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A versatile route to the synthesis of 1-substituted β-carbolines by a single step Pictet–Spengler cyclization

Mei-Lin Yang,[a] Ping-Chung Kuo,[b] Amooru G. Damu,[a] Ren-Jie Chang,[a] Wen-Fei Chiou[c] and Tian-Shung Wu[a,c,*]

[a]*Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan, ROC*
[b]*Department of Biotechnology, National Formosa University, Yunlin 632, Taiwan, ROC*
[c]*National Research Institute of Chinese Medicine, Taipei 112, Taiwan, ROC*

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Abstract—A one-step conversion of L-tryptophan and activated aldehydes (1,2-dicarbonyl compounds) directly to 1-substituted β-carbolines without formation of the tetrahydro derivatives under modified Pictet–Spengler conditions was described. Moreover, a practical application for the synthesis of a natural 1-substituted β-carboline, luzongerine A, isolated from *Illigera luzonensis* was also successfully carried out utilizing this protocol. The effects of synthetic compounds **11** and **11a** on nitric oxide (NO) production in LPS/IFN-γ stimulated RAW 264.7 macrophage cells were evaluated in vitro. They displayed significant dose-dependent inhibition of inducible nitric oxide synthase (iNOS).

1. Introduction

Pyrido[3,4-*b*]indoles, commonly known as β-carbolines represent a deeply investigated family of indole alkaloids that possess a wide diversity of important biological activities, particularly concerning the central nervous system.[1] Due to their unique rigid heterocyclic skeleton, many β-carbolines are known to bind with high affinity to benzodiazepine (BzR), serotonin, and dopamine receptors sites and to inhibit monoamine oxidase A.[2] It has also been reported that the medicinal activities of β-carbolines were improved by the introduction of appropriate susbstitutions into position 1.[3] Also, 1-substituted β-carbolines widely exist in nature, and there have been many methodologies concerning their syntheses.[4] For almost one century, the Pictet–Spengler reaction has remained as one of the most powerful methods for the formation of this ring system via C–C bond formation using tryptophan as the starting material.[2] In general, this reaction can be characterized by the formation of an iminium salt after an acid-catalyzed condensation of tryptophan and tryptamine derivatives with an aldehyde and then *endo* cyclization is effected between a carbon nucleophile of a sufficiently reactive aromatic moiety and the activated iminium ion resulting in an *N*-heterocyclic ring via a new C–C bond.[5] Over the years, several groups have studied the detail mechanistic aspects of this reaction and it is interesting to note that the method still continues to be a significant focus of research as chemists continue to improve upon the methodology by applying new reaction conditions.[6] In order to investigate the structure–activity relationship of a series of iNOS inhibitors, we required a robust and general synthetic methodology for the preparation of 1-alkyl or aryl substituted β-carboline nucleus. In this context, Behforouz and co-workers described a useful approach for the preparation of 1-acetyl β-carboline derivatives via acid mediated coupling of methyl ester of DL-tryptophan and pyruvaldehyde.[7] The ease of this one-pot oxidation reaction prompted us to investigate the scope and synthesis of variety of 1-substituted β-carboline derivatives. As a result, we recognized the conversion of L-tryptophan and phenylglyoxal directly to 1-substituted β-carbolines in the presence of acid via a single step Pictet–Spengler reaction. This strategy improved the scope of the Pictet–Spengler cyclization and allows product diversification at C-1 by the use of inexpensive and commercially available L-tryptophan and activated aldehydes like pyruvaldehyde and phenylglyoxal. Herein we wish to describe this single step synthetic methodology, which would be an alternative for the preparation of 1-substituted β-carbolines, and successful application of this reaction to the synthesis of naturally occurring 1-substituted β-carbolines.

2. Results and discussions

By virtue of the readily synthetic availability, L-tryptophan was chosen as the model substrate. Initial attention was

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* Corresponding author. Tel.: +886 6 2747538; fax: +886 6 2740552; e-mail: tswu@mail.ncku.edu.tw

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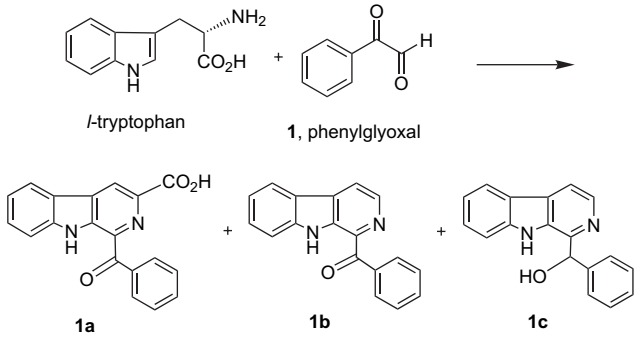
focused on the use of phenylglyoxal as an electrophile employing its cyclocondensation with L-tryptophan in H_2SO_4 as model reaction leading to **1a**, **1b**, and **1c** as the major products (Scheme 1). The transformation was next explored using H_2SO_4, *p*-TsOH, and HCl as acids, different solvent systems (MeOH, acetone, MeCN, THF, 1,4-dioxane, DMSO, and DMF), reaction temperatures, reaction time, and equivalents of L-tryptophan. A representative collection of results is summarized in Table 1. Among these conditions, addition of *p*-TsOH to the mixture of L-tryptophan and phenylglyoxal in MeOH at 50 °C for 2 h (entry 7) provided the best results, and it is common in acid-catalytic reactions that mild conditions were preferred. Temperature was found to have a profound effect on the reaction course, a decrease in the reaction temperature lengthened the reaction time (entries 5 and 6). In process of optimization of yields, it was found that 1.3 equiv of L-tryptophan was necessary to ensure the complete conversion of phenylglyoxal to **1a–c** in satisfactory yields. Overall yields of **1b** and **1c** are 80%, and under oxidation condition, **1c** could be easily converted into **1b**. A decrease in the polarity of solvent, by using acetone, MeCN, THF, or 1,4-dioxane (entries 11–14), resulted in the quite lower conversion of L-tryptophan probably due to its poor solubility and thus low yield of products. No product was obtained using polar aprotic solvents, such as DMF and DMSO in Pictet–Spengler condensation under optimized reaction conditions. In every case, the crude product obtained after work up was easily purified by silica gel chromatography using the mixture of *n*-hexane and ethyl acetate as an eluent and characterized by mass and NMR spectroscopic methods.

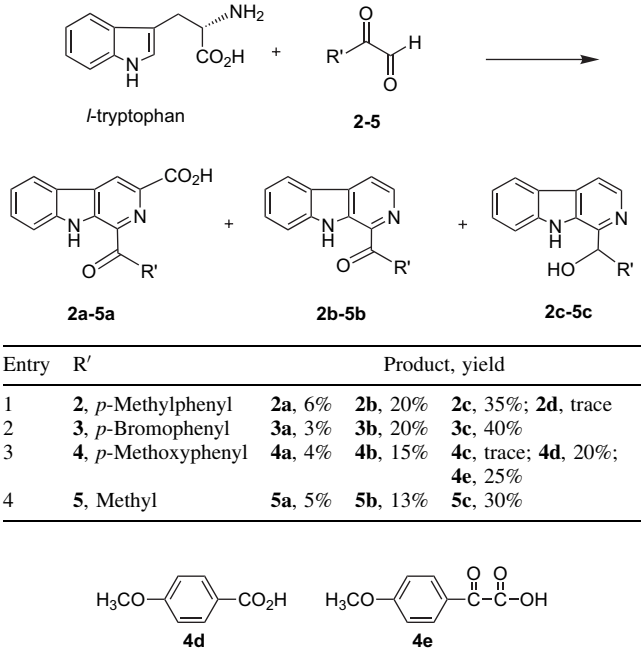


Scheme 1. Condensation between L-tryptophan and phenylglyoxal.

Having established a useful set of reaction conditions, these optimized conditions were subsequently applied to reactions of L-tryptophan with aliphatic and otherwise functionalized aromatic aldehydes, generally furnishing of products as shown in Table 2. However, no general conclusions on the electronic effects could be deduced. Both the electron-releasing (**2** and **5**) and electron-withdrawing (**3**) groups gave similar yields of the two major products. In the first entry of Table 2, a minor product **2d** was also confirmed with the yield of less than 1%. With these selected electrophiles, both HCl and *p*-TsOH catalyses afforded similar yields. Surprisingly, only a trace amount of **4c** was obtained in the case of **4** (electron-releasing group). It may be due to the formation of oxidized products **4d** and **4e**, which interrupt the successive cyclization and aromatization.

In general, the classical Pictet–Spengler reaction is a two-step method and involves acid-catalyzed condensation of an aliphatic amine attached to a sufficiently reactive aromatic

Table 2. Preparation of 1-substituted β-carboline derivatives by direct condensation of L-tryptophan with selected activated aldehydes



Entry	R′	Product, yield		
1	**2**, *p*-Methylphenyl	**2a**, 6%	**2b**, 20%	**2c**, 35%; **2d**, trace
2	**3**, *p*-Bromophenyl	**3a**, 3%	**3b**, 20%	**3c**, 40%
3	**4**, *p*-Methoxyphenyl	**4a**, 4%	**4b**, 15%	**4c**, trace; **4d**, 20%; **4e**, 25%
4	**5**, Methyl	**5a**, 5%	**5b**, 13%	**5c**, 30%

Table 1. Optimization of reaction involving preparation of 1-substituted β-carbolines using L-tryptophan and phenylglyoxal under different Pictet–Spengler protocols

Entry	Acid (equiv)	L-Tryptophan (equiv)	Solvent	Conditions (*T* (°C), time (h))	Yield (%)		
					1a	**1b**	**1c**
1	H_2SO_4 (1)	1.1[a]	MeOH	rt, 24	3	2	4
2	H_2SO_4 (1)	1.1[a]	MeOH	50 °C, 2	3	3	5
3	HCl (1)	1.1[a]	MeOH	50 °C, 2	5	7	10
4	*p*-TsOH (1)	1.1[a]	MeOH	50 °C, 2	5	6	9
5	HCl (1)	1.3	MeOH	50 °C, 2	4	32	42
6	HCl (1)	1.3	MeOH	rt, 48	5	24	35
7	*p*-TsOH (1)	1.3	MeOH	50 °C, 2	4	35	45
8	H_2SO_4 (1)	1.3	MeOH	50 °C, 2	12	13	20
9	HCl (1)	1.3	MeOH	50 °C, 2	6	28	35
10	*p*-TsOH (1)	1.3	MeOH	50 °C, 2	5	20	25
11	HCl (1)	1.3	Acetone	50 °C, 2	5	6	11
12	HCl (1)	1.3	MeCN	50 °C, 2	14	3	15
13	HCl (1)	1.3	THF	50 °C, 2	2	3	6
14	HCl (1)	1.3	1,4-Dioxane	50 °C, 2	12	2	17

[a] The phenylglyoxal was monitored by HPLC and not completely consumed.

nucleus with aldehydes.[5] In the first step an imine is formed, which may be activated by acids and in the second step *endo* cyclization is affected between a carbon nucleophile of a sufficiently reactive aromatic moiety and the activated iminium ion resulting in an *N*-heterocyclic ring via a new C–C bond and forming tetrahydro-β-carboline (THBC), which on dehydrogenation leads to β-carboline.[8] However, in our experiments, treatment of L-tryptophan with *p*-tolylglyoxal under acidic conditions did not produce the tetrahydro-β-carbolines but rather afforded directly a dehydrogenated β-carboline product **2c** and its oxidized product **2b** as major along with minor amounts of **2a** and **2d** in a single step.[9] These observations can be rationalized by the mechanism proposed in Figure 1. In the presence of acid, the aldehyde is activated to allow nucleophilic attack of tryptophan forming tetrahydro-β-carboline-3-carboxylic acid intermediate **6**. Successive dehydrogenation and oxidative decarboxylation as described in the literature[10] leads to the β-carboline **2b** as major product accompanied by a minor product **2a**. However, if the intermediate **7** tautomerizes in the acidic condition, **2c** could be afforded through an enol intermediate. The other minor product **2d** could be rationalized through decarboxylation process occurred at the expense of an oxazolidine-5-one intermediate **9** formed by intramolecular cyclization of the Schiff's base **8**,[11] followed by an intermediate **10** prone to cyclize to form hydration product **2d**.

Encouraged by our success on 1-substituted β-carbolines synthesis, we have applied the developed methodology for the synthesis of luzongerine A (**11**),[12] a minor 1-substituted β-carboline, isolated from *Illigera luzonensis*. Compound **11** was prepared under the optimized reaction conditions using **4** and 5-methoxy-tryptophan as the starting materials (Scheme 2). As a result, compound **11** was afforded in 40% yield along with a minor product **11a** in 5% yield. Physical and spectral data of synthetic sample coincided well with those of the isolated one. Thus, the described method is applicable to the synthesis of the natural 1-substituted β-carbolines.

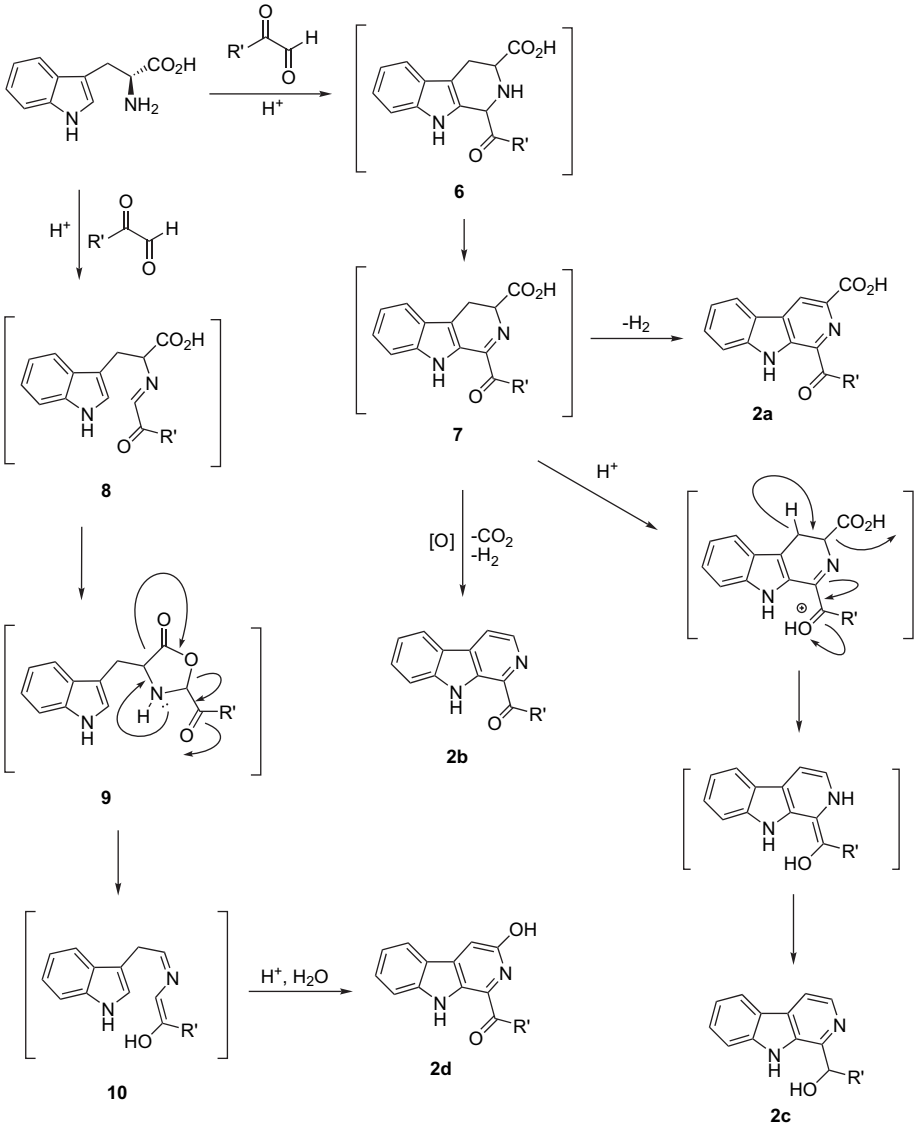
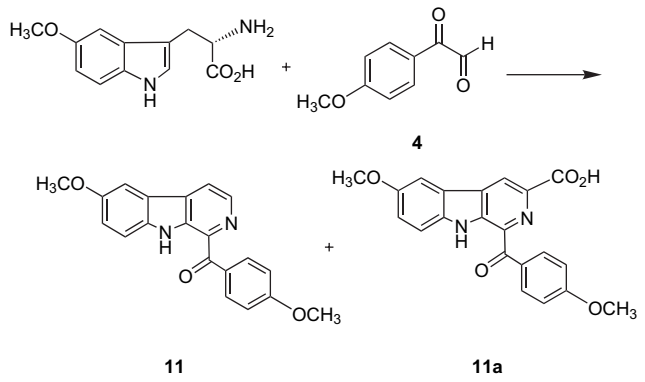


Figure 1. Proposed mechanism for **2a–d** formation.



Scheme 2. Synthesis of **11** and **11a** by the reported modified Pictet–Spengler method.

Table 3. Effects of tested compounds on LPS/IFN-γ-induced nitrite production of RAW 264.7 macrophage cells

Compound	IC_{50} (μM)	Potency
11	12.67±2.39	3.2×
11a	18.39±6.09	2.2×
Aminoguanidine (iNOS inhibitor)	40.96±5.04	1.0×

Nitric oxide (NO) is a molecular messenger that is synthesized by nitric oxide synthase (NOS) enzymes. NO is implicated in a variety of physiological and pathological processes.[13] Excessive NO generated by inducible nitric oxide synthase (iNOS) is known to be an important mediator of acute and chronic inflammations.[14] Naturally occurring 1-substituted β-carboline alkaloids were shown to inhibit the expression of iNOS in various cell systems.[15] Thus, the inhibition effects of the synthetic analogues on the generation of NO were examined in LPS/IFN-γ stimulated RAW 264.7 macrophages according to the method reported in the literature.[16] Tested compounds **11** and **11a** (1, 3, 10, and 30 μM) alone did not affect basal nitrite production, however, significantly and dose-dependently suppressed LPS/IFN-γ stimulated nitrite accumulation with IC_{50} values of 12.67±2.39 and 18.39±6.09 μM, respectively, as shown in Table 3. The cytotoxic effects of the synthetic compounds were measured using the MTT assay; no detectable cytotoxicity was observed at all the concentrations tested (1, 3, 10, and 30 μM) and the viability effects of treated cells were all greater than 95%.

3. Conclusions

In conclusion, a new application of Pictet–Spengler reaction for the synthesis of 1-substituted β-carboline derivatives has been developed. A variety of aryl and alkyl substituted activated aldehydes undergoes this process and allows product diversification at C-1 in overall good yields. This strategy improved the scope of Pictet–Spengler cyclization, giving access directly to a new family of β-carbolines in a single step without the need of aromatization step. Compounds **11** and **11a** displayed significant iNOS inhibition activity. Thus, the method met our criteria for simplicity and generality and has provided us with a vehicle for the preparation of a diverse set of 1-substituted β-carboline derivatives for a SAR evaluation. Currently work is in progress in our lab with several second generation substrates designed on the basis of our new concept for the Pictet–Spengler reaction and will be reported soon.

4. Experimental

4.1. General

Melting points were measured on a Yanaco MP-S3 micro melting point apparatus and are uncorrected. IR spectra were obtained with a Shimadzu FT-IR DR-8011 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on Bruker Avance-300 NMR spectrometers, with tetramethylsilane (TMS) as internal standard. EI and HREIMS spectra were recorded on a VG 70-250 S spectrometer. FAB and HRFABMS were measured on a Jeol JMS-700 mass spectrometer. Elementary Analyses were performed on an Elementar vario EL III analyzer.

4.2. Typical preparation procedure of 1a–5c

To a stirred suspension of 0.174 g (0.854 mmol, 1.3 equiv) of L-tryptophan in 1.0 equiv of *p*-toluenesulfonic acid monohydrate, 0.09 g (0.657 mmol, 1.0 equiv) of phenylglyoxal monohydrate was added. The resulting solution was stirred at 50 °C for 2 h, and the phenylglyoxal was monitored by HPLC to be completely consumed. The reaction mixture was poured into water and the precipitate was filtered and purified by silica gel column chromatography eluted with a gradient of *n*-hexane and ethyl acetate to afford **1a**, **1b**, and **1c**. Compounds **2a**–**5c** were prepared with the similar procedures.

4.2.1. 1-Benzoyl-9*H*-β-carboline-3-carboxylic acid (1a). Yellow powder (EtOAc–MeOH), mp 281–283 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.97 (1H, br s, D_2O exchangeable, CO_2H), 12.40 (1H, br s, D_2O exchangeable, NH), 9.17 (1H, s, H-4), 8.48 (1H, d, J=7.8 Hz, H-5), 8.40 (2H, d, J=7.4 Hz, H-2′ and -6′), 7.85 (1H, d, J=8.2 Hz, H-8), 7.72–7.57 (4H, m, H-7, -3′, -4′, and -5′), 7.37 (1H, t, J=7.5 Hz, H-6); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 192.8, 166.6, 142.3, 137.1, 136.9, 136.4, 135.9, 133.0, 131.7, 131.4, 129.6, 128.3, 122.4, 121.2, 120.7, 120.7, 113.5; EIMS *m*/*z* 316 $[M]^+$ (43), 271 (24), 242 (23), 105 (38), 77 (100); HREIMS *m*/*z* 316.0850 $[M]^+$ (calcd for $C_{19}H_{12}N_2O_3$, 316.0848). Anal. Calcd for $C_{19}H_{12}N_2O_3$: C, 72.15; H, 3.82; N, 8.86. Found: C, 72.16; H, 3.80; N, 8.90.

4.2.2. (9*H*-β-Carbolin-1-yl)-phenyl-methanone (1b). Yellow needle (EtOAc), mp 135–138 °C; IR (KBr) ν_{max} 3432, 1642, 1621 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.06 (1H, br s, D_2O exchangeable, NH), 8.53 (1H, d, J=4.7 Hz, H-3), 8.46 (1H, d, J=4.7 Hz, H-4), 8.33 (1H, d, J=7.8 Hz, H-5), 8.18 (2H, d, J=7.5 Hz, H-2′ and -6′), 7.81 (1H, d, J=8.3 Hz, H-8), 7.69–7.55 (4H, m, H-7, -3′, -4′, and -5′), 7.32 (1H, t, J=7.8 Hz, H-6); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 194.1, 141.9, 137.7, 137.3, 136.5, 136.1, 132.4, 131.2, 131.0, 129.4, 128.1, 121.9, 120.4, 120.3, 119.0, 113.2; EIMS *m*/*z* 272 $[M]^+$ (100), 244 (91), 167 (23), 149 (50); HREIMS *m*/*z* 272.0953 $[M]^+$ (calcd for $C_{18}H_{12}N_2O$, 272.0950). Anal. Calcd for $C_{18}H_{12}N_2O$: C, 79.39; H, 4.44; N, 10.29. Found: C, 79.54; H, 4.40; N, 10.33.

4.2.3. (9*H*-β-Carbolin-1-yl)-phenyl-methanol (1c). Yellow powder (EtOAc), mp 141–144 °C; IR (KBr) ν_{max} 3352, 1627 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.26 (1H, br s, D_2O exchangeable, NH), 8.23 (1H, d, J=5.0 Hz, H-3), 8.17 (1H, d, J=7.8 Hz, H-5), 7.98 (1H, d, J=5.0 Hz, H-4), 7.72 (1H, d, J=8.2 Hz, H-8), 7.60 (2H, d, J=7.4 Hz, H-2′ and -6′), 7.50 (1H, t, J=7.5 Hz, H-7), 7.29–7.17 (3H, m, H-3′, -5′, and -6), 6.51 (1H, d, J=3.8 Hz, OH), 6.14 (1H, d, J=3.8 Hz, C*H*OH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 147.7, 144.0, 140.9, 137.1, 132.4, 128.9, 128.2, 127.2, 126.5, 121.6, 120.6, 119.4, 113.8, 112.8, 76.1. EIMS m/z 274 $[M]^+$ (50), 255 (100); HREIMS m/z 274.1109 $[M]^+$ (calcd for $C_{18}H_{14}N_2O$, 274.1106). Anal. Calcd for $C_{18}H_{14}N_2O$: C, 78.81; H, 5.14; N, 10.21. Found: C, 78.62; H, 5.24; N, 10.07.

4.2.4. 1-(4-Methyl-benzoyl)-9*H*-β-carboline-3-carboxylic acid (2a). Yellow powder (EtOAc–MeOH), mp 259–260 °C; IR (KBr) ν_{max} 3256, 1766, 1731, 1639, 1620 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.35 (1H, br s, D_2O exchangeable, NH), 9.15 (1H, s, H-4), 8.46 (1H, d, J=7.7 Hz, H-5), 8.34 (2H, d, J=7.6 Hz, H-2′ and -6′), 7.83 (1H, d, J=7.9 Hz, H-8), 7.63 (1H, t, J=7.5 Hz, H-7), 7.38 (2H, d, J=7.8 Hz, H-3′ and -5′), 7.35 (1H, t, J=7.5 Hz, H-6), 2.42 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 192.2, 166.7, 143.4, 142.2, 136.8, 136.4, 136.1, 134.4, 131.6, 131.5, 129.5, 128.9, 122.4, 121.1, 120.7, 120.5, 113.4, 21.4; EIMS m/z 330 $[M]^+$ (100), 315 (35), 302 (18), 285 (79), 271 (22), 258 (70); HREIMS m/z 330.1007 $[M]^+$ (calcd for $C_{20}H_{14}N_2O_3$, 330.1004). Anal. Calcd for $C_{20}H_{14}N_2O_3$: C, 72.72; H, 4.27; N, 8.48. Found: C, 72.91; H, 4.35; N, 8.51.

4.2.5. (9*H*-β-Carbolin-1-yl)-*p*-tolyl-methanone (2b). Yellow needle (EtOAc), mp 158–161 °C; IR (KBr) ν_{max} 3395, 1621, 1604 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.02 (1H, br s, D_2O exchangeable, NH), 8.52 (1H, d, J=5.0 Hz, H-3), 8.44 (1H, d, J=5.0 Hz, H-4), 8.32 (1H, d, J=7.6 Hz, H-5), 8.14 (2H, d, J=8.0 Hz, H-2′ and -6′), 7.79 (1H, d, J=7.9 Hz, H-8), 7.60 (1H, dd, J=7.9, 7.4 Hz, H-7), 7.38 (2H, d, J=8.0 Hz, H-3′ and -5′), 7.31 (1H, dd, J=7.6, 7.4 Hz, H-6), 2.42 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 193.4, 142.9, 141.8, 137.3, 136.8, 135.9, 134.9, 131.2, 131.1, 129.1, 128.8, 122.0, 120.3, 120.2, 118.9, 113.1, 21.4; EIMS m/z 286 $[M]^+$ (100), 271 (74), 258 (100); HREIMS m/z 286.1102 $[M]^+$ (calcd for $C_{19}H_{14}N_2O$, 286.1106). Anal. Calcd for $C_{19}H_{14}N_2O$: C, 79.70; H, 4.93; N, 9.78. Found: C, 79.92; H, 5.03; N, 9.84.

4.2.6. (9*H*-β-Carbolin-1-yl)-*p*-tolyl-methanol (2c). Yellow powder (EtOAc), mp 184–185 °C; IR (KBr) ν_{max} 3360, 1625 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.23 (1H, br s, D_2O exchangeable, NH), 8.22 (1H, d, J=5.2 Hz, H-3), 8.17 (1H, d, J=7.6 Hz, H-5), 7.97 (1H, d, J=5.2 Hz, H-4), 7.72 (1H, d, J=7.9 Hz, H-8), 7.50 (1H, dd, J=7.9, 7.4 Hz, H-7), 7.47 (2H, d, J=7.7 Hz, H-2′ and -6′), 7.19 (1H, dd, J=7.6, 7.4 Hz, H-6), 7.07 (2H, d, J=7.7 Hz, H-3′ and -5′), 6.46 (1H, d, J=4.0 Hz, OH), 6.10 (1H, d, J=4.0 Hz, C*H*OH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 147.8, 140.9, 140.8, 137.0, 136.1, 132.3, 128.7, 128.7, 128.0, 126.4, 121.5, 120.5, 119.2, 113.7, 112.7, 75.8, 20.8; EIMS m/z 288 $[M]^+$ (100), 269 (56), 255 (92); HREIMS m/z 288.1265 $[M]^+$ (calcd for $C_{19}H_{16}N_2O$, 288.1263). Anal. Calcd for $C_{19}H_{16}N_2O$: C, 79.14; H, 5.59; N, 9.72. Found: C, 79.27; H, 5.66; N, 9.72.

4.2.7. (3-Hydroxy-9*H*-β-carbolin-1-yl)-*p*-tolyl-methanone (2d). Orange powder (EtOAc), mp 171–175 °C; IR (KBr) ν_{max} 3360, 1659, 1631 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.56 (1H, br s, D_2O exchangeable, NH), 10.33 (1H, br s, D_2O exchangeable, OH), 8.21 (1H, d, J=8.6 Hz, H-5), 8.18 (2H, d, J=8.4 Hz, H-2′ and -6′), 7.69 (1H, s, H-4), 7.67 (1H, d, J=8.1 Hz, H-8), 7.53 (1H, t, J=7.5 Hz, H-7), 7.36 (2H, d, J=8.1 Hz, H-3′ and -5′), 7.20 (1H, t, J=7.5 Hz, H-6), 2.41 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 192.6, 154.8, 143.4, 142.7, 136.0, 135.2, 132.9, 132.0, 131.2, 129.4, 128.8, 122.3, 120.0, 119.6, 112.8, 104.6, 21.4; EIMS m/z 302 $[M]^+$ (100), 287 (34); HREIMS m/z 302.1058 $[M]^+$ (calcd for $C_{19}H_{14}N_2O_2$, 302.1055).

4.2.8. 1-(4-Bromo-benzoyl)-9*H*-β-carboline-3-carboxylic acid (3a). Yellow powder (EtOAc–MeOH), mp 290–291 °C; IR (KBr) ν_{max} 3276, 1731, 1640, 1621 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.41 (1H, br s, D_2O exchangeable, NH), 9.16 (1H, s, H-4), 8.47 (1H, d, J=7.8 Hz, H-5), 8.34 (2H, d, J=8.2 Hz, H-2′ and -6′), 7.84 (1H, d, J=8.8 Hz, H-8), 7.81 (2H, d, J=8.2 Hz, H-3′ and -5′), 7.65 (1H, t, J=7.5 Hz, H-7), 7.37 (1H, t, J=7.5 Hz, H-6); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 191.9, 166.7, 142.4, 137.0, 136.6, 136.2, 135.5, 133.5, 131.9, 131.5, 129.8, 127.3, 122.6, 121.4, 121.0, 120.8, 113.6; EIMS m/z 396 $[M+2]^+$ (22), 394 $[M]^+$ (23), 349 (13), 322 (12), 271 (12), 241 (12); HREIMS m/z 393.9957 $[M]^+$ (calcd for $C_{19}H_{11}N_2O_3Br$, 393.9953).

4.2.9. (4-Bromo-phenyl)-(9*H*-β-carbolin-1-yl)-methanone (3b). Yellow needle (EtOAc), mp 194–196 °C; IR (KBr) ν_{max} 3389, 1644 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.09 (1H, br s, D_2O exchangeable, NH), 8.51 (1H, d, J=4.9 Hz, H-3), 8.44 (1H, d, J=4.9 Hz, H-4), 8.31 (1H, d, J=7.8 Hz, H-5), 8.15 (2H, d, J=8.5 Hz, H-2′ and -6′), 7.82 (1H, d, J=8.2 Hz, H-8), 7.77 (2H, d, J=8.5 Hz, H-3′ and -5′), 7.60 (1H, t, J=7.5 Hz, H-7), 7.31 (1H, t, J=7.5 Hz, H-6); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 192.8, 141.9, 137.4, 136.6, 136.0, 133.0, 131.3, 131.2, 129.2, 126.5, 122.0, 120.4, 120.2, 119.3, 113.2; EIMS m/z 352 $[M+2]^+$ (17), 350 $[M]^+$ (18), 322 (14), 279 (27), 185 (71), 183 (79), 167 (44), 149 (100); HREIMS m/z 350.0055 $[M]^+$ (calcd for $C_{18}H_{11}N_2OBr$, 350.0055). Anal. Calcd for $C_{18}H_{11}N_2OBr$: C, 61.56; H, 3.16; N, 7.98. Found: C, 61.76; H, 3.21; N, 7.94.

4.2.10. (4-Bromo-phenyl)-(9*H*-β-carbolin-1-yl)-methanol (3c). Yellow powder (EtOAc), mp 157–159 °C; IR (KBr) ν_{max} 3441, 1628 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.27 (1H, br s, D_2O exchangeable, NH), 8.23 (1H, d, J=5.3 Hz, H-3), 8.17 (1H, d, J=7.6 Hz, H-5), 7.99 (1H, d, J=5.3 Hz, H-4), 7.71 (1H, d, J=8.0 Hz, H-8), 7.55 (2H, d, J=8.5 Hz, H-2′ and -6′), 7.51 (1H, dd, J=8.0, 7.5 Hz, H-7), 7.47 (2H, d, J=8.5 Hz, H-3′ and -5′), 7.20 (1H, dd, J=7.6, 7.5 Hz, H-6), 6.62 (1H, d, J=4.1 Hz, OH), 6.14 (1H, d, J=4.1 Hz, C*H*OH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 147.1, 143.3, 140.9, 137.1, 132.4, 131.1, 129.0, 128.7, 128.2, 121.6, 120.6, 120.3, 119.4, 114.0, 112.7, 75.2; EIMS m/z 354 $[M+2]^+$ (34), 352 $[M]^+$ (35), 335 (24),

333 (22), 255 (100); HREIMS *m/z* 352.0211 $[M]^+$ (calcd for $C_{18}H_{13}N_2OBr$, 352.0211). Anal. Calcd for $C_{18}H_{13}N_2OBr$: C, 61.21; H, 3.71; N, 7.93. Found: C, 61.18; H, 3.81; N, 7.72.

4.2.11. 1-(4-Methoxy-benzoyl)-9*H*-β-carboline-3-carboxylic acid (4a). Yellow powder (EtOAc–MeOH), mp 264–266 °C; IR (KBr) ν_{max} 3273, 1703 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.33 (1H, br s, D_2O exchangeable, NH), 9.14 (1H, s, H-4), 8.53 (2H, d, *J*=8.8 Hz, H-2′ and -6′), 8.46 (1H, d, *J*=7.6 Hz, H-5), 7.82 (1H, d, *J*=8.2 Hz, H-8), 7.63 (1H, dd, *J*=8.2, 7.8 Hz, H-7), 7.35 (1H, dd, *J*=7.8, 7.6 Hz, H-6), 7.13 (2H, d, *J*=8.8 Hz, H-3′ and -5′), 3.89 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 190.6, 166.7, 163.4, 142.2, 136.8, 136.6, 136.3, 134.0, 131.4, 129.5, 129.5, 122.4, 121.0, 120.7, 120.4, 113.8, 113.4, 55.8; EIMS *m/z* 346 $[M]^+$ (100), 317 (15), 302 (20), 299 (21), 274 (32), 135 (82); HREIMS *m/z* 346.0951 $[M]^+$ (calcd for $C_{20}H_{14}N_2O_4$, 346.0954). Anal. Calcd for $C_{20}H_{14}N_2O_4$: C, 69.36; H, 4.07; N, 8.09. Found: C, 69.59; H, 4.10; N, 8.07.

4.2.12. (9*H*-β-Carbolin-1-yl)-(4-methoxy-phenyl)-methanone (4b). Yellow needle (EtOAc), mp 185–187 °C; IR (KBr) ν_{max} 3423, 1597 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.98 (1H, br s, D_2O exchangeable, NH), 8.52 (1H, d, *J*=4.8 Hz, H-3), 8.41 (1H, d, *J*=4.8 Hz, H-4), 8.31 (2H, d, *J*=8.9 Hz, H-2′ and -6′), 8.30 (1H, d, *J*=7.7 Hz, H-5), 7.79 (1H, d, *J*=8.0 Hz, H-8), 7.59 (1H, dd, *J*=8.0, 7.4 Hz, H-7), 7.29 (1H, dd, *J*=7.7, 7.4 Hz, H-6), 7.10 (2H, d, *J*=8.9 Hz, H-3′ and -5′), 3.87 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 191.8, 163.0, 141.8, 137.2, 137.1, 135.9, 133.6, 131.0, 130.0, 129.0, 122.0, 120.3, 118.6, 113.6, 113.1, 55.7; EIMS *m/z* 302 $[M]^+$ (100), 273 (75), 135 (41); HREIMS *m/z* 302.1056 $[M]^+$ (calcd for $C_{19}H_{14}N_2O_2$, 302.1055). Anal. Calcd for $C_{19}H_{14}N_2O_2$: C, 75.48; H, 4.67; N, 9.27. Found: C, 75.48; H, 4.66; N, 9.27.

4.2.13. (9*H*-β-Carbolin-1-yl)-(4-methoxy-phenyl)-methanol (4c). Yellow syrup; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.22 (1H, br s, D_2O exchangeable, NH), 8.21 (1H, d, *J*=5.2 Hz, H-3), 8.17 (1H, d, *J*=7.6 Hz, H-5), 7.96 (1H, d, *J*=5.2 Hz, H-4), 7.71 (1H, d, *J*=7.8 Hz, H-8), 7.49 (1H, dd, *J*=7.8, 7.4 Hz, H-7), 7.48 (2H, d, *J*=8.6 Hz, H-2′ and -6′), 7.19 (1H, dd, *J*=7.6, 7.4 Hz, H-6), 6.82 (2H, d, *J*=8.6 Hz, H-3′ and -5′), 6.41 (1H, d, *J*=3.9 Hz, OH), 6.08 (1H, d, *J*=3.9 Hz, C*H*OH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 158.5, 148.0, 140.8, 137.0, 136.0, 132.3, 128.8, 128.1, 127.7, 121.5, 120.6, 119.3, 113.7, 113.6, 112.7, 75.6, 55.2; EIMS *m/z* 304 $[M]^+$ (100), 285 (98), 272 (76), 255 (73), 242 (30); HREIMS *m/z* 304.1215 $[M]^+$ (calcd for $C_{19}H_{16}N_2O_2$, 304.1212).

4.2.14. 4-Methoxy-benzoic acid (4d). Yellow powder (benzene), mp 190–192 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.60 (1H, br s, D_2O exchangeable, OH), 7.88 (2H, d, *J*=8.7 Hz, H-2′ and -6′), 7.00 (2H, d, *J*=8.7 Hz, H-3′ and -5′), 3.81 (3H, s, OCH_3).

4.2.15. (4-Methoxy-phenyl)-oxo-acetic acid (4e). Yellow powder (benzene), mp 223–226 °C; IR (KBr) ν_{max} 1651, 1608 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.82 (2H, d, *J*=8.6 Hz, H-2′ and -6′), 7.02 (2H, d, *J*=8.6 Hz, H-3′ and -5′), 3.82 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 193.5, 169.7, 163.4, 131.6, 127.0, 114.1, 55.7.

4.2.16. 1-Acetyl-9*H*-β-carboline-3-carboxylic acid (5a). Yellow powder (EtOAc–MeOH), mp 292 °C (dec); ^1H NMR (DMSO-d_6, 300 MHz) δ 12.23 (1H, br s, D_2O exchangeable, NH), 9.14 (1H, s, H-4), 8.44 (1H, d, *J*=8.0 Hz, H-5), 7.84 (1H, d, *J*=8.0 Hz, H-8), 7.62 (1H, t, *J*=8.0 Hz, H-7), 7.34 (1H, t, *J*=8.0 Hz, H-6), 2.87 (3H, s, $COCH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 201.3, 166.5, 142.5, 136.5, 135.3, 135.2, 131.7, 129.5, 122.4, 121.2, 121.2, 120.4, 113.6, 26.0; EIMS *m/z* 254 $[M]^+$ (100), 236 (16), 210 (32), 194 (39), 182 (35); HREIMS *m/z* 254.0688 $[M]^+$ (calcd for $C_{14}H_{10}N_2O_3$, 254.0691).

4.2.17. 1-(9*H*-β-Carbolin-1-yl)-ethanone (5b). Yellow needle (EtOAc), mp 207–209 °C; IR (KBr) ν_{max} 3333, 1669 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.88 (1H, br s, D_2O exchangeable, NH), 8.50 (1H, d, *J*=4.8 Hz, H-3), 8.43 (1H, d, *J*=4.8 Hz, H-4), 8.29 (1H, d, *J*=7.8 Hz, H-5), 7.80 (1H, d, *J*=8.1 Hz, H-8), 7.58 (1H, t, *J*=7.8 Hz, H-7), 7.29 (1H, t, *J*=7.8 Hz, H-6), 2.79 (3H, s, $COCH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 201.5, 142.0, 137.6, 136.1, 134.2, 131.1, 129.1, 122.0, 120.4, 120.0, 119.6, 113.3, 26.1; EIMS *m/z* 210 $[M]^+$ (98), 182 (52), 168 (100), 140 (35); HREIMS *m/z* 210.0796 $[M]^+$ (calcd for $C_{13}H_{10}N_2O$, 210.0793).

4.2.18. 1-(9*H*-β-Carbolin-1-yl)-ethanol (5c). Yellow powder (EtOAc), mp 168–170 °C; IR (KBr) ν_{max} 3288, 1628 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.22 (1H, br s, D_2O exchangeable, NH), 8.22 (1H, d, *J*=5.1 Hz, H-3), 8.17 (1H, d, *J*=8.4 Hz, H-5), 7.98 (1H, d, *J*=5.1 Hz, H-4), 7.68 (1H, d, *J*=8.1 Hz, H-8), 7.50 (1H, t, *J*=8.1 Hz, H-7), 7.20 (1H, t, *J*=7.5 Hz, H-6), 5.68 (1H, d, *J*=4.5 Hz, OH), 5.19 (1H, qd, *J*=6.5, 4.5 Hz, C*H*OH), 1.54 (3H, d, *J*=6.5 Hz, $COCH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 148.9, 140.7, 136.8, 132.5, 128.5, 128.0, 121.6, 120.7, 119.3, 113.7, 112.6, 69.5, 23.1; EIMS *m/z* 212 $[M]^+$ (75), 193 (100), 184 (56), 168 (77).

4.3. Preparation of (6-methoxy-9*H*-β-carbolin-1-yl)-(4-methoxy-phenyl)-methanone (11)

To a stirred suspension of 0.093 g (0.397 mmol, 1.3 equiv) of 5-methoxy-tryptophan in 1.0 equiv of *p*-toluenesulfonic acid, 0.050 g (0.305 mmol, 1.0 equiv) of **4** was added. The resulting solution was stirred at 50 °C for 2 h. The reaction mixture was poured into water and the precipitate was filtered and purified by silica gel column chromatography eluted with a gradient of *n*-hexane and ethyl acetate to afford **11** and **11a**, respectively. Compound **11**: yellow needle (EtOAc), mp 135–138 °C; IR (KBr) ν_{max} 3432, 1642, 1621 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.82 (1H, br s, D_2O exchangeable, NH), 8.48 (1H, d, *J*=5.0 Hz, H-3), 8.41 (1H, d, *J*=5.0 Hz, H-4), 8.31 (2H, d, *J*=8.8 Hz, H-2′ and -6′), 7.87 (1H, d, *J*=2.4 Hz, H-5), 7.69 (1H, d, *J*=8.9 Hz, H-7), 7.24 (1H, dd, *J*=8.9, 2.4 Hz, H-8), 7.11 (2H, d, *J*=8.8 Hz, H-3′ and -5′), 3.87 (6H, s, $OCH_3\times2$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 191.8, 162.9, 154.1, 137.2, 136.6, 136.3, 133.6, 131.0, 130.0, 120.7, 119.0, 118.8, 113.9, 113.6, 103.8, 55.8, 55.7; EIMS *m/z* 332 $[M]^+$ (100), 317 (10), 303 (25), 289 (28); HREIMS *m/z* 332.1163 $[M]^+$ (calcd for $C_{20}H_{16}N_2O_3$, 332.1161). Anal. Calcd for $C_{20}H_{16}N_2O_3$: C, 72.28; H, 4.85; N, 8.43. Found: C, 71.97; H, 4.96; N, 8.30. Compound **11a**: yellow needle

(EtOAc–MeOH), mp 135–138 °C; IR (KBr) ν_{max} 3432, 1642, 1621 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.18 (1H, br s, D_2O exchangeable, NH), 9.16 (1H, s, H-4), 8.54 (2H, d, J=8.8 Hz, H-2′ and -6′), 8.07 (1H, d, J=2.1 Hz, H-5), 7.72 (1H, d, J=8.9 Hz, H-8), 7.27 (1H, dd, J=8.9, 2.1 Hz, H-7), 7.12 (2H, d, J=8.8 Hz, H-3′ and -5′), 3.89 (3H, s, OCH_3), 3.88 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 190.6, 166.8, 163.4, 154.7, 137.1, 136.9, 136.6, 135.6, 134.0, 131.6, 131.3, 129.6, 121.3, 120.7, 119.5, 114.3, 114.0, 113.8, 104.2, 55.9, 55.8; EIMS m/z 376 $[M]^+$ (100), 332 (46), 301 (14), 224 (16); HREIMS m/z 376.1056 $[M]^+$ (calcd for $C_{21}H_{16}N_2O_5$, 376.1059).

4.4. Bioassay

4.4.1. Cell culture. Raw 264.7 cells (American Type Culture Collection ATCC, TIB 71, Rockville, MD) suspending in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), penicillin (100 U mL^{-1}), and streptomycin (100 μg/mL) were seeded onto 96-well plated (Corning-Costar). LPS (1 μg/mL) plus IFN-γ (50 U/mL) was added to the medium for 24 h to stimulate NO production. Tested compounds and iNOS inhibitor (aminoguanidine) were added together with LPS/IFN-γ.[13]

4.4.2. Nitrite measurement. Nitrite formation, an indicator of NO synthesis, was measured by adding 100 μL of Griess reagent (1% sulfanilamide and 0.1% naphthylenediamine in 5% phosphoric acid) to 100 μL samples of medium. The optical density at 550 nm (OD_{550}) was measured with a microplate reader. Concentrations were calculated by comparison with OD_{550} of standard solutions of sodium nitrite prepared in culture medium.

4.4.3. Cell viability. Cell viability was assessed by the mitochondria-dependent reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to formazan. The extent of reduction of MTT to formazan within cells was quantitated by measurement of OD_{570} against OD_{630}.

4.4.4. Statistical evaluation. The results are expressed as mean±SE and NO production is indicated as absolute concentrations in micromolars. Computation of 50% inhibitory concentration (IC_{50}) and the slope of regression line were computer-assisted (PHARM/PCS v.4.2).

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