Synthesis and Biological Evaluation of Nitric Oxide-Donating Thalidomide Analogues as Anticancer Agents

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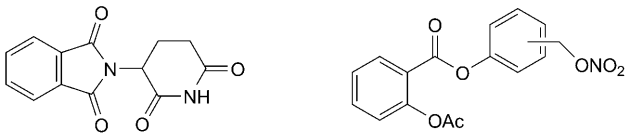
In search of more potent anticancer agents, 15 nitric oxide (NO)-donating thalidomide analogues, **6a**, **6b**, **8a**–**8e**, and **13a**–**13h**, were designed and synthesized. Cytotoxicity of these compounds was evaluated *in vitro* against three human tumor cell lines (HepG2, A549, and PC-3). The results indicated that **13a**–**13d** exhibited notable anticancer activities comparable to or stronger than that of 5-fluorouracil (5-FU). Structure–activity relationships were also discussed, based on the experimental data obtained. Generally, the cytotoxic activity of target compounds is closely related to the type of NO donors, and the length of the spacers connecting to NO donors also appears important for the bioactivities.

Introduction. – Thalidomide (*Fig.*) was a sedative and/or hypnotic drug in the 1950s, which was withdrawn from the market when its teratogenicity was discovered [1]. However, basic research on thalidomide did not come to a halt, and in earlier 1990s thalidomide proved to be an immunomodulatory agent [2][3]. In response to this, numerous researches have been carried out, especially for the treatment of tumors [4–9].



Thalidomide NO-ASA

Figure. *The structures of thalidomide and NO-ASA*

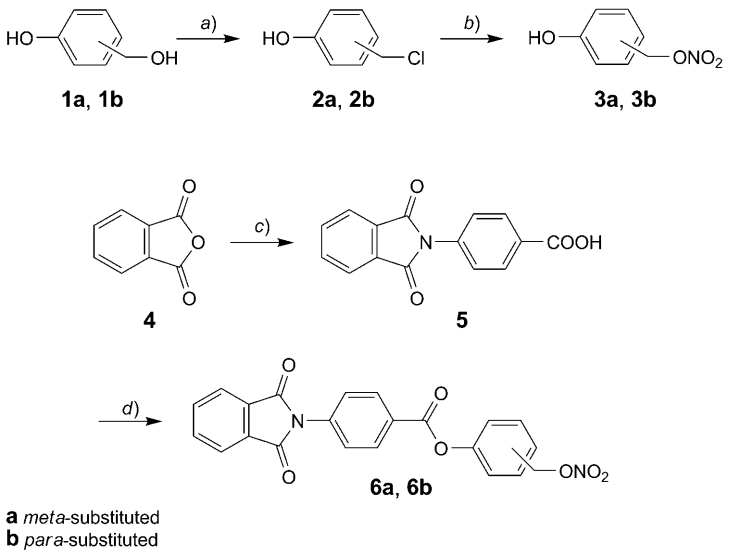
Nitric oxide (NO), naturally synthesized from L-arginine by the action of NO synthase (NOS), is a key signaling molecule involved in the regulation of many physiological processes. NO can also be generated from synthetic NO-releasing compounds, such as nitrate, furoxan, diazeniumdiolate, and others [10][11]. During recent years, NO-releasing derivatives have currently come into focus on the treatment of cancer, inflammation, and vascular diseases [12–14]. Among these compounds, NO-donating aspirin (NO-ASA; *Fig.*) is a representative drug for the treatment of cancer.

In studies *in vitro*, NO-ASA inhibited the growth of colon, prostate, lung, and breast cancer cell lines 10–6000-fold relative to their parent ASA [15–17].

Hybridization of two bioactive compounds is now accepted as an effective strategy for designing ligands, inhibitors, and other types of drugs. Our group has implemented the approach in drug discovery and achieved some promising results with varying compound classes [18][19]. With these in mind, and also based on the diverse bioactivities of the above-mentioned thalidomide as well as NO-ASA, we designed and synthesized 15 NO-donating thalidomide analogues. In this article, we describe the synthesis, *in vitro* cytotoxicity, and structure–activity relationships (SAR) of the target compounds.

Results and Discussion. – 1. *Synthesis.* Compounds **6a** and **6b** were prepared according to the procedures illustrated in *Scheme 1*. The appropriate (hydroxymethyl)phenols, **1a** and **1b**, were transformed into the corresponding chloro derivatives **2a** and **2b**, respectively, by chlorination with $SOCl_2$ in anhydrous $CHCl_3$. The subsequent reaction with $AgNO_3$ in MeCN gave the nitrates **3a** and **3b**, which reacted with acyl halide of **5** in anhydrous CH_2Cl_2 to afford **6a** and **6b**, respectively. Compound **5** was prepared from phthalic anhydride **4**, which was condensed with 4-aminobenzoic acid in refluxing AcOH to give **5**.

Scheme 1



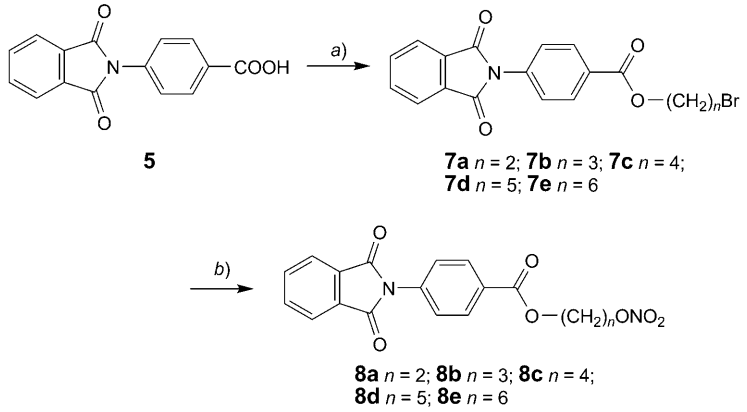
a) $SOCl_2$, $CHCl_3$, 0°, 0.5 h. *b*) $AgNO_3$, MeCN, 70°, 12 h. *c*) 4-Aminobenzoic acid, AcOH, reflux, 3 h. *d*) 1. $SOCl_2$, DMF (cat.), reflux, 1 h; 2. **3a**,**3b**, $CHCl_3$, r.t., 2 h.

To investigate the influence of replacing the aryl residue in the NO-donor moiety of **6a** and **6b** by an alkyl group on anticancer activity, compounds **8a**–**8e** were prepared.

The synthesis of **8a**–**8e** is depicted in *Scheme 2*. Compound **5** was first treated with dibromoalkanes bearing two to six C-atoms in the presence of Et_3N in acetone to

generate **7a**–**7e** in 70–88% yields. The subsequent reaction with $AgNO_3$ in MeCN produced the nitrates **8a**–**8e**.

Scheme 2



5

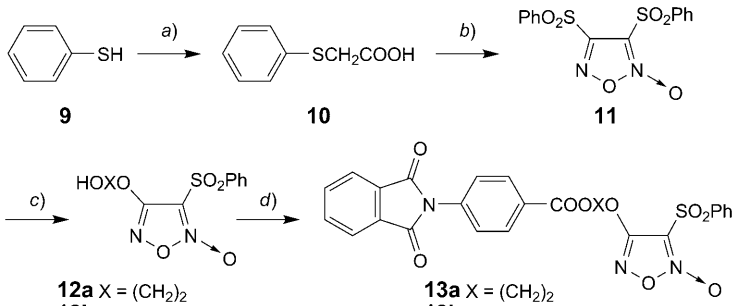
7a *n* = 2; **7b** *n* = 3; **7c** *n* = 4; **7d** *n* = 5; **7e** *n* = 6

8a *n* = 2; **8b** *n* = 3; **8c** *n* = 4; **8d** *n* = 5; **8e** *n* = 6

a) Br–$(CH_2)_n$–Br (n = 2–6), Et_3N, reflux, 6 h. *b*) $AgNO_3$, MeCN, 70°, 4 h.

Subsequently, to compare the effects of a furoxan (= forazan 2-oxide) with those of a nitrate on anticancer activity, compounds **13a**–**13h** were synthesized directly from **5** as depicted in *Scheme 3*.

Scheme 3



9 **10** **11**

12a X = $(CH_2)_2$
12b X = $(CH_2)_3$
12c X = $CH(Me)(CH_2)_2$
12d X = $(CH_2)_4$
12e X = $CH_2CH{=}CHCH_2$
12f X = CH_2CCCH_2
12g X = $(CH_2)_5$
12h X = $(CH_2)_2O(CH_2)_2$

13a X = $(CH_2)_2$
13b X = $(CH_2)_3$
13c X = $CH(Me)(CH_2)_2$
13d X = $(CH_2)_4$
13e X = $CH_2CH{=}CHCH_2$
13f X = CH_2CCCH_2
13g X = $(CH_2)_5$
13h X = $(CH_2)_2O(CH_2)_2$

a) 1. NaOH (aq.), $ClCH_2CO_2H$, 140°, 2 h; 2. 6N HCl. *b*) 1. 30% H_2O_2, AcOH, r.t., 3 h; 2. fuming HNO_3, 90°, 4 h. *c*) HO–X–OH, 50% NaOH, THF, r.t., 2 h. *d*) **5**, 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI), 4-(dimethylamino)pyridine (DMAP), CH_2Cl_2, r.t., 24 h.

The substituted furoxans were prepared in a three-step sequence. The starting thiophenol (**9**) was converted to 2-(phenylsulfanyl)acetic acid (**10**) by treatment with

$ClCH_2CO_2H$. Compound **10** was oxidized with 30% H_2O_2 solution, followed by treatment with fuming HNO_3 to give bis(phenylsulfonyl)furoxans **11** [20]. The latter were then converted to various mono(phenylsulfonyl)furoxans **12a**–**12h** by treatment with corresponding diol. Finally, the resulting furoxans were reacted with **5** to give **13a**–**13h**.

2. *Cytotoxicity Evaluation.* The pharmacological results, *i.e.*, the cytotoxic activities against HepG2 (human hepatoma cells), A549 (human lung carcinoma cells), and PC-3 (human prostate cancer cells), were summarized in the *Table.*

Table. *Cytotoxic Activity* (IC_{50} [μM]) *of Target Compounds against Human Tumor Cell Lines*

Compound	HepG2	PC-3	A549
6a	25.9 ± 2.5	38.5 ± 1.9	32.6 ± 1.4
6b	28.2 ± 1.6	27.8 ± 0.7	37.5 ± 1.7
8a	> 100	> 100	> 100
8b	> 100	> 100	> 100
8c	23.9 ± 2.0	> 100	> 100
8d	> 100	> 100	> 100
8e	> 100	> 100	> 100
13a	30.3 ± 1.9	24.8 ± 2.6	35.8 ± 1.1
13b	19.6 ± 1.7	13.5 ± 1.3	16.8 ± 1.0
13c	12.8 ± 2.1	7.2 ± 2.2	10.4 ± 1.1
13d	24.9 ± 1.8	27.4 ± 1.2	28.6 ± 2.9
13e	41.9 ± 2.2	30.4 ± 1.1	34.2 ± 0.5
13f	39.5 ± 2.1	33.5 ± 3.1	41.9 ± 2.6
13g	46.3 ± 1.4	47.7 ± 2.1	38.1 ± 2.5
13h	44.6 ± 1.9	41.5 ± 0.8	49.2 ± 0.7
Thalidomide	52.9 ± 1.3	33.4 ± 1.3	47.5 ± 1.8
5-FU[a])	19.8 ± 1.15	20.4 ± 1.25	25.3 ± 0.63

[a]) 5-Fluorouracil, used as a positive control. The data were the mean ± SD obtained from three independent experiments.

As can be seen from the *Table*, most target compounds showed certain anticancer activities against the tumor cells (HepG2, A549, and PC-3) as compared with the control 5-fluorouracil (5-FU). Among these compounds, **13a** and **13d** were as potent as 5-FU, while **13b** and **13c** were even more potent than 5-FU.

According to the activities mentioned above, some structure–activity relationships of target compounds can be inferred. First, the glutarimide ring of thalidomide is not essential for cytotoxicity against tumor cells lines, since substitution of the glutarimide moiety by phenyl ring does not produce biologically inactive analogues. Furthermore, the results in the *Table* indicate the bioactivity of target compounds is closely related to the type of NO donors. The compounds **13a**–**13d** with furoxan moiety displayed more potent anticancer activities than those with nitrate groups. Additionally, the length of the spacers connecting to NO donors also appears important for the bioactivities. Compounds **13b** and **13c** with a C_3 spacer was more potent than **13a** or **13d** with shorter or longer spacers, respectively. As a matter of fact, **13c** showed the highest anticancer activity than others.

Conclusions. – In search of more potent anticancer agents, we have designed and synthesized 15 NO-donating thalidomide analogues, **6a** and **6b**, **8a**–**8e**, and **13a**–**13h**. Preliminary screening experiments revealed that **13a**–**13d** showed anticancer activities similar to or better than that of 5-fluorouracil (5-FU). Further *in vivo* biological evaluation and mechanistic studies on this novel class of anticancer agents are currently in progress and will be reported in due course.

Experimental Part

1. *General.* Reagents were from *Shanghai Chemical Reagent Company* and used without further purification. Column chromatography (CC): silica gel *60* (SiO_2; 200–300 mesh). TLC: SiO_2 *60 F254* plates (250 μm; *Qingdao Ocean Chemical Company*, P. R. China). M.p.: *RDCSY-I* melting point apparatus; uncorrected. IR Spectra: *Shimadzu FTIR-8400S* spectrometer; in cm^{-1}. ^{1}H-NMR Spectra: *Bruker ACF-300Q* apparatus at 300 MHz, in $CDCl_3$ unless otherwise indicated; δ in ppm rel. to Me_4Si, J in Hz. MS: *Hewlett-Packard 1100 LC/MSD* spectrometer; in *m/z*. Elemental analysis: *Elementar Vario EL III* instrument.

3-Hydroxybenzyl Chloride (**2a**). $SOCl_2$ (0.22 ml, 12.0 mmol) was added dropwise to a cold soln. of *3-(hydroxymethyl)phenol* (**1a**; 496 mg, 4.0 mmol) in $CHCl_3$ (14 ml). The mixture was stirred for 1 h at r.t. The $CHCl_3$ layer was washed with H_2O and dried (Na_2SO_4), and the solvent was removed to afford **2a** (306 mg, 54%). Yellow oil. ESI-MS: 143 ($[M+H]^+$).

4-Hydroxybenzyl Chloride (**2b**). As described for **2a**, with $SOCl_2$ (0.22 ml, 12.0 mmol) and 4-(hydroxymethyl)phenol (**1b**; 496 mg, 4.0 mmol): **2b** (273 mg, 48%). Yellow oil. ESI-MS: 143 ($[M+H]^+$).

3-Hydroxybenzyl Nitrate (**3a**). A soln. of **2a** (284 mg, 2.00 mmol) in a small amount of MeCN was added to a stirred soln. of $AgNO_3$ (680 mg, 4.00 mmol) in MeCN (8 ml). Stirring was continued for 1 h at r.t. in the dark, and then the precipitate was filtered off, and the solvent was evaporated. The crude product was purified by CC (SiO_2; AcOEt/petroleum ether (PE) 1:1) to give **3a** (118 mg, 35%). Yellow oil. ESI-MS: 170 ($[M+H]^+$).

4-Hydroxybenzyl Nitrate (**3b**). As described for **3a**, with **2b** (284 mg, 2.00 mmol) and $AgNO_3$ (680 mg, 4.00 mmol): **3b** (91 mg, 27%). Yellow oil. ESI-MS: 170 ($[M+H]^+$).

*4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoic Acid* (**5**). A soln. of 4-aminobenzoic acid (10 g, 72.9 mmol) and phthalic anhydride (10 g, 67.5 mmol) in glacial AcOH (50 ml) and was heated to reflux for 1 h. Compound **5** solidified upon cooling. An almost white powder appeared that was filtered and washed twice with H_2O (50 ml), to give **5** (15.48 g, 80%). White powder. M.p. 293–294°. ^{1}H-NMR ((D_6)DMSO): 13.71–12.92 (br. *s*, 1 H); 8.09 (*d*, J = 8.7, 2 H); 8.01–7.91 (*m*, 4 H); 7.62 (*d*, J = 8.4, 2 H). ESI-MS: 266 ($[M-H]^-$).

*3-(Nitrooxymethyl)phenyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**6a**). A soln. of **5** (267 mg, 1.00 mmol) in freshly dist. $SOCl_2$ (4 ml) and cat. amount of DMF was vigorously stirred under reflux for 1 h. Then, the solvent was carefully evaporated at reduced pressure, and a soln. of **3a** (200 mg, 1.20 mmol) in CH_2Cl_2 (10 ml) was added. The mixture was stirred for 30 min at r.t., then poured in H_2O (20 ml) and extracted with CH_2Cl_2 (3 × 15 ml). The org. layer was dried (Na_2SO_4) and evaporated at reduced pressure to give **6a** (196 mg, 47%). M.p. 122–126°. IR (KBr): 1778, 1762, 1733, 1718, 1635. ^{1}H-NMR: 8.05 (*d*, J = 8.7, 2 H); 7.96–7.91 (*m*, 4 H); 7.86 (*d*, J = 9.3, 2 H); 7.54–7.23 (*m*, 4 H); 5.41 (*s*, 2 H). ESI-MS: 419 ($[M+H]^+$). Anal. calc. for $C_{22}H_{14}N_2O_7$: C 63.16, H 3.37, N 6.70; found: C 63.37, H 3.56, N 6.51.

*4-(Nitrooxymethyl)phenyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**6b**). As described for **6a**, with **3b** (1.20 mmol) and **5** (267 mg, 1.00 mmol): **6b** (176 mg, 42%). M.p. 101–104°. IR (KBr): 1781, 1759, 1738, 1712, 1633. ^{1}H-NMR: 8.01 (*d*, J = 9.0, 2 H), 7.98–7.87 (*m*, 4 H); 7.87 (*d*, J = 9.0, 2 H); 7.49–7.28 (*m*, 4 H); 5.47 (*s*, 2 H). ESI-MS: 419 ($[M+H]^+$). Anal. calc. for $C_{22}H_{14}N_2O_7$: C 63.16, H 3.37, N 6.70; found: C 63.30, H 3.19, N 6.44.

*2-Bromoethyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**7a**). To a stirred soln. of **5** (534 mg, 2.00 mmol) in acetone (6 ml), 1,2-dibromoethane (744 mg, 4.00 mmol) and Et_3N (0.4 ml) in acetone were added dropwise. The mixture was heated at reflux for 4 h and cooled to r.t. Solvent and

excess Et_3N were evaporated *in vacuo*, and the residue was purified by CC (SiO_2; AcOEt/PE 1:5) to give **7a** (610 mg, 83%). M.p. 195–198°. ^{1}H-NMR: 8.18 (*d*, *J* = 7.8, 2 H); 8.05–8.00 (*m*, 4 H); 7.66 (*d*, *J* = 7.8, 2 H); 4.69 (*t*, *J* = 6.0, 2 H); 3.65 (*t*, *J* = 6.0, 2 H).

*3-Bromopropyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**7b**). As described for **7a**, with 1,3-dibromopropane (800 mg, 4.00 mmol) and **5** (534 mg, 2.00 mmol): **7b** (302 mg, 78%). M.p. 172–174°. ^{1}H-NMR: 8.19 (*d*, *J* = 7.8, 2 H); 7.99–7.83 (*m*, 4 H); 7.61 (*d*, *J* = 7.8, 2 H); 4.57 (*t*, *J* = 6.0, 2 H); 3.58 (*t*, *J* = 6.3, 2 H); 2.43–2.34 (*m*, 2 H).

*4-Bromobutyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**7c**). As described for **7a**, with 1,4-dibromobutane (856 mg, 4.00 mmol) and **5** (534 mg, 2.00 mmol): **7c** (706 mg, 88%). M.p. 138–140°. ^{1}H-NMR: 8.18 (*d*, *J* = 8.4, 2 H); 7.99–7.81 (*m*, 4 H); 7.61 (*d*, *J* = 8.7, 2 H); 4.39 (*t*, *J* = 6.0, 2 H); 3.49 (*t*, *J* = 6.0, 2 H); 2.10–1.94 (*m*, 2 H).

*5-Bromopentyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**7d**). As described for **7a**, with 1,5-dibromopentane (912 mg, 4.00 mmol) and **5** (534 mg, 2.00 mmol): **7d** (680 mg, 82%). M.p. 103–105°. ^{1}H-NMR: 8.18 (*d*, *J* = 8.7, 2 H); 8.00–7.81 (*m*, 4 H); 7.61 (*d*, *J* = 8.7, 2 H); 4.37 (*t*, *J* = 6.3, 2 H); 3.45 (*t*, *J* = 6.6, 2 H); 2.00–1.78 (*m*, 4 H); 1.68–1.60 (*m*, 4 H).

*6-Bromohexyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**7e**). As described for **7a**, with 1,6-dibromohexane (968 mg, 4.00 mmol) and **5** (534 mg, 2.00 mmol): **7e** (600 mg, 70%). M.p. 126–128°. ^{1}H-NMR: 8.17 (*d*, *J* = 8.4, 2 H); 7.99–7.81 (*m*, 4 H); 7.26 (*d*, *J* = 9.0, 2 H); 4.36 (*t*, *J* = 6.6, 2 H); 3.43 (*t*, *J* = 6.6, 2 H); 1.93–1.79 (*m*, 4 H); 1.60–1.52 (*m*, 4 H).

*2-(Nitrooxy)ethyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**8a**). To a suspension of **7a** (373 mg, 1.00 mmol) in MeCN (10 ml), $AgNO_3$ (340 mg, 2.00 mmol) was added, and the mixture was refluxed for 4 h. The precipitate was filtered off, and the solvent was carefully evaporated. The residue was taken up in AcOEt, washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The crude residue was purified by CC (SiO_2; AcOEt/PE 1:5) to afford **8a** (146 mg, 41%) as a pale-yellow solid that was further purified by twice crystallizations with 95% EtOH. M.p. 173–175°. IR (KBr): 1758, 1740, 1698, 1633. ^{1}H-NMR: 8.19 (*d*, *J* = 8.7, 2 H); 8.00–7.83 (*m*, 4 H); 7.63 (*d*, *J* = 9.0, 2 H); 4.74 (*t*, *J* = 6.0, 2 H); 4.64 (*t*, *J* = 6.0, 2 H). ESI-MS: 357 ($[M+H]^+$). Anal. calc. for $C_{17}H_{12}N_2O_7$: C 57.31, H 3.39, N 7.86; found: C 57.65, H 3.55, N 7.56.

*3-(Nitrooxy)propyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**8b**). As described for **8a**, with $AgNO_3$ (340 mg, 2.00 mmol) and **7b** (387 mg, 1.00 mmol): **8b** (193 mg, 52%). M.p. 142–144°. IR (KBr): 1789, 1732, 1701, 1629. ^{1}H-NMR: 8.18 (*d*, *J* = 8.4, 2 H); 7.99–7.81 (*m*, 4 H); 7.62 (*d*, *J* = 9.3, 2 H); 4.65 (*t*, *J* = 6.3, 2 H); 4.47 (*t*, *J* = 6.0, 2 H); 2.28–2.20 (*m*, 2 H). ESI-MS: 371 ($[M+H]^+$). Anal. calc. for $C_{18}H_{14}N_2O_7$: C 58.38, H 3.81, N 7.56; found: C 58.10, H 3.57, N 7.78.

*4-(Nitrooxy)butyl 4-(1,3-Dioxo-2,3-dihydro-1*H*-2-isoindolyl)benzoate* (**8c**). As described for **7a**, with $AgNO_3$ (340 mg, 2.00 mmol) and **7c** (401 mg, 1.00 mmol): **8c** (177 mg, 46%). M.p. 118–119°. IR (KBr): 1770, 1729, 1710, 1643. ^{1}H-NMR: 8.24 (*d*, *J* = 8.7, 2 H); 7.98–7.82 (*m*, 4 H); 7.62 (*d*, *J* = 9.0, 2 H); 4.56 (*t*, *J* = 6.6, 2 H); 4.28 (*t*, *J* = 6.3, 2 H); 2.09–2.02 (*m*, 4 H). ESI-MS: 385 ($[M+H]^+$). Anal. calc. for $C_{19}H_{16}N_2O_7$: C 59.38, H 4.20, N 7.29; found: C 59.14, H 3.88, N 7.57.

*5-(Nitrooxy)pentyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl) benzoate* (**8d**). As described for **7a**, with $AgNO_3$ (340 mg, 2.00 mmol) and **7d** (415 mg, 1.00 mmol): **8d** (195 mg, 49%). M.p. 91–94°. IR (KBr): 1792, 1736, 1708, 1656. ^{1}H-NMR: 8.17 (*d*, *J* = 8.4, 2 H); 7.98–7.82 (*m*, 4 H); 7.63 (*d*, *J* = 8.4, 2 H); 4.55 (*t*, *J* = 6.0, 2 H); 4.40 (*t*, *J* = 6.0, 2 H); 2.28–2.20 (*m*, 4 H); 1.62–1.56 (*m*, 2 H). ESI-MS: 399 ($[M+H]^+$). Anal. calc. for $C_{20}H_{18}N_2O_7$: C 60.30, H 4.55, N 7.03; found: C 60.46, H 4.69, N 6.87.

*6-(Nitrooxy)hexyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl) benzoate* (**8e**). As described for **7a**, with $AgNO_3$ (340 mg, 2.00 mmol) and **7e** (429 mg, 1.00 mmol): **8e** (181 mg, 44%). M.p. 78–80°. IR (KBr): 1788, 1737, 1703, 1659. ^{1}H-NMR: 8.18 (*d*, *J* = 8.4, 2 H); 7.99–7.81 (*m*, 4 H); 7.60 (*d*, *J* = 8.7, 2 H); 4.71 (*t*, *J* = 6.3, 2 H,); 4.36 (*t*, *J* = 6.3, 2 H); 1.80–1.78 (*m*, 4 H); 1.60–1.51 (*m*, 4 H). ESI-MS: 413 ($[M+H]^+$). Anal. calc. for $C_{21}H_{20}N_2O_7$: C 61.16, H 4.89, N 6.79; found: C 61.32, H 4.81, N 6.69.

2-(Phenylsulfanyl)acetic Acid (**10**). *Benzenethiol* (**9**; 24.2 g, 0.22 mol) and NaOH (8.8 g, 0.22 mol) were dissolved in 95% EtOH (110 ml) and added to a soln. of $ClCH_2CO_2H$ (22.7 g, 0.24 mol) and Na_2CO_3 (12.7 g, 0.12 mol) in H_2O (50 ml). The mixture was stirred for *ca.* 3 h at r.t. and heated to reflux for 1 h, cooled to r.t., and HCl was added to the soln. until pH 2. After removal of EtOH *in vacuo*, white crystals of **10** were formed in a 92% yield (34 g). M.p. 60–62°. ESI-MS: 167 ($[M-H]^+$).

3,4-Bis(phenylsulfonyl)-1,2,5-oxadizole 2-Oxide (**11**). To a soln. of **10** (13.4 g, 0.08 mol) in glacial AcOH (50 ml) was added 30% H_2O_2 aq. soln. The mixture was stirred for *ca.* 3 h at r.t., fuming HNO_3 (32 ml, 0.172 mol) was added dropwise, and the mixture was stirred for *ca.* 0.5 h at 90°. After cooling to r.t., white crystals of **11** were formed in 76% yield (22.2 g). M.p. 154–156°. ^{1}H-NMR: 8.19–8.15 (*m*, 4 H); 7.84–7.63 (*m*, 6 H).

2-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]ethanol (**12a**). To a stirred soln. of **11** (732 mg, 2.00 mmol) in THF (20 ml) was first added glycol (248 mg, 4.0 mmol) and then, keeping the temp. at 25°, a 50% NaOH soln. (*w*/*w*; 0.35 g, 4.00 mmol). The mixture was kept under stirring for 3 h at r.t. The solvent was removed *in vacuo*, and the residue was treated with H_2O and extracted with CH_2Cl_2. The combined org. layers were dried (Na_2SO_4) and concentrated *in vacuo*. The concentrated soln. was treated with charcoal, filtered, and then evaporated *in vacuo* to give **12a** (372 mg, 65%). White solid. M.p. 118–119°. ^{1}H-NMR: 8.09 (*d*, *J* = 8.7, 2 H); 7.77–7.60 (*m*, 3 H); 4.72 (*t*, *J* = 6.0, 2 H); 3.80 (*t*, *J* = 6.9, 2 H); 2.12–2.07 (br. *s*, 1 H).

3-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]propan-1-ol (**12b**). As described for **12a**, with propane-1,3-diol (304 mg, 4.0 mmol) and **11** (732 mg, 2.00 mmol): **12b** (432 mg, 72%). M.p. 105–106°. ^{1}H-NMR: 8.01 (*d*, *J* = 7.8, 2 H); 7.91–7.75 (*m*, 3 H); 4.54 (*t*, *J* = 5.4, 2 H); 3.86 (*t*, *J* = 6.0, 2 H); 2.45–2.36 (*m*, 2 H); 2.08–2.01 (br. *s*, 1 H).

1-Methyl-3-[2-oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]propan-1-ol (**12c**). As described for **12a**, with butane-1,3-diol (360 mg, 4.0 mmol) and **11** (732 mg, 2.00 mmol): **12c** (522 mg, 83%). M.p. 104–107°. ^{1}H-NMR: 8.05 (*d*, *J* = 7.5, 2 H); 7.79–7.60 (*m*, 3 H); 4.52 (*t*, *J* = 6.3, 2 H); 4.13–4.07 (*m*, 1 H); 2.22–2.13 (*m*, 2 H); 2.07–1.95 (br. *s*, 1 H); 1.31 (*d*, *J* = 6.0, 3 H).

4-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]butan-1-ol (**12d**). As described for **12a**, with butane-1,4-diol (360 mg, 4.0 mmol) and **11** (732 mg, 2.00 mmol): **12d** (428 mg, 68%). M.p. 61–64°. ^{1}H-NMR: 8.04 (*d*, *J* = 7.8, 2 H); 7.59–7.44 (*m*, 3 H); 4.48 (*t*, *J* = 6.0, 2 H); 3.82 (*t*, *J* = 6.0, 2 H); 2.11–2.05 (br. *s*, 1 H); 2.03–1.98 (*m*, 2 H); 1.79–1.74 (*m*, 2 H).

4-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]but-2-en-1-ol (**12e**). As described for **12a**, with but-2-ene-1,4-diol (352 mg, 4.0 mmol) and **11** (732 mg, 2.00 mmol): **12e** (456 mg, 73%). M.p. 72–74°. ^{1}H-NMR: 8.06 (*d*, *J* = 7.5, 2 H); 7.66–7.60 (*m*, 3 H); 6.05–5.99 (*m*, 1 H); 5.86–5.78 (*m*, 1 H); 5.13 (*t*, *J* = 5.1, 2 H); 4.32 (*t*, *J* = 6.3, 2 H); 2.07–2.03 (br. *s*, 1 H).

4-[2-Oxido-4-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]but-2-yn-1-ol (**12f**). As described for **12a**, with but-2-yne-1,4-diol (344 mg, 4.0 mmol) and **11** (732 mg, 2.00 mmol): **12f** (372 mg, 60%). M.p. 110–112°. ^{1}H-NMR: 8.03 (*d*, *J* = 7.8, 2 H); 7.73–7.65 (*m*, 3 H); 5.11 (*s*, 2 H); 4.35 (*s*, 2 H); 2.05–2.01 (br. *s*, 1 H).

5-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]pentan-1-ol (**12g**). As described for **12a**, with pentane-1,5-diol (416 mg, 4.0 mmol) and **11** (732 mg, 2.00 mmol): **12g** (460 mg, 71%). M.p. 63–67°. ^{1}H-NMR: 8.05 (*d*, *J* = 7.8, 2 H); 7.78–7.64 (*m*, 3 H); 4.44 (*t*, *J* = 6.3, 2 H); 3.70 (*t*, *J* = 6.0, 2 H); 2.13–2.09 (br. *s*, 1 H); 1.96–1.88 (*m*, 2 H); 1.70–1.63 (*m*, 4 H).

2-{2-[2-Oxido-4-(phenylsulfonyl)-1,2,5-oxadizol-2-yloxy]ethoxy}ethanol (**12h**). As described for **12a**, with diethylene glycol (424 mg, 4.0 mmol) and **11** (732 mg, 2.00 mmol): **12g** (342 mg, 52%). M.p. 58–60°. ^{1}H-NMR: 8.09 (*d*, *J* = 7.8, 2 H); 7.79–7.60 (*m*, 3 H); 4.59 (*t*, *J* = 4.4, 2 H); 3.95 (*t*, *J* = 4.4, 2 H); 3.79 (*t*, *J* = 4.4, 2 H); 3.70 (*t*, *J* = 7.8, 2 H); 2.08–2.03 (br. *s*, 1 H).

*2-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]ethyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**13a**). To a stirred soln. of **5** (265 mg, 1.00 mmol) in THF (8.0 ml) was added EDCI (183 mg, 1.00 mmol) and DMAP (306 mg, 3 mmol). The mixture was stirred for 1 h in ice-water bath. Then, **12a** (286 mg, 1.00 mmol) was added, and the mixture was stirred at r.t. overnight. Solvent was put into ice-H_2O, and the H_2O layer was extracted with AcOEt. The combined org. layers were dried (Na_2SO_4) and evaporated *in vacuo*. The product was purified by CC (SiO_2; AcOEt/PE 1:2): **13a** (380 mg, 71%). M.p. 195–196°. IR (KBr): 1780, 1744, 1705, 1370, 1158. ^{1}H-NMR: 8.09–8.00 (*m*, 6 H); 7.82–7.62 (*m*, 7 H); 4.87 (*t*, *J* = 6.6, 2 H); 4.76 (*t*, *J* = 6.0, 2 H). ESI-MS: 536 ($[M+H]^+$). Anal. calc. for $C_{25}H_{17}N_3O_9S$: C 56.07, H 3.20, N 7.85; found: C 56.19, H 3.12, N 7.73.

*3-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]propyl 4-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)benzoate* (**13b**). As described for **13a**, with **5** (265 mg, 1.00 mmol) and **12b** (286 mg, 1.00 mmol): **13b** (307 mg, 56%). M.p. 185–187°. IR (KBr): 1771, 1745, 1714, 1365, 1156. ^{1}H-NMR: 8.09–8.03 (*m*, 6 H);

7.78–7.59 (*m*, 7 H); 4.65(*t*, *J* = 6.0, 2 H); 4.25 (*t*, *J* = 6.6, 2 H); 2.52–2.45 (*m*, 2 H). ESI-MS: 550 ([*M* + H]$^+$). Anal. calc. for $C_{26}H_{19}N_3O_9S$: C 56.83, H 3.49, N 7.65; found: C 57.11, H 3.57, N 7.39.

*1-Methyl-3-[2-oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]propyl 4-(2,3-Dihydro-1,3-dioxo-1*H-*isoindol-2-yl)benzoate* (**13c**). As described for **13a**, with **5** (265 mg, 1.00 mmol) and **12c** (300 mg, 1.00 mmol): **13c** (366 mg, 65%). M.p. 163–165°. IR (KBr): 1786, 1739, 1700, 1311, 1169. ^{1}H-NMR: 8.11–7.99 (*m*, 6 H); 7.82–7.59 (*m*, 7 H); 5.46–5.39 (*m*, 1 H); 4.58 (*t*, *J* = 6.6, 2 H); 2.34–2.27 (*m*, 2 H); 1.57 (*d*, *J* = 6.3, 3 H). ESI-MS: 564 ([*M* + H]$^+$). Anal. calc. for $C_{27}H_{21}N_3O_9S$: C 57.55, H 3.76, N 7.46; found: C 57.43, H 3.60, N 7.50.

*4-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]butyl 4-(2,3-Dihydro-1,3-dioxo-1*H-*isoindol-2-yl)benzoate* (**13d**). As described for **13a**, with **5** (265 mg, 1.00 mmol) and **12d** (300 mg, 1.00 mmol): **13d** (333 mg, 59%). M.p. 152–154°. IR (KBr): 1780, 1728, 1705, 1366, 1189. ^{1}H-NMR: 8.08–7.97 (*m*, 6 H); 7.85–7.61 (*m*, 7 H); 4.52 (*t*, *J* = 6.0, 2 H); 4.46 (*m*, *J* = 6.0, 2 H); 2.07–1.99 (*m*, 4 H). ESI-MS: 564 ([*M* + H]$^+$). Anal. calc. for $C_{27}H_{21}N_3O_9S$: C 57.55, H 3.76, N 7.46; found: C 57.39, H 3.92, N 7.32.

*4-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]but-2-enyl 4-(2,3-Dihydro-1,3-dioxo-1*H-*isoindol-2-yl)benzoate* (**13e**). As described for **13a**, with **5** (265 mg, 1.00 mmol) and **12e** (298 mg, 1.00 mmol): **13e** (269 mg, 48%). M.p. 149–150°. IR (KBr): 1782, 1743, 1687, 1368, 1142. ^{1}H-NMR: 7.99–7.83 (*m*, 6 H); 7.82–7.60 (*m*, 7 H); 6.09–5.94 (*m*, 2 H); 5.15 (*d*, *J* = 5.7, 2 H); 4.35 (*d*, *J* = 5.4, 2 H). ESI-MS: 562 ([*M* + H]$^+$). Anal. calc. for $C_{27}H_{19}N_3O_9S$: C 57.75, H 3.41, N 7.48; found: C 57.82, H 3.47, N 7.48.

*4-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]but-2-ynyl 4-(2,3-Dihydro-1,3-dioxo-1*H-*isoindol-2-yl)benzoate* (**13f**). As described for **13a**, with **5** (265 mg, 1.00 mmol) and **12f** (296 mg, 1.00 mmol): **13f** (314 mg, 56%). M.p. 134–136°. IR (KBr): 1767, 1735, 1782, 1392, 1188. ^{1}H-NMR: 8.15–8.05 (*m*, 6 H); 7.84–7.63 (*m*, 6 H); 5.14 (*s*, 2 H); 4.88 (*s*, 2 H). ESI-MS: 560 ([*M* + H]$^+$). Anal. calc. for $C_{27}H_{17}N_3O_9S$: C 57.96, H 3.06, N 7.51; found: C 57.75, H 3.30, N 7.29.

*5-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]pentyl 4-(2,3-Dihydro-1,3-dioxo-1*H-*isoindol-2-yl)benzoate* (**13g**). As described for **13a**, with **5** (265 mg, 1.00 mmol) and **12g** (314 mg, 1.00 mmol): **13g** (381 mg, 66%). M.p. 114 116°. IR (KBr): 1779, 1746, 1695, 1348, 1167. ^{1}H-NMR: 8.10–8.01 (*m*, 6 H); 7.84–7.61 (*m*, 7 H); 4.56 (*t*, *J* = 6.3, 2 H); 4.35 (*t*, *J* = 6.2, 2 H); 1.98–1.92 (*m*, 4 H); 1.58–1.52 (*m*, 2 H). ESI-MS: 578 ([*M* + H]$^+$). Anal. calc. for $C_{28}H_{23}N_3O_9S$: C 58.23, H 4.01, N 7.28; found: C 58.21, H 4.14, N 7.27.

*2-{2-[2-Oxido-3-(phenylsulfonyl)-1,2,5-oxadizol-4-yloxy]ethoxy}ethyl 4-(2,3-Dihydro-1,3-dioxo-1*H-*isoindol-2-yl)benzoate* (**13h**). As described for **13a**, with **5** (265 mg, 1.00 mmol) and **12h** (316 mg, 1.00 mmol): **13h** (353 mg, 61%). M.p. 85–87°. IR (KBr): 1782, 1738, 1700, 1373, 1163. ^{1}H-NMR: 8.18–7.96 (*m*, 6 H); 7.84–7.56 (*m*, 7 H); 4.60 (*t*, *J* = 4.5, 2 H); 4.55 (*t*, *J* = 4.2, 2 H); 4.00–3.93 (*m*, 4 H). ESI-MS: 580 ([*M* + H]$^+$). Anal. calc. for $C_{27}H_{21}N_3O_{10}S$: C 55.96, H 3.65, N 7.25; found: C 56.04, H 3.81, N 7.11.

2. *Cytotoxicity Assays.* All of the prepared compounds were tested for their *in vitro* anticancer activities against HepG2, A549, and PC-3 cells by MTT-based assay [21] with some modifications.

Briefly, cells were cultured in *RPMI 1640* medium, supplemented with 10% fetal calf serum at 37° with 5% CO_2. For experiments, cells were plated in 96-well plates (10^5 cells/well for adherent cells or 0.3×10^6 cells/well for suspended cells in 100 µl medium). After 24 h, the compounds (10^{-4}–10^{-8} M) dissolved in DMSO (5%) were added to each well and incubated for 3 d (72 h). Control groups received the same amount of DMSO. 5-Fluorouracil (5-FU) was used as positive control. At the end of 72-h incubation, the medium in each well was replaced by fresh medium (200 µl) containing 0.5 mg/ml of MTT. Three h later, the formazan product of MTT reduction was dissolved in DMSO, and absorbance was measured using an ELISA micro-plate reader. Growth of tumoral cells was quantitated by the ability of living cells to reduce the yellow dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide (MTT) to a purple formazan product. Drug effect was quantified as the percentage of control absorbance of reduced dye at 590 nm. In all experiments, three replicate wells were used for each drug concentration, and each assay was carried out at least three times. The results of this experiment were summarized in the *Table*.

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