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Novel solanesylpiperazinotriamines as well as their *N*-aryl-substituted analogs were synthesized starting from solanesol through a multistep procedure. Their structures were confirmed by IR, ^{1}H NMR, MS, and elemental analysis. The preliminary results indicated that these novel derivatives were preferentially toxic against tumor cells, and at non-cytotoxic concentration, the synergistic effect of solanesyltriamines (**3a** and **3c**) was even superior to that of *N*,*N*′-bis(3,4-dimethoxybenzyl)-*N*-solanesylethylenediamine. Interestingly, the cytotoxicity of solanesylpiperazinotriamine derivatives was markedly enhanced when conjugating with aryl pharmacophores (**7**–**9**).

Keywords: solanesol; solanesylpiperazinotriamine; antitumor; synergistic effect

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1. Introduction

Solanesol (**1**, Figure 1), all-*E* conformational polyprenol extracted from tobacco leaves, is an important naturally occurring compound, which has been considered as membrane and cell components in the early development of life [1], and it acts as biosynthetic precursors of many thousands of complex isoprenoids as well as prenylated proteins and carbohydrates [2,3]. In fact, many clinical agents containing polyprenyl fragments, such as C_OQ_{10} and vitamin K_2, exhibit various pharmacological activities (e.g. quenching of free radicals, participating in the electron transport process for respiration, promoting normal clotting of the blood) [4–6]. Thus, structural modifications based on solanesol will be of attractive synthetic effort for the indispensable natural product. In the past years, a series of potent agents containing isoprenoid side chains of different lengths have been synthesized with promising blocking Pgp-mediated drug transport, which resulted in the multidrug resistance (MDR) of many clinical anticancer drugs [7]. Among them, a solanesol derivative, *N*,*N*′-bis(3,4-dimethoxybenzyl)-*N*-solanesylethylenediamine (SDB; Figure 1), is found to potently overcome the MDR in tumor models and sensitize tumor cells to conventional anticancer drugs [8,9].

Previous work in our laboratory involved evaluating the cytotoxicity of solanesol derivatives conjugated with various pharmacological groups via either ester or acyl chemical bond [10,11]. Unfortunately, most of them possessed undesirable pharmacological properties against the tested cell lines. Recently, prompted by the MDR-reverse activity

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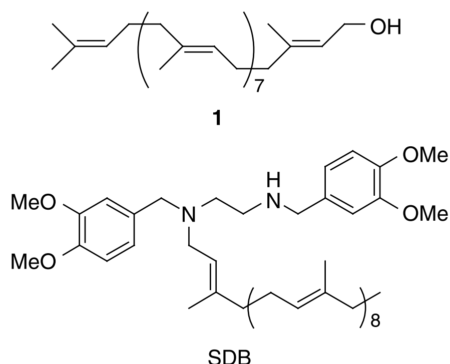

Figure 1. Chemical structures of compounds **1** and SDB.

of SDB on clinical drugs, we have synthesized a series of solanesyltriamines and evaluated the synergistic effects on anticancer drugs. The preliminary results indicated that these solanesol derivatives were preferentially toxic for several tumor cells, and at non-cytotoxic concentrations, they could sensitize cells to vincristine (VCR) [12]. In order to further explore the optimal structure of solanesol derivatives, we report, in this paper, the synthesis and cytotoxicity of novel solanesyltriamine analogs, and investigate the synergistic effects of target compounds on clinical anticancer drug.

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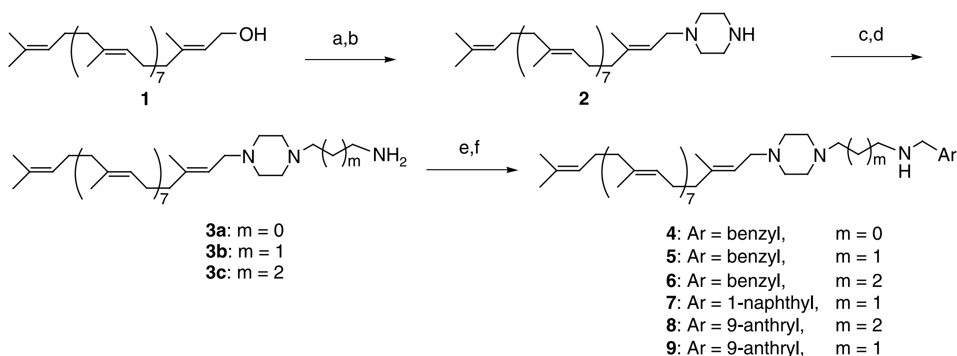
2. Results and discussion

2.1 Synthesis

Because of the intrinsic disadvantage of amide in biological conditions, solanesol derivatives conjoining with pharmacophore via ester or acyl chemical bond exhibited less cancer cell line inhibitory activities [10,11]. On the other hand, little data have been reported on the cytotoxicity of solanesyltriamine derivatives with C—N bond as a linkage. Moreover, we introduce piperazine unit, which is known as a privileged substructure in a great number of bioactive agents, as the linking element. Thus, a series of solanesylpiperazinoalkyltriamine analogs were prepared through an effective strategy described below.

The synthesis of target compounds is outlined in Scheme 1.

Compound **2** was readily prepared according to the literature [13,14]. Solanesol (extracted from tobacco leaves, $>90\%$ HPLC purity) was bromized by PBr_3 in anhydrous petroleum ether and then bromide was reacted with piperazine via the nucleophilic substitution process to afford compound **2** in a mixture of C_2H_5OH–isopropyl alcohol. Compound **3** was synthesized through the Gabriel reaction procedure as previously reported



Scheme 1. Reagents and conditions: (a) PBr_3, anhydrous petroleum ether, 0–5°C, 90%; (b) piperazine, C_2H_5OH–isopropyl alcohol, r.t., 61.5%; (c) *N*-bromoalkylphthalimide, K_2CO_3–CH_3CN, reflux, 63.3–79.5%; (d) $H_2NNH_2 \cdot H_2O$, C_2H_5OH, reflux, 73.3–84.6%; (e) aldehyde, CH_2Cl_2–CH_3OH, r.t.; (f) $NaBH_4$, CH_2Cl_2–CH_3OH, r.t. 65–78%.

[12]: compound **2** (15 mmol) was reacted with *N*-bromoalkylphthalimide (18 mmol) for 16 h using K_2CO_3 as a catalyst. Then, the intermediate and hydrazine hydrate (12 mmol) in 30 ml of ethanol were heated to reflux until the reaction was complete (monitored by thin-layer chromatography, TLC) and the hot solution was concentrated *in vacuo*. After cooling to ambient temperature, the residue was suspended in 10% ammonia and extracted with $CHCl_3$, and then dried with K_2CO_3. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, $V_{CHCl_3}:V_{CH_3OH}:V_{NH_3 \cdot H_2O} = 5:1:0.06$) to afford compounds **3a**–**3c** (white solid) in good yield (73.3–84.6%). Finally, the target compounds **4**–**9** were conveniently prepared through reductive amination of **3a**–**3c** with the corresponding aldehydes.

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2.2 Biological activity

2.2.1 Cytotoxicity

As shown in Table 1, the *in vitro* antitumor activities of the target compounds were investigated by the standard MTT assay against L1210 (murine leukemia) and HeLa cells. In general, the IC_{50} values of tested compounds on L1210 cells were smaller than those on HeLa cells. As with L1210 cells, the inhibitory activity of *N*-aryl-substituted derivatives was stronger than that of their respective parent compounds (e.g. **4** vs. **3a**, **6** vs. **3c**) except for compound **5**, which was weaker than **3b**. Moreover, the *N*-anthryl substitution derivatives (**8** and **9**) exerted significant antitumor activity among all the compounds tested.

Table 1. The *in vitro* cytotoxicity of synthetic compounds against tumor cell lines.

	IC_{50} (μM)	
	L1210	HeLa
3a	11.21 ± 3.64	–
3b	4.40 ± 0.41	–
3c	17.08 ± 0.48	–
4	3.48 ± 0.23	6.77 ± 0.76
5	28.40 ± 6.36	>100
6	11.27 ± 4.55	>100
7	4.59 ± 0.59	7.98 ± 1.72
8	3.45 ± 0.55	9.10 ± 2.23
9	2.83 ± 0.83	17.55 ± 0.45
SDB	>100	–
N-Butyl-9-anthracene	14.60 ± 1.35	–

Note: L1210, murine leukemia; HeLa, human cervical carcinoma.

2.2.2 Synergistic effect

Figure 2 illustrated the synergistic effects of selected compounds on clinical antitumor agent vs. the reference compound SDB. As shown in Figure 2, the inhibitory behavior of compounds **3a** and **3c** in combination with VCR resembled the model of the reference compound SDB, and exhibited a superior synergistic effect than that of SDB. As with **3b**, the inhibitory rate soared markedly along with gradually increasing concentrations, which displayed a different manner. Interestingly, at non-cytotoxic concentration (e.g. 1 μmol/l), the inhibitory rates of compounds **3a** and **3c**

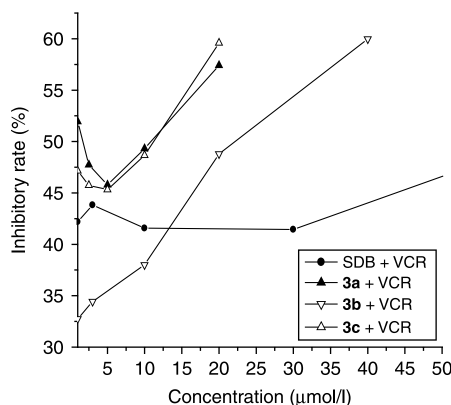

Figure 2. The corporate synergistic effects of compounds **3a**–**3c**. Cells were treated with VCR alone or with VCR in combination with SDB and **3a**–**3c**, respectively, and incubated for 48 h. Inhibitory rate was determined by the MTT test. Tested compounds were performed at 1 μmol/l except VCR, 2 μmol/l. Data are mean ± SE of three independent experiments.

were evidently higher than those of SDB. Especially, **3a** markedly enhanced the curative effect of VCR.

The antitumor activity of *N*-aryl-substituted solanesol derivatives (except for **5** and **6**) was generally higher than the reference compound *N*-butyl-9-anthracene, which was derived from the anthracene nucleus and an alkyl chain. The results may be due to the presence of a solanesyl fragment resulting in the different synergistic effects of solanesol derivatives.

3. Conclusion

In summary, a series of solanesylpiperazinoalkylamines as well as their *N*-aryl-substituted derivatives were prepared and investigated for their antitumor activities. The preliminary results indicated that these novel derivatives exerted preferentially toxic effect on tumor cells when administered alone and that, at non-cytotoxic concentration, the synergistic effect of solanesyltriamines (**3a** and **3c**) was even superior to that of SDB. When conjugated with aryl pharmacophores (e.g. **7–9**), the cytotoxicity was evidently enhanced. Detailed studies are required to determine the mechanism of synergy by which solanesol derivatives sensitize tumor cells to anticancer drugs, and the role of the solanesol fragment on the cytotoxic profiles is also worthy of further investigation, which are in progress in our laboratories.

4. Experimental

4.1 General experimental procedures

The reagents used were purchased from commercial sources and were employed without further purification. Solanesol (>90% HPLC purity) was extracted from tobacco leaves in our laboratory. Reactions were monitored by TLC using silica gel GF254 (Haiyang Chemical Company, Qingdao, China) with UV light (254 nm) for visualization. Column chromatography was performed over silica gel (200–300 mesh) purchased from Haiyang Chemical Company.

The ^1H NMR spectra were recorded on a Bruker AV-400 instrument using $CDCl_3$ as the solvent. Chemical shifts are reported in parts per million (δ). The IR spectra were obtained by means of the KBr pellet technique on an Anatar-360 spectrometer. The MS spectra were performed on an Esquire 3000 LC-MS instrument and the elemental analyses were taken on a PE-2400 analyzer.

4.2 General synthesis procedure of compounds 3a–3c

A mixture of compound **2** (15 mmol), *N*-bromoalkylphthalimide (18 mmol), and K_2CO_3 (73.9 mmol) in 50 ml of acetonitrile was heated to reflux for 16 h. The hot suspension was filtered and the filtrate was concentrated under reduced pressure to give the phthalimide intermediate. Then, the intermediate and hydrazine hydrate (12 mmol) in 30 ml of ethanol were heated to reflux for 3 h. The hot solution was concentrated *in vacuo*. The residue was suspended in 10% ammonia and extracted with $CHCl_3$, dried on anhydrous K_2CO_3. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography to afford compounds **3a–3c**, respectively.

4.2.1 N^1-Solanesyl-N^4-(2-aminoethyl)-piperazine (3a)

IR (KBr) ν_{max}: 3421, 2920, 1655 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.30 (br s, 1H), 4.89 (m, 8H), 3.23 (br, 2H, $-NH_2$), 2.99 (br s, 2H), 2.86 (br s, 2H), 2.68–2.60 (m, 8H), 1.87–1.75 (m, 34H), 1.45 (s, 3H), 1.37 (s, 27H); ESI-MS *m/z*: 743.0 $[M+H]^+$; Elemental analysis: Found: C, 82.02%; H, 11.49%; N, 5.48%; calcd for $C_{51}H_{87}N_3 \cdot 0.5H_2O$: C, 82.13%; H, 11.81%; N, 5.63%.

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4.2.2 N^1-Solanesyl-N^4-(3-aminopropyl)-piperazine (**3b**)

IR (KBr) ν_{max}: 3350, 2960, 1665 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.26 (br s, 1H), 5.11 (m, 8H), 2.97 (d, $J = 6.9$ Hz, 2H), 2.85 (t, $J = 6.4$ Hz, 2H), 2.60 (m, 8H), 2.46 (t, $J = 6.9$ Hz, 2H), 2.08–1.98 (m, 34H), 1.73 (t, 2H), 1.64 (s, 3H), 1.60 (s, 27H); ESI-MS *m/z*: 757.0 $[M+H]^+$; Elemental analysis: Found: C, 76.37%; H, 11.76%, N, 5.31%; calcd for $C_{52}H_{89}N_3 \cdot 3.5H_2O$: C, 76.23%; H, 11.81%; N, 5.29%.

4.2.3 N^1-Solanesyl-N^4-(4-aminobutyl)-piperazine (**3c**)

IR (KBr) ν_{max}: 3345, 2924, 1664 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.25 (t, $J = 6.4$ Hz, 1H), 5.11 (m, 8H), 4.14 (br s, 2H, —NH_2), 3.00 (d, $J = 6.7$ Hz, 2H), 2.80 (t, $J = 5.9$ Hz, 2H), 2.52 (br s, 8H), 2.40 (t, $J = 6.3$ Hz, 2H), 2.07–1.60 (m, 34H), 1.69 (s, 2H), 1.65 (s, 3H), 1.61 (s, 27H); ESI-MS *m/z*: 770.9 $[M+H]^+$; Elemental analysis: Found: C, 80.89%; H, 11.65%; N, 5.28%; calcd for $C_{53}H_{91}N_3 \cdot H_2O$: C, 80.75%; H, 11.89%; N, 5.33%.

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4.3 General synthesis procedure of target compounds 4–9

To a stirred solution of amines **3a**–**3c** (12 mmol) in 25% $CH_3OH-CH_2Cl_2$ (10 ml), respective aldehydes (10 mmol) were added in 25% $CH_3OH-CH_2Cl_2$ (10 ml) under N_2. The mixture was stirred at room temperature overnight until the imine formation was complete (monitored by TLC). The solvent was evaporated *in vacuo* to give the crude imine, which was used for reduction without further purification.

$NaBH_4$ (30 mmol) was added in small portions to the solution of crude imine in 50% $CH_3OH-CH_2Cl_2$ (20 ml) at 0°C. The mixture was stirred at room temperature overnight and then concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (50 ml) and washed three times with 50 ml of aqueous NaOH (5%). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to provide the secondary amines **4**–**9**.

4.3.1 N^1-Solanesyl-N^4-(N'-benzyl-2-aminoethyl)-piperazine (**4**)

IR (KBr) ν_{max}: 3315, 2924, 1668, 1453, 1382, 1153 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.32 (m, 5H), 5.27 (t, $J = 6.5$ Hz, 1H), 5.11 (m, 8H), 3.77 (s, 2H), 3.42 (br s, 1H), 2.99 (d, $J = 6.6$ Hz, 2H), 2.72 (t, $J = 5.2$ Hz, 2H), 2.52 (t, $J = 5.3$ Hz, 2H), 2.50 (br s, 8H), 2.06–1.98 (m, 32H), 1.68 (s, 3H), 1.60 (s, 27H); ESI-MS *m/z*: 832.9 $[M+H]^+$; Elemental analysis: Found: C, 78.91%; H, 11.48%; N, 4.88%; calcd for $C_{58}H_{93}N_3 \cdot 3H_2O$: C, 78.59%; H, 11.26%; N, 4.74%.

4.3.2 N^1-Solanesyl-N^4-(N'-benzyl-3-aminopropyl)-piperazine (**5**)

IR (KBr) ν_{max}: 3301, 2924, 1667, 1449, 1382, 1153 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.34 (m, 5H), 5.24 (t, $J = 6.5$ Hz, 1H), 5.11 (m, 8H), 3.83 (s, 2H), 3.45 (br s, 1H), 2.93 (d, $J = 6.9$ Hz, 2H), 2.75 (t, $J = 6.4$ Hz, 2H), 2.44 (t, $J = 6.9$ Hz, 2H), 2.49 (br s, 8H), 2.06–1.98 (m, 34H), 1.68 (s, 3H), 1.60 (s, 27H); ESI-MS *m/z*: 846.9 $[M+H]^+$; Elemental analysis: Found: C, 79.64%; H, 11.64%; N, 4.85%; calcd for $C_{59}H_{95}N_3 \cdot 2.5H_2O$: C, 79.49%; H, 11.31%; N, 4.71%.

4.3.3 N^1-Solanesyl-N^4-(N'-benzyl-4-aminobutyl)-piperazine (**6**)

IR (KBr) ν_{max}: 3324, 2926, 1665, 1451, 1381, 1153 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.32 (m, 5H), 5.26 (t, $J = 6.5$ Hz, 1H), 5.11 (m, 8H), 3.79 (s, 2H), 3.11 (br s, 1H), 2.96 (d, $J = 6.8$ Hz, 2H), 2.65 (t, 2H), 2.47 (br s, 8H), 2.35 (t, $J = 6.12$ Hz, 2H), 2.06–1.98 (m, 36H), 1.63 (s, 3H), 1.54 (s, 27H); ESI-MS *m/z*: 860.9 $[M+H]^+$; Elemental analysis: Found: C, 78.61%; H,

10.96%; N, 4.83%; calcd for $C_{60}H_{97}N_3 \cdot 3H_2O$: C, 78.80%; H, 11.35%; N, 4.59%.

*4.3.4 N^1-Solanesyl-N^4-(N'-1-naphthylmethyl-3-aminopropyl)-piperazine (**7**)*

IR (KBr) ν_{max}: 3336, 2925, 1664, 1449, 1380, 1153 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.01 (br s, 1H), 7.87 (d, $J = 7.9$ Hz, 1H), 7.78 (d, $J = 8.1$ Hz, 1H), 7.54–7.43 (m, 4H), 5.22 (t, $J = 6.5$ Hz, 1H), 5.11 (m, 8H), 4.27 (s, 2H), 3.88 (br s, 1H), 2.89 (br s, 2H), 2.87 (t, $J = 5.8$ Hz, 2H), 2.51 (br s, 8H), 2.43 (t, $J = 6.9$ Hz, 2H), 2.08–1.98 (m, 34H), 1.68 (s, 3H), 1.60 (s, 27H); ESI-MS *m/z*: 896.9 $[M+H]^+$; Elemental analysis: Found: C, 79.84%; H, 10.78%; N, 4.74%; calcd for $C_{63}H_{97}N_3 \cdot 2.8H_2O$: C, 79.91%; H, 10.32%; N, 4.44%.

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*4.3.5 N^1-Solanesyl-N^4-(N'-9-anthrylmethyl-4-aminobutyl)-piperazine (**8**)*

IR (KBr) ν_{max}: 3273, 2924, 1669, 1447, 1382, 1155 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.49 (s, 1H), 8.38 (d, $J = 8.8$ Hz, 2H), 8.05 (d, $J = 8.3$ Hz, 2H), 7.61–7.51 (m, 4H), 5.13 (br s, 9H), 4.90 (s, 2H), 3.11 (br s, 2H), 2.78 (t, 2H), 2.43 (br s, 8H), 2.40 (t, $J = 6.1$ Hz, 2H), 2.01–1.86 (m, 34H), 1.69 (s, 3H), 1.61 (s, 27H); ESI-MS *m/z*: 946.9 $[M+H]^+$; Elemental analysis: Found: C, 81.39%; H, 10.62%; N, 4.56%; calcd for $C_{67}H_{99}N_3 \cdot 2.2H_2O$: C, 81.60%; H, 10.57%; N, 4.26%.

*4.3.6 N^1-Solanesyl-N^4-(N'-9-anthrylmethyl-3-aminopropyl)-piperazine (**9**)*

IR (KBr) ν_{max}: 3307, 2926, 1669, 1446, 1382, 1155 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.40 (s, 1H), 8.34 (d, $J = 8.9$ Hz, 2H), 8.00 (d, $J = 8.2$ Hz, 2H), 7.55–7.44 (m, 4H), 5.25 (br t, 1H), 5.11 (m, 8H), 4.73 (s, 2H), 3.41 (br s, 1H), 2.95 (d, $J = 6.9$ Hz, 2H), 2.89 (t, $J = 6.5$ Hz, 2H), 2.45 (br s, 8H), 2.36 (t, $J = 7.5$ Hz, 2H), 2.06–1.98 (m, 36H), 1.68 (s, 3H), 1.60 (s, 27H); ESI-MS *m/z*: 960.8 $[M+H]^+$; Elemental analysis: Found: C, 80.42%; H, 10.52%; N, 4.49%; calcd for $C_{68}H_{101}N_3 \cdot 3H_2O$: C, 80.50%; H, 10.63%; N, 4.14%.

4.4 Cell culture

Tumor cell lines (L1210, murine leukemia; HeLa, human cervical carcinoma) used in this study were purchased from the Cell Bank of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. All cell lines were maintained in RPMI-1640 culture medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C, 5% CO_2 in a humidified atmosphere.

4.5 Cell proliferation assay

The toxicity of novel compounds was determined by the MTT assay. Cells (5×10^3 in 100 μl of the culture medium) were plated into a 96-well plate. After 16 h, the cells were treated with increasing concentrations of novel compounds. For the synergistic tests, the cells were treated with increasing concentrations of VCR in the presence of solanesyltriamines **3** or SDB. After the drug exposure was completed, 20 μl of aqueous MTT solution was added into each well for an additional 3 h. Formazan was dissolved in acidified DMSO, and the absorbency at $\lambda = 540$ nm was measured on a microplate reader.

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