the prodrug **6** demonstrated excellent aqueous solubility, and therefore, it was selected for in vivo antitumor activity study. Preliminary studies showed that compound **6** was well-tolerated in mice receiving daily intravenous injection of a saline solution at 10 mg/kg, and this dose was chosen for antitumor efficacy study. To this end, a variant of breast cancer MCF-7 cells designated as MCF-7^{ER-}, which does not express estrogen receptor (ER),[30] was subcutaneously implanted at the right flank of BALB/c nude mice. When the tumor sizes reached ~100 mm^3 on day 16, the tumor-bearing mice were

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randomized and treated with either vehicle or a saline solution of **6** at 10 mg/kg once a day for 28 days. The tumor volumes from the drug-treated mice were significantly smaller than those just receiving vehicle starting from 23 days post-treatment (Figure 7A). At the end of the treatment, the average tumor volume of drug-treated was 65% of vehicle-treated. Throughout the treatment, the body weights of the treated-mice were not different from those receiving vehicle (Figure 7B), suggesting **6** did not display overt toxicity in mice.

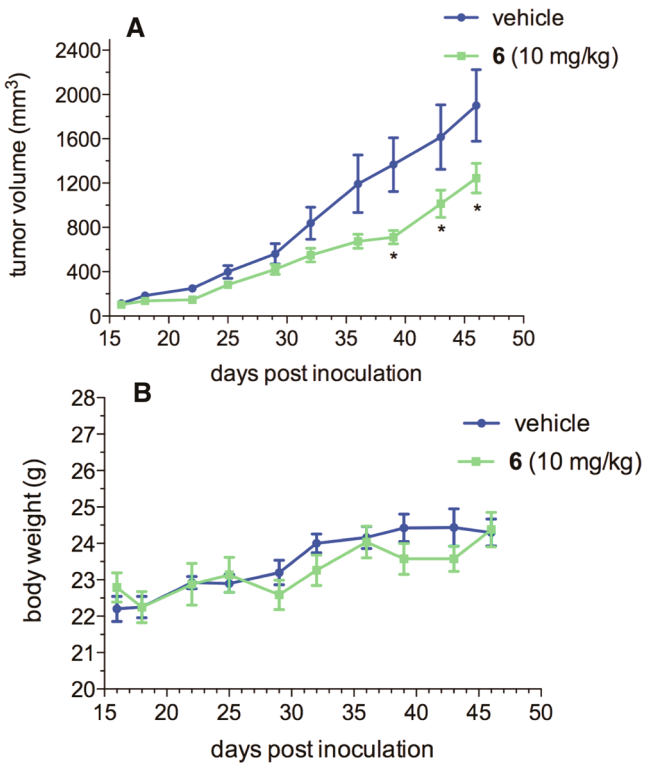

Figure 7. In vivo antitumor activity of compound **6** in BALB/c nude mice with MCF-7^{ER-} xenograft. The female BALB/c nude mice were xenografted with MCF-7^{ER-} at the right flanks. When the average tumor volume reached ~100 mm^3 (on day 16), the mice were randomized to receive either **6** in saline or just saline (n = 8) once a day for 28 days (qd × 28). The tumor volume (A) and body weight (B) were measured twice a week during the treatment period. The student t test was used to evaluate significance. *P < 0.05.

In summary, we described our discovery of a highly water-soluble compound **6** as an inhibitor of CREB-mediated gene transcription. Unexpectedly, we found that compound **6** was a novel type of prodrug to give **12** as the actual active species under physiological conditions, which entails a long-range intramolecular *O*,*N*-acyl transfer. Compounds **6** and **12** displayed potent growth inhibitory activities against various cancer cell lines, and the former also exhibited in vivo antitumor activity without overt toxicity. This novel type of prodrug strategy may be applicable to other structures with spatially arranged hydroxyl and amide groups to improve their aqueous solubility such as those found in naphthoquinone amides[31] and myxochelin A and its derivatives.[32,33]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional synthetic and biological evaluation procedures. Detailed compound characterization data and a CIF file for the crystal structure of **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; CREB, cyclic-AMP-response element-binding protein; CBP, CREB-binding protein; DEAD, diethyl azodicarboxylate; DIPEA, diisoproylethylamine; DMEM, Dulbecco's Modified Eagle Medium; ER, estrogen receptor; FBS, fetal bovine serum; KID, kinase-inducible domain; KIX, KID-interacting; PBS, phosphate buffered saline; MS, mass spectroscopy; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; NMR, nuclear magnetic resonance; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PTEN, phosphatase and tensin homologue; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SD, standard deviation; $t_{1/2}$, half-life

■ REFERENCES

(1) Xiao, X.; Li, B. X.; Mitton, B.; Ikeda, A.; Sakamoto, K. M. Targeting CREB for cancer therapy: friend or foe. *Curr. Cancer Drug Targets* **2010**, *10*, 384−391.

(2) Shaywitz, A. J.; Greenberg, M. E. CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu. Rev. Biochem.* **1999**, *68*, 821−861.

(3) Mayr, B.; Montminy, M. Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 599−609.

(4) Gonzalez, G. A.; Montminy, M. R. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* **1989**, *59*, 675−80.

(5) Cardinaux, J. R.; Notis, J. C.; Zhang, Q.; Vo, N.; Craig, J. C.; Fass, D. M.; Brennan, R. G.; Goodman, R. H. Recruitment of CREB binding protein is sufficient for CREB-mediated gene activation. *Mol. Cell. Biol.* **2000**, *20*, 1546−52.

(6) Hagiwara, M.; Alberts, A.; Brindle, P.; Meinkoth, J.; Feramisco, J.; Deng, T.; Karin, M.; Shenolikar, S.; Montminy, M. Transcriptional attenuation following cAMP induction requires PP-1-mediated dephosphorylation of CREB. *Cell* **1992**, *70*, 105−113.

(7) Wadzinski, B. E.; Wheat, W. H.; Jaspers, S.; Peruski, L. F.; Lickteig, R. L.; Johnson, G. L.; Klemm, D. J. Nuclear-protein phosphatase-2A dephosphorylates protein kinase A phosphorylated CREB and regulates CREB transcriptional stimulation. *Mol. Cell. Biol.* **1993**, *13*, 2822−2834.

(8) Gu, T.; Zhang, Z.; Wang, J.; Guo, J.; Shen, W. H.; Yin, Y. CREB is a novel nuclear target of PTEN phosphatase. *Cancer Res.* **2011**, *71*, 2821−5.
(9) Chhabra, A.; Fernando, H.; Watkins, G.; Mansel, R. E.; Jiang, W. G. Expression of transcription factor CREB1 in human breast cancer and its correlation with prognosis. *Oncol. Rep.* **2007**, *18*, 953−958.
(10) Zhang, S.; Chen, L.; Cui, B.; Chuang, H.-Y.; Yu, J.; Wang-Rodriguez, J.; Tang, L.; Chen, G.; Basak, G. W.; Kipps, T. J. ROR1 is expressed in human breast cancer and associated with enhanced tumor-cell growth. *PLoS One* **2012**, *7*, e31127.
(11) Wu, W.; Zhau, H. E.; Huang, W. C.; Iqbal, S.; Habib, F. K.; Sartor, O.; Cvitanovic, L.; Marshall, F. F.; Xu, Z.; Chung, L. W. K. cAMP-responsive element-binding protein regulates vascular endothelial growth factor expression: implication in human prostate cancer bone metastasis. *Oncogene* **2007**, *26*, 5070−5077.
(12) Seo, H. S.; Liu, D. D.; Bekele, B. N.; Kim, M. K.; Pisters, K.; Lippman, S. M.; Wistuba, I. I.; Koo, J. S. Cyclic AMP response element-binding protein overexpression: A feature associated with negative prognosis in never smokers with non-small cell lung cancer. *Cancer Res.* **2008**, *68*, 6065−6073.
(13) Tan, X.; Wang, S.; Zhu, L.; Wu, C.; Yin, B.; Zhao, J.; Yuan, J.; Qiang, B.; Peng, X. cAMP response element-binding protein promotes gliomagenesis by modulating the expression of oncogenic microRNA-23a. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 15805−10.
(14) Crans-Vargas, H. N.; Landaw, E. M.; Bhatia, S.; Sandusky, G.; Moore, T. B.; Sakamoto, K. M. Expression of cyclic adenosine monophosphate response-element binding protein in acute leukemia. *Blood* **2002**, *99*, 2617−2619.
(15) Conkright, M. D.; Montminy, M. CREB: the unindicted cancer co-conspirator. *Trends Cell Biol.* **2005**, *15*, 457−459.
(16) Radhakrishnan, I.; Perez-Alvarado, G. C.; Parker, D.; Dyson, H. J.; Montminy, M. R.; Wright, P. E. Solution structure of the KIX domain of CBP bound to the transactivation domain of CREB: A model for activator:Coactivator interactions. *Cell* **1997**, *91*, 741−752.
(17) Li, B. X.; Xiao, X. Discovery of a small-molecule inhibitor of the KIX-KID interaction. *ChemBioChem* **2009**, *10*, 2721−2724.
(18) Li, B. X.; Yamanaka, K.; Xiao, X. Structure-activity relationship studies of naphthol AS-E and its derivatives as anticancer agents by inhibiting CREB-mediated gene transcription. *Bioorg. Med. Chem.* **2012**, *20*, 6811−20.
(19) Xie, F.; Li, B. X.; Broussard, C.; Xiao, X. Identification, synthesis and evaluation of substituted benzofurazans as inhibitors of CREB-mediated gene transcription. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5371−5.
(20) Jiang, M.; Li, B. X.; Xie, F.; Delaney, F.; Xiao, X. Design, synthesis, and biological evaluation of conformationally constrained analogues of naphthol AS-E as inhibitors of CREB-mediated gene transcription. *J. Med. Chem.* **2012**, *55*, 4020−4.
(21) Lodge, J. M.; Rettenmaier, T. J.; Wells, J. A.; Pomerantz, W. C.; Mapp, A. K. FP tethering: a screening technique to rapidly identify compounds that disrupt protein−protein interactions. *MedChemComm* **2014**, *5*, 370−375.
(22) Kam, S. T.; Matier, W. L.; Mai, K. X.; Barcelon-Yang, C.; Borgman, R. J.; O'Donnell, J. P.; Stampfli, H. F.; Sum, C. Y.; Anderson, W. G.; Gorczynski, R. J.; Lee, R. J. [(Arylcarbonyl)oxy]-propanolamines. 1. Novel beta-blockers with ultrashort duration of action. *J. Med. Chem.* **1984**, *27*, 1007−16.
(23) The identity of the minor peak eluted at ∼13.0 min is currently unknown. By comparing with a standard sample, we excluded that it is the acid part after hydrolysis of **6** (i.e., the Boc-deprotected acid **10**).
(24) The actual yield of this conversion should be higher than 50% because some of the product was lost during the $CHCl_3$ washing step (see Supporting Information). We did not attempt to recover this portion of compound **12**.
(25) Hayashi, Y.; Skwarczynski, M.; Hamada, Y.; Sohma, Y.; Kimura, T.; Kiso, Y. A novel approach of water-soluble paclitaxel prodrug with no auxiliary and no byproduct: design and synthesis of isotaxel. *J. Med. Chem.* **2003**, *46*, 3782−4.
(26) Irwin, W. J.; Belaid, K. A. Drug-delivery by ion-exchange. Hydrolysis and rearrangement of ester pro-drugs of propranolol. *Int. J. Pharm.* **1988**, *46*, 57−67.
(27) Quigley, J. M.; Jordan, C. G. M.; Timoney, R. F. The synthesis, hydrolysis kinetics and lipophilicity of O-acyl esters of propranolol. *Int. J. Pharm.* **1994**, *101*, 145−163.
(28) Oliyai, R. Prodrugs of peptides and peptidomimetics for improved formulation and delivery. *Adv. Drug Delivery Rev.* **1996**, *19*, 275−286.
(29) Best, J. L.; Amezcua, C. A.; Mayr, B.; Flechner, L.; Murawsky, C. M.; Emerson, B.; Zor, T.; Gardner, K. H.; Montminy, M. Identification of small-molecule antagonists that inhibit an activator: coactivator interaction. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17622−17627.
(30) This MCF-7 subline was established at Crownbio, Inc., and did not express estrogen receptor (ER). In contrast to the parental MCF-7, the tumor growth of this subline in mice does not require estrogen supplement.
(31) Kongkathip, B.; Akkarasamiyo, S.; Hasitapan, K.; Sittikul, P.; Boonyalai, N.; Kongkathip, N. Synthesis of novel naphthoquinone aliphatic amides and esters and their anticancer evaluation. *Eur. J. Med. Chem.* **2013**, *60*, 271−84.
(32) Miyanaga, S.; Obata, T.; Onaka, H.; Fujita, T.; Saito, N.; Sakurai, H.; Saiki, I.; Furumai, T.; Igarashi, Y. Absolute configuration and antitumor activity of myxochelin A produced by Nonomuraea pusilla TP-A0861. *J. Antibiot.* **2006**, *59*, 698−703.
(33) Miyanaga, S.; Sakurai, H.; Saiki, I.; Onaka, H.; Igarashi, Y. Synthesis and evaluation of myxochelin analogues as antimetastatic agents. *Bioorg. Med. Chem.* **2009**, *17*, 2724−32.