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Article

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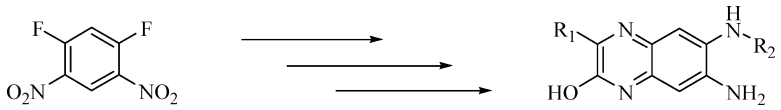

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Parallel Approach for Solution-Phase Synthesis of 2-Quinoxalinol Analogues and Their Inhibition of LPS-Induced TNF-α Release on Mouse Macrophages in Vitro

Liang Zhang, Gang Liu,* Suo-De Zhang, Hong-Zhen Yang, Li Li, Xiang-Hong Wu, Junli Yu, Bin-Bin Kou, Song Xu, Jing Li, Gang-Chun Sun, Ya-Fei Ji, and Gui-Fang Cheng

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A parallel solution-phase synthesis of 2-quinoxalinol analogues is described. The key step—simultaneous reductions of *m*-Ar$(NO_2)_2$ to *m*-Ar$(NH_2)_2$ was investigated extensively. We obtained preliminary pharmacological activity of those analogues for the inhibition of LPS-induced TNF-α release on mouse macrophage in vitro. Two compounds revealed inhibitory activity, with IC_{50} values of 0.40 μM (7-amino-6-[(3-methoxypropyl)amino]-3-methyl-2-quinoxalinol) and 2.2 μM (7-amino-6-[(3-butoxypropyl)amino]-3-methyl-2-quinoxalinol), respectively.

Introduction

Combinatorial generation of diverse chemical libraries has been well-practiced for drug lead discovery and optimization during the past decade. Development of methodologies for synthesis of pharmacological scaffold libraries has been one of the attractive goals. Particularly, time- and labor-efficient generation of diverse "lead-like" libraries is currently necessary to tremendously accelerate sample collection for high-throughput screening. *o*-Fluoronitrobenzene is a versatile reagent for the construction of benzofused heterocycles via a solid-phase synthetic approach (Figure 1).[1] 1,5-Difluoro-2,4-dinitrobenzene **1** is a symmetrical scaffold with chemical activities similar to *o*-fluoronitrobenzene, but more structural diversity can be introduced through asymmetrical nucleophilic substitutions. After simultaneous reduction of the two nitro groups, many kinds of heterocyclic scaffold compounds could be generated. Therefore, we have launched a scaffold-directed project using **1** for heterocyclic compound library synthesis. Compound **1** has been used for constructing a nitroaromatic library[2] and tricyclic imidazo[4,5-*g*]quinoxalin-6-one analogues.[3] Herein, we report our initial studies of quantitatively reductive conditions of *m*-Ar$(NO_2)_2$, parallel solution-phase synthesis of 2-quinoxalinol analogues **4**, and their novel biological activity represented by the inhibition of LPS-induced TNF-α release on mouse macrophages in vitro.

2-Quinoxalinol is a 2-hydroxylized quinoxaline whose analogues have shown such biological activities as glutamate blocker,[4] treatment of sensorineural smell disorders,[5] DNA topoisomerase (Topo) II beta-inhibitor,[6] antimycobacterial activity,[7] and selective inhibitor of I kappa B kinase.[8] To our knowledge, a 2-quinoxalinol library has not yet been reported.

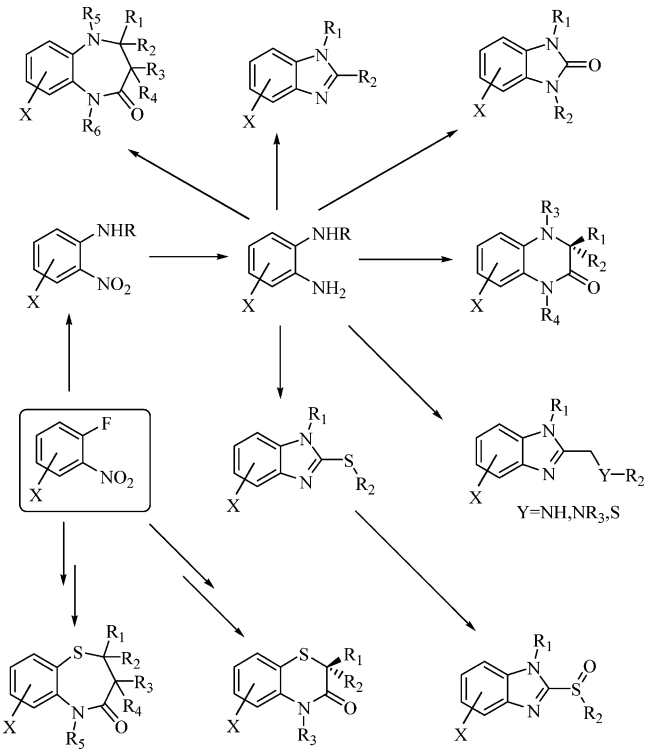

Figure 1. Diverse scaffolds have been generated by utilization of *o*-fluoronitrobenzene.

Results and Discussion

The synthetic route is outlined in Scheme 1. Starting from **1**, two fluoro atoms were quantitatively substituted by the nucleophilic amino acid alkyl ester and subsequent alkyl-

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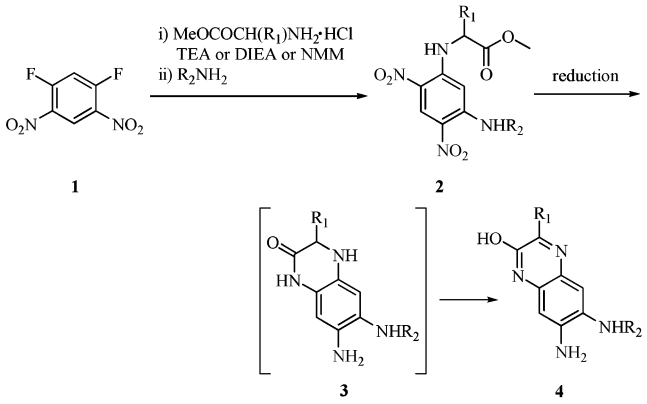
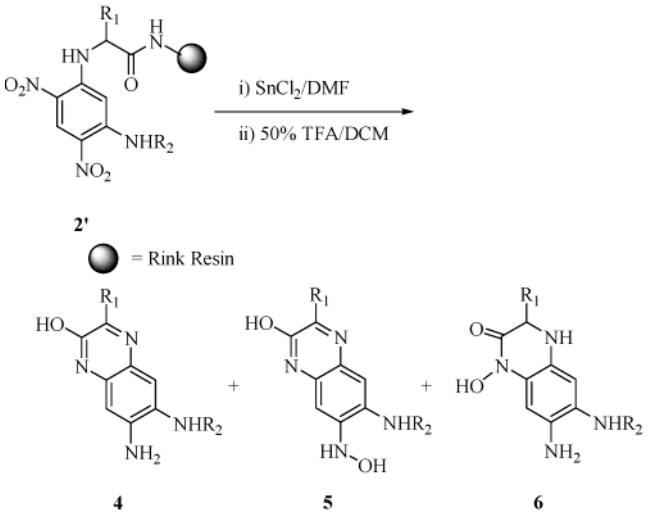
Scheme 1. Synthesis of 2-Quinoxalinol from 1,5-Difluoro-2,4-dinitrobenzene



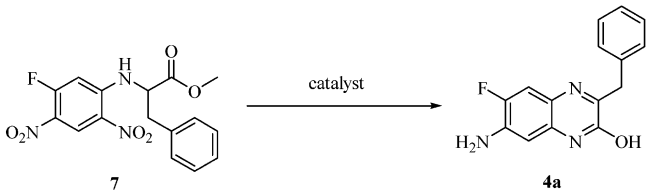
amine to give compound **2** in the presence of an organic base, such as triethylamine (TEA), diisopropylethylamine (DIPEA), or *N*-methylmorpholine (NMM), as in a previous report.[2] To compare with alkylamine (R_2 resource), the nucleophilicity of the amino acid alkyl ester (R_1 resource) is weaker than the former. Thus, the latter is used to carry out the primary substitution, and the alkylamine is used in a secondary substitution without any unanticipated byproduct.

Catalytic reduction of $Ar-NO_2$ into $Ar-NH_2$ has been studied very well and reviewed,[9] including common methods for Fe + AcOH, Zn + NaOH, Fe + HCl, Sn + HCl, H_2−Raney Ni, H_2-PtO_2, H_2−Pd/C, and N_2H_4−Pd/C. Particularly, ammonium formate in the presence of Pd/C is a convenient method. Simultaneously converting of *m*-$Ar(NO_2)_2$ into *m*-$Ar(NH_2)_2$ with >90% yield has also been reported in the presence of a catalyst, such as $Rh_6(CO)_{16}$ in $THF/NEt_3/H_2O$,[10] $Ti(SO_4)_2$ (2.4 wt %) and H_2SO_4(35%)/H_2O;[11] $NaBH_4$, $FeCl_2$,[12] H_2, *trans*-$PdPy_2Cl_2$ in EtOH;[13] excess Zn, $HCOONH_4$/MeOH,[14] H_2, Pd/C (cat.)/EtOH,[15] 5 atm H_2, Pd/C (cat.) in EtOH;[16] HCOOH−Pd/C,[17] $SnCl_2$ in HCl/EtOH[18] etc. Taking advantage of simple separation from side product(s) and excessive reactant(s), initially we expected all the substitutions and reduction could be performed on solid support, as reported by Mazurov.[3] Similar experiments of *o*-fluoronitrobenzene reduction were followed by utilization of an acid-liable resin, such as Rink resin[1] and PAL resin[3] with 2.0 M $SnCl_2$/DMF in the presence of NMM or DIPEA (Table 1, entry 5). However, the reduction of *m*-$Ar(NO_2)_2$ to *m*-$Ar(NH_2)_2$ on solid phase was unsuccessful in our experiments,

Scheme 2. Reduction Using $SnCl_2$ on Solid-Phase Generally Produced Two Hydroxyamino Byproducts



Scheme 3. Typical Reduction for Selecting Catalyst in Solution Phase



since the reaction was partially stopped at the hydroxyamino step, which gave us a mixture, including the anticipated compound **4**, and one of two hydroxyamino compounds **5** or **6**, on the basis of LC−MS analysis results (Scheme 2.). This side reaction was also found in solution-phase synthesis. Thus, selection and optimization of soluble reductants and catalysts were necessary (Table 1.). When $SnCl_2$ was dissolved in AcOH/HCl/DCM,[19] the reduction was completed in solution phase (Table 1, entry 6); however, such a strong acid condition definitely limits many acid-sensitive groups and common acid-liable resins. Instead of $SnCl_2$, $Na_2S_2O_4$,[20] Na_2S,[23] $KBH_4/SnCl_2$,[21] and $NH_2NH_2\cdot H_2O$[22] were also studied, as in Scheme 3. The reduction with Na_2S, $KBH_4/SnCl_2$, and $NH_2NH_2\cdot H_2O$ did not give satisfactory results (entries 2−4).

$Na_2S_2O_4$ demonstrated complete reduction in 50% H_2O/THF in the presence of tetrabutylammonium iodide (TBAI), with a long reaction time (generally requires 96 h, entry 1). Using this mild method, 10 designed compounds carrying

Table 1. Soluble Reductive Catalyst and Their Reactions

entry	reductant	catalyst	solvent (v/v)	time (h)	temp (°C)	conversion (by HPLC)[a]
1	$Na_2S_2O_4$[b]	TBAI	H_2O/THF (1:1)	96	reflux	complete
2	Na_2S[c]	TBAI	H_2O/EtOH (1:1)	4	reflux	no reaction
3	$KBH_4/SnCl_2$[d]		EtOH	40 min	r.t.	very low yield
4	$NH_2NH_2\cdot H_2O$[e]	Pd/C	$ClCH_2CH_2Cl$/EtOH (1:1)	8	~50 to 60	side reaction[f]
5	$SnCl_2$[g]		DMF	18	25	incomplete
6	$SnCl_2$[h]		HOAc/HCl/DCM (1:0.35:1)	45min	reflux	complete

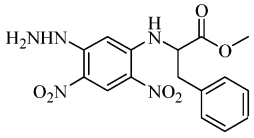
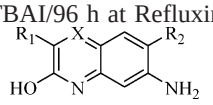
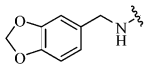
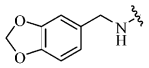
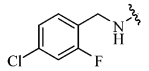
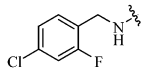
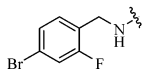
[a] Purity based on the integration area on HPLC (the detection at 254 nm). [b] See ref 20. [c] See ref 23. [d] See ref 21. [e] See ref 22. [f] Only a byproduct  was obtained. [g] See ref 3. [h] See ref 18.

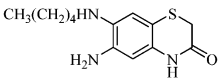
Table 2. Reduction of Aromatic *m*-Dinitro Compounds with $Na_2S_2O_4$/THF/H_2O/TBAI/96 h at Refluxing Temperature[a]



Entry	Product	R_1	R_2	X	HPLC Purity (%)[b]
1	4a	$PhCH_2$	F	N	82.10
2	4b	CH_2OH	F	N	88.05
3	4c	$CH_2CH_2SCH_3$	F	N	63.60
4	4d	$PhCH_2$		N	92.00
5	4e	CH_2OH		N	81.00
6	4f	$PhCH_2$		N	85.50
7	4g	CH_2OH		N	75.00
8	4h	CH_2OH		N	Trace
9	4i	$PhCH_2$	NH_2	N	85.00
10	4j	H	$CH_3(CH_2)_4NH$	S	Trace[c]

[a] TBAI: tetrabutyl ammonium Iodide

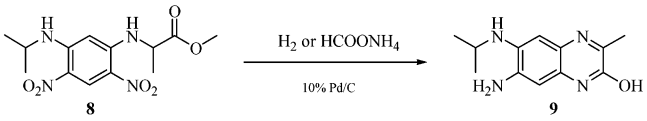
[b] Purity based on the integration area on HPLC (the detection at 254 nm)

[c] The structure of anticipated compound: 

strong acid-sensitive groups were prepared (Table 2). Two cases (entries 8 and 10) gave very low yields for some unknown reasons. It did not give the anticipated results on Rink resin; however, that might be the polar solvent making the resin shrink.

Because of the failure of efforts for solid-phase synthesis of anticipated compounds from **1**, a parallel solution-phase synthetic manner then was considered as an alternative approach. Because a Pd/C catalyst is commercially available and can be conveniently removed from the reaction system through parallel filtration, H_2 and $HCOONH_4$ were selected as the hydrogen resource. For an efficient reduction condition, a set of experiments is detailed in Table 3. Scheme 4 shows a representative example that is the most difficult to be done, based on our experience with the alanine-substituted case. H_2/Pd/C (entry 9) gave quantitative reduction and further cyclization to form 2-quinoxalinol. However, this method required a H_2 generation apparatus. $HCOONH_4$/Pd/C

Scheme 4. Example of Reduction by 10% Pd/C Catalyst



8 **9**

was systematically studied. Obviously, water is necessary for $HCOONH_4$ dissolution. When $CHCl_3$ replaced water, the reduction was not observed, even with the aid of sonication (entry 7). A polar solvent (EtOH, butanol, and DMF) was also important to successfully perform this reaction, whereas H_2O/$CHCl_3$ replacement resulted in no reaction, even in the presence of a phase-transfer catalyst (TBAB) (entry 4). Butanol and DMF were demonstrated to be candidate solvents (entries 2 and 3); however, they are difficult to remove. EtOH with water in a low volume ratio presented a good solvent system (entry 1), although it led to the uncompleted reduction as increasing the volume ratio of H_2O/EtOH to 1:50 (entry 5). Sonication greatly improved this reaction, taking advantage of a short reaction time and lower reaction temperature (entries 6 and 8).

In summary of all the investigated conditions, $HCOONH_4$/Pd/C was finally chosen for solution-phase synthesis of a 2-quinoxalinol library because of several key points: (1) quantitative reduction of *m*-$Ar(NO_2)_2$ to *m*-$Ar(NH_2)_2$ over a range of temperatures, 55−65 °C, or at room temperature with the aid of sonication; (2) no additional apparatus is required to generate H_2; (3) the solid catalyst (Pd/C) is easily removed by filtration; (4) saving a step of removal of excessive $HCOONH_4$ that was decomposed into volatile H_2, NH_3, and CO_2. The limitation of this method is that HCOOH-sensitive building blocks are not used. Moreover, partial debenzylation generally occurs when benzylamine is used as a building block. Sixty-four 2-quinoxalinol (2-hydroxyquinoxaline) analogues, listed in Table 4, were synthesized and characterized by an auto 300-MHz NMR and an auto fast LC−MS/MS.

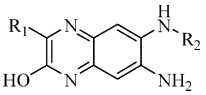
Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine and is relative to inflammatory diseases, such as rheumatoid arthritis, psoriatic arthritis, and inflammatory bowel disease.[24] One of TNF-α's release pathways in vivo is to directly stimulate blood monocytes and tissue macrophages by bacterial toxins such as lipopolysaccharide (LPS).[25] Inhibition of macrophages' massively releasing

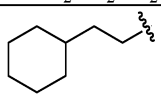
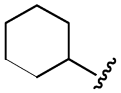
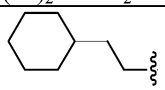
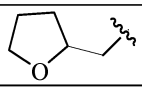
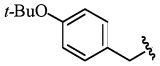
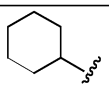
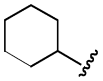
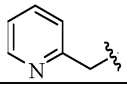
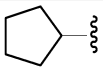
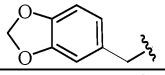
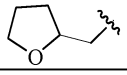
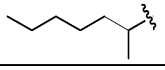
Table 3. Optimized Conditions of Simultaneously Reducing Two Nitro Groups by H_2/Pd/C at 3 Bar and $HCOONH_4$/Pd/C

entry	substrate/Pd/C (10%) (mmol/g)	solvent (v/v)	time (h)	temp (°C)	conversion (by HPLC)[d]
1	1.0/0.08	H_2O/EtOH (1:4)	3	75	complete
2	1.0/0.16	H_2O/DMF (1:50)	3	55	complete
3	1.0/0.12	H_2O/butanol (1:50)	1	55	complete
4	1.0/0.12	H_2O/$CHCl_3$ (1:1) (TBAB)[a]	3	55	no reaction
5	1.0/0.12	H_2O/EtOH (1:50)	6	65	incomplete
6	1.0/0.12	H_2O/EtOH (1:100)[b]	10 min	r.t.	complete
7	1.0/0.12	$CHCl_3$/EtOH (1:1)[b]	4	70	no reaction
8	1.0/0.12	H_2O/DMF (1:10)[b]	20 min	r.t.	complete
9[c]	1.0/0.10	THF/EtOH/(2%)H_2SO_4 (1:4:0.3)	12	∼50 to 60	complete

[a] TBAB: tetrabutylammonium bromide. [b] The reaction is aided by ultrasound. [c] H_2/Pd/C at 3 bar. [d] Purity based on the integration area on HPLC (the detection at 254 nm).

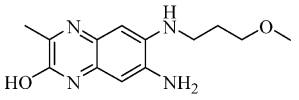
Table 4. Novel Synthetic Compounds



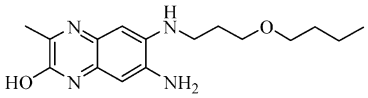
No.	R_1	R_2	No.	R_1	R_2
12	CH_3	3,4-(MeO)$PhCH_2CH_2$	43	$CH_3SCH_2CH_2$	$CH_3OCH_2CH_2CH_2$
13	CH_3	$PhCH_2CH_2CH_2$	44	$CH_3SCH_2CH_2$	*n*-C_7H_{15}
14	CH_3	$CH_3(CH_2)_{11}$	45	$CH_3SCH_2CH_2$	$PhCH_2CH_2CH_2$
15	CH_3	$CH_3(CH_2)_4$	46	$CH_3SCH_2CH_2$	$(Ph)_2CHCH_2$
16	CH_3	$CH_3(CH_2)_8$	47	$CH_3SCH_2CH_2$	$(Ph)_2CHCH_2CH_2$
17	CH_3	$CH_3CH_2CH_2$	48	$CH_3SCH_2CH_2$	$CH_3(CH_2)_5$
18	CH_3	$PhCH_2CH_2$	49	$CH_3SCH_2CH_2$	$CH_3CH_2CH_2$
19	CH_3OCOCH_2	*n*-$BuOCH_2CH_2CH_2$	50	$PhCH_2$	3,4-(MeO)$PhCH_2CH_2$
20	CH_3OCOCH_2	$PhCH_2CH_2CH_2$	51	$PhCH_2$	$PhCH_2$
21	CH_3OCOCH_2		52	$PhCH_2$	$CH_3OCH_2CH_2CH_2$
22	CH_3OCOCH_2	$PhCH_2CH_2$	53	$PhCH_2$	
23	H	*n*-$BuOCH_2CH_2CH_2$	54	$PhCH_2$	*n*-C_6H_{13}
24	H	$CH_3(CH_2)_4$	55	$PhCH_2$	*n*-C_9H_{19}
25	H	$CH_3CH_2CH_2$	56	$PhCH_2$	$CH_3CH_2CH_2$
26	H	$(CH_3)_2CHCH_2CH_2$	57	$PhCH_2$	*o*-CH_3OPhCH_2
27	H	$(Ph)_2CHCH_2$	58	$PhCH_2$	*p*-CH_3PhCH_2
28	H	$CH_3OCH_2CH_2CH_2$	59	$PhCH_2$	$PhCH_2CH_2$
29	H	$EtOCH_2CH_2CH_2$	60	CH_2OH	3,4-(MeO)$PhCH_2CH_2$
30	H	3,4-(MeO)$PhCH_2CH_2$	61	CH_2OH	$CH_3OCH_2CH_2CH_2$
31	H	$(Ph)_2CHCH_2CH_2$	62	CH_2OH	*n*-$BuOCH_2CH_2CH_2$
32	H		63	CH_2OH	*n*-$C_{12}H_{25}$
33	H		64	CH_2OH	*n*-C_5H_{11}
34	$(CH_3)_2CHCH_2$	3,4-(MeO)$PhCH_2CH_2$	65	CH_2OH	$PhCH_2CH_2$
35	$(CH_3)_2CHCH_2$	*o*-CF_3PhCH_2	66		
36	$(CH_3)_2CHCH_2$	$PhCH_2$	67	$(CH_3)_2CH$	
37	$(CH_3)_2CHCH_2$	$CH_3(CH_2)_{11}$	68	$(CH_3)_2CH$	
38	$(CH_3)_2CHCH_2$	$(CH_3)_2CH$	69	$(CH_3)_2CH$	$PhCH_2CH_2CH_2$
39	$(CH_3)_2CHCH_2$	$CH_3(CH_2)_8$	70	$(CH_3)_2CH$	
40	$(CH_3)_2CHCH_2$	$PhCH_2CH_2$	71	$(CH_3)_2CH$	n-C_7H_{15}
41	$(CH_3)_2CHCH_2$		72	$(CH_3)_2CH$	
42	$(CH_3)_2CHCH_2$		73	$(CH_3)_2CH$	3,4-(MeO)$PhCH_2CH_2$

TNF-α in the presence of LPS in vitro, therefore, has been well-defined as a screening method for the discovery of antiinflammatory drugs.[26] Herein, we have evaluated only synthetic novel compounds, and we have found that two of them show inhibitory activities of TNF-α release in vitro with interesting structure−activity relationships (SAR). The IC_{50} values are 3.976×10^{-7} M (**10**) and 2.226×10^{-6} M (**11**), respectively. R_1 is defined as a methyl group and R_2

required an alkyl−oxygen−ether chain. Their further detailed SAR is being studied.



10



11

Conclusion

We have developed a practical and efficient solution-phase method for the synthesis of 2-hydroxyquinoxaline analogues. Preliminary pharmacological evaluation of the synthesized library in vitro shows that two (**10** and **11**) of them revealed an inhibitory activity against TNF-α release, with IC_{50} values of 3.976×10^{-7} M and 2.226×10^{-6} M, respectively.

Experimental Section

All amino acid methyl esters were purchased from Chem-Impex International, Inc. (Wood Dale, IL). All alkylamines were purchased from Acros Organics (Geel, Belgium). All organic solvents were redistilled after a standard drying procedure. HPLC analysis was performed on a Shimadzu apparatus equipped with a SPD-10A VP detector, LC-10AT VP pump, and DGU-12A degasser and eluting with a gradient system of 5/95 to 95/5 acetonitrile/H_2O with a buffer consisting of 0.05% TFA over 5 min at 1 mL/min and detected by UV at 254 nm. Auto LC−MS/MS analysis was performed on a Thermo Finnigan, LCQ Advantage mass spectrometer equipped with a Gilson 322 pump, Gilson UV/vis-152 detector, Gilson 215 liquid handler, and a fluent splitter(LC gradient, flow rate, detected wavelength is as same as above. 5% eluent was split into MS system). The column employed was a Kromasil C18 column (4.6 μm, 4.6 × 50 mm) from DIKMA. Mass spectra were recorded in positive ion mode using electrospray ionization. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Varian Mercury 300 spectrometer at 300 MHz. Chemical shifts are reported as δ values (ppm). The parallel synthesis was carried out on an H+P Labortechnik GmbH parallel synthesizer equipped with reflux cooler 96.16 and DLSB-20/40 low temperature (max, −40 °C) liquid circulator by Zheng-Zou Great Wall Ke-Mao Corp.

General Procedure for the Synthesis of 7-Amino-6-alkylamino-2-quinoxalinol Derivatives by H_2 Reduction via Pd/C Catalyst. To a stirred solution of **1** (204 mg, 1.0 mmol) in THF (8 mL) was added DIPEA (4.0 mmol) and $NH_2CH(R_1)COOMe$·HCl (1.0 mmol). The mixture was vigorously stirred at room temperature for 3 h and then, with added alkylamine (1.0 mmol), for an additional 15 h. The solvent was removed under reduced pressure to give compound **2** (compound **2** was used directly or extracted with $CHCl_3$, washed with 2% HCl). The above two nucleophilic replacements were traced by a fast LC−MS system until all of **1** was changed to the anticipated compound. Compound **2** was then dissolved in a mixed solvent of THF (5 mL), EtOH (20 mL) and 2% H_2SO_4 (1.5 mL), followed by the addition of 175 mg of 10% Pd/C. This was subjected to hydrogenation at ∼2.8 to 3.0 bar at 40 °C for 12 h. The catalyst was filtered off and washed with THF. The combined filtrate was concentrated in vacuo, and water (50 mL) was added to the residue. This was extracted with ethyl acetate (2 × 50 mL) and then washed with saturated $NaHCO_3$ (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed in vacuo to give product **4**. The collected compounds were characterized by the fast LC−MS/MS system and ^{1}H NMR.

General Procedure for the Synthesis of 7-Amino-6-alkylamino-2-quinoxalinol Derivatives by $HCOONH_4$ Reduction via the Pd/C Catalyst. Compound **2** (1.0 mmol) was dissolved in ethanol (40 mL) and water (10 mL), DMF (10 mL) and water (0.2 mL), or butanol (10 mL) and water (0.2 mL), followed by the addition of $HCOONH_4$ (1.26 g) and 10% Pd/C (0.12 g). The mixture was heated at 55−65 °C for 3 h with vigorously stirring. The catalyst was filtered off and the filtrate was concentrated in vacuo. Water (50 mL) was added to the residue, and this was extracted by ethyl acetate (3 × 20 mL). The organic layer was washed with saturated NaCl (3 × 20 mL) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to give compound **4**.

Typical Procedure for the Synthesis of 7-Amino-6-alkylamino-2-quinoxalinol Derivatives by $Na_2S_2O_4$. To a stirred solution of compound **2** (0.14 mmol) and a catalytic amount of tetrabutylammonium iodide (TBAI) in THF (6 mL)/H_2O (6 mL) was added sodium dithionite (0.06 g, 0.34 mmol) over 10 h. The mixture was then refluxed for 96 h. The solvent turned to red from yellow and eventually to fluorescent yellow. The organic layer was separated, and the water layer was extracted with EtOAc (3 × 4 mL). The combined organic layers were washed with saturated NaCl (3 × 5 mL) and dried over anhydrous $MgSO_4$, and the solvent was removed in vacuo to give product **4**.

The Assay of Inhibition of LPS-Induced TNF-α Release on Mouse Macrophage in Vitro. Peritoneal cells were harvested from male mice (C57BL6/J) in vivo ∼3 to 4 days after injection of brewer thioglycollate medium (5 mL/100 g body weight). Peritoneal cells then were collected, mixed, and seeded into 24-well plates (Costar) at a cell density of 1×10^6 cells/mL in a total volume of 0.5 mL/well. RPMI-1640 medium was supplemented with 5% (v/v) newborn calf serum, 100 units/mL penicillin, and 100 mg/mL streptomycin. After settlement for ∼2 to 3 h, nonadherent cells were washed away by D-Hanks balanced salt solution. Almost all of the adherent cells were macrophages, as assessed by a giemsa staining assay. The macrophages were continuously cultured in 0.5 mL/well of complete RPMI-1640 medium in the presence of 2 μg/mL LPS and a series of diluted compounds for 24 h. Total TNF-α in the supernatant was measured by using a murine TNF-α ELISA Kit (Diaclone Research) according to the protocol provided by the manu-

facturer. Cell viability was examined by trypan blue exclusion. All incubation procedures were carried out with 5% CO_2 in humidified air at 37 °C.

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Supporting Information Available. Full characterization of all synthetic compounds is available as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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