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Synthesis, in vitro anti-inflammatory and cytotoxic evaluation, and mechanism of action studies of 1-benzoyl-β-carboline and 1-benzoyl-3-carboxy-β-carboline derivatives

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ABSTRACT

In the present study, various 1-substituted and 1,3-disubstituted β-carboline derivatives were synthesized by a modified single-step Pictet–Spengler reaction. The compounds were examined for cytotoxicity and anti-inflammatory activity, as measured by the inhibition of prostaglandin E_2 (PGE_2) production and nitric oxide (NO) production. While only two compounds (**28** and **31**) showed marginal cytotoxicity against four human cancer cell lines, most of the tested compounds exhibited potent inhibitory activity of both NO and PGE_2 production. Moreover, compounds **6** and **16** significantly reduced the expression of inducible nitric oxide synthase (*i*NOS) and cyclooxygenase 2 (COX2), suggesting that β-carboline analogs can inhibit NO and PGE_2 production at the translational level. In addition, several of the β-carboline derivatives (**1**, **2**, **4**–**8**, **11**, **13**, **22**, **25**, **27**, **31**, and **41**–**43**) displayed significant inhibitory activity of superoxide anion (O_2^-) generation or elastase release compared to the reference compound, with **6** being the most potent. *N*-Formyl-L-methionyl-phenylalanine (FMLP)-induced phosphorylation of *c-Jun N*-terminal kinase (JNK) and protein kinase B (AKT) were also inhibited by **6**, suggesting that it suppresses human neutrophil functions by inhibiting the activation of JNK and AKT signaling pathways. Therefore, the synthetic 1-benzoyl-3-carboxy β-carboline analogs may have great potential to be developed as anti-inflammatory agents.

1. Introduction

Sustained nitric oxide (NO) release by inducible nitric oxide synthase (*i*NOS) and prostaglandin E_2 (PGE_2) production by cyclooxygenase 2 (COX2) have been implicated as mediators of inflammation and are induced by bacterial lipopolysaccharide (LPS) or immunological stimuli.[1,2] It has been reported that excess production of NO and PGE_2 by macrophages and other cells exposed to endotoxins may contribute to septic shock, cerebral injury, myocardical ischemia, diabetes, arteriosclerosis, and other local or systemic inflammatory disorders.[2,3] Thus, inhibition of NO synthesis and PGE_2 production stands as an important therapeutic goal. On the other hand, overwhelming activation of neutrophils is known to play important roles in the pathogenesis of various diseases, such as rheumatoid arthritis, ischemia, reperfusion injury, chronic obstructive pulmonary disease, and asthma.[4,5] In response to diverse stimuli, activated neutrophils secrete a series of cytotoxins, such as superoxide anion (O_2^-), a precursor of other reactive oxygen species, and elastase, a granule protease. Therefore, it is crucial to retain O_2^- production and elastase release in physiological conditions, while potentiating these functions in infected tissues and organs. Currently, only a few agents are available in clinical practice that can directly modulate neutrophil proinflammatory responses.

Natural and synthetic products containing a β-carboline pharmacophore exhibit a wide range of important bioactivities, particularly on the central nervous system.[6,7] Due to their unique rigid heterocyclic skeleton, many β-carbolines bind with high affinity to benzodiazepine,[8] serotonin,[6] and dopamine[9] receptor sites and inhibit monoamine oxidase A.[10] The reported biological effects of this class of compounds include sedative,[11] antithrombotic,[12] anti-HIV,[13] and DNA-targeting properties,[14] as well as suppression of CDK,[15,16] topoisomerase,[17] and IκK.[18] For example, flazin[19] and

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flazinamide[20] possess anti-HIV activity, while dichotomine C exhibits inhibitory effects against the release of β-hexosaminidase, TNF-α, and IL-4 from RBL-2H3 cells.[21] With appropriate substituents at the 3-position, β-carboline derivatives also showed cytotoxic activity against human tumor cell lines.[22–24]

As a continuing study of bioactive 1-substituted β-carbolines, we synthesized more novel 1-substituted and 1,3-disubstituted β-carboline derivatives, using the previously reported single-step Pictet–Spengler reaction.[25] We report the synthesis, cytotoxic and anti-inflammatory evaluation, as well as the structure–activity relationship (SAR) analysis of these derivatives in the present paper.

2. Chemistry

The synthetic methods used to obtain compounds **1–39** by single-step Pictet–Spengler reaction of L-tryptophan with different functionalized phenylglyoxal derivatives were described in our previous report as shown in Figure 1.[25] The yields of the synthetic β-carboline compounds are listed in Table 1. The 1-benzoyl-3-carboxy-β-carboline products **1–8** and **11–13** were obtained in 2–6% yields, while **9** and **10** were present in trace amounts, insufficient to be isolated and characterized. These compounds displayed very strong affinity for silica gel, considering that chromatography eluting with highly polar solvent systems, such as mixtures of EtOAc and MeOH, were still barely able to elute and purify these compounds.

The decarboxylated 1-benzoyl-β-carboline analogs **14–22** and **24–25** were obtained in moderate yields (~20%), while **23** and **26** were available in only trace amounts. Generally, these compounds could be purified by chromatography with medium polar solvent systems. Compounds **27**, **28**, and **31**, were obtained by reduction of the ketone group in yields of 45%, 35%, and 35%, respectively.

In addition, as shown in Figure 2, pyruvic aldehyde was condensed with L-tryptophan or L-tryptophanylamide to yield β-carboline derivatives **40–42** and **43**, respectively. Moreover, compound **44** was obtained by coupling between 4-methoxyphenylglyoxal and tryptophan methyl ester. While compounds **40–42** were obtained in similar yields to those previously reported,[25] changing the carboxylic acid group to amide and ester functionalities significantly improved the yields in the modified Pictet–Spengler condensation reactions, as was seen with **43** and **44**. Not only were β-carbolines **43** and **44** produced in higher yields, but the condensation reactions afforded only one product, due to the difference of the 3-substituent in the β-carboline basic skeleton.

Table 1
Yields of differently substituted β-carbolines **1–44**

	Products, yield		
$R_1 = R_2 = R_3 = H$	**1**, 4%	**14**, 35%	**27**, 45%
$R_1 = CH_3$, $R_2 = R_3 = H$	**2**, 6%	**15**, 20%	**28**, 35%
$R_2 = CH_3$, $R_1 = R_3 = H$	**3**, 5%	**16**, 18%	**29**, trace
$R_3 = CH_3$, $R_1 = R_2 = H$	**4**, 8%	**17**, 22%	**30**, trace
$R_1 = Br$, $R_2 = R_3 = H$	**5**, 3%	**18**, 20%	**31**, 35%
$R_2 = Br$, $R_1 = R_3 = H$	**6**, 5%	**19**, 18%	**32**, trace
$R_3 = Br$, $R_1 = R_2 = H$	**7**, 4%	**20**, 20%	**33**, trace
$R_1 = OCH_3$, $R_2 = R_3 = H$	**8**, 4%	**21**, 15%	**34**, trace
$R_2 = OCH_3$, $R_1 = R_3 = H$	**9**, trace	**22**, 15%	**35**, trace
$R_3 = OCH_3$, $R_1 = R_2 = H$	**10**, trace	**23**, trace	**36**, trace
$R_1 = NO_2$, $R_2 = R_3 = H$	**11**, 2%	**24**, 10%	**37**, trace
$R_2 = NO_2$, $R_1 = R_3 = H$	**12**, trace	**25**, 10%	**38**, trace
$R_3 = NO_2$, $R_1 = R_2 = H$	**13**, 2%	**26**, trace	**39**, trace
	40, 5%	**41**, 13%	**42**, 30%
	43, 55%		
	44, 50%		

3. Results and discussion

The novel 1-substituted and 1,3-disubstituted β-carboline derivatives (**1–2**, **4–8**, **11**, **13–22**, **24–25**, **27–28**, **31**, and **40–44**) were first tested against four different human cancer cell lines: A549 (lung), DU145 (prostate), KB (nasopharyngeal), and KB-VIN (its vincristine-resistant subline) using paclitaxel as a positive control (Table 2). Overall, the newly obtained β-carboline derivatives did not show strong cytotoxicity. Except for **27**, **28**, and **31**, the new derivatives did not exhibit cytotoxicity against any of the four tested cell lines at concentrations less than 10 μg/mL. Comparing the potency of active **28** and **31** with inactive **2**/**15** and **5**/**18**, respectively, we found that a 3-carboxylic acid and 1′-ketone were detrimental, while a 1′-hydroxy moiety was necessary for the compounds to exhibit cytotoxicity. The three active compounds differed structurally only in the substituents on the pendant phenyl ring. Compound **28** with a *para*-methyl group showed the best activity (GI_{50}: 2.68–7.68 μg/mL, 9.29–26.6 μM). Interestingly, these three compounds showed better activity against KB-VIN than KB.

The synthetic β-carboline derivatives were then examined for inhibition of PGE_2 production, which was stimulated by lipopolysaccharide in interferon-γ (LPS/IFN-γ)-stimulated macrophages. The data are displayed in Table 3. Among the 1-benzoyl-3-carboxy-β-carboline derivatives (**1–8**, **11**, and **13**), compounds **2**, **3**, **5**, and **6**, with methyl and bromide at the *para*- and *meta*-positions

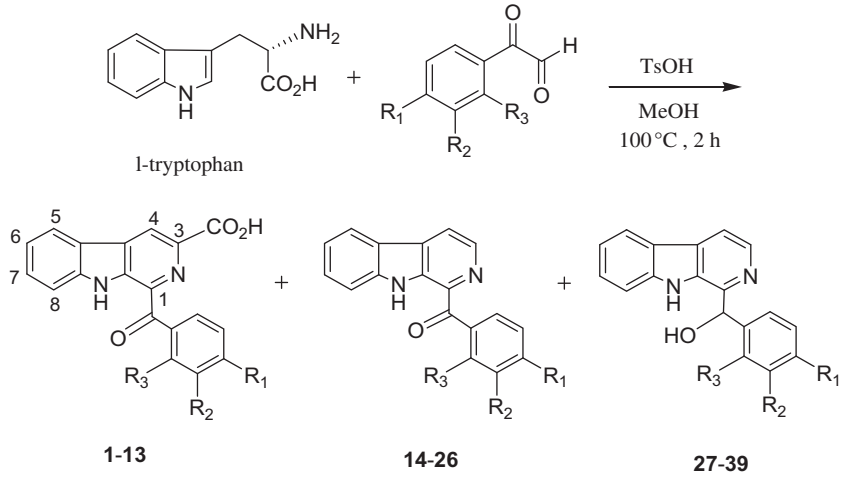

Figure 1. Synthesis of differently substituted β-carbolines **1–39**.

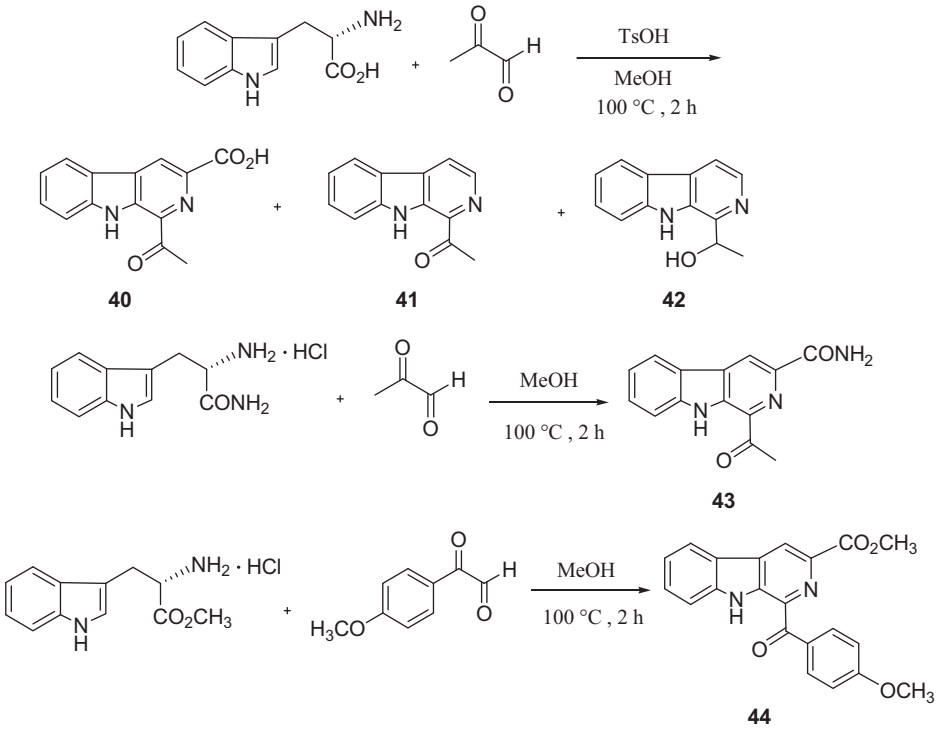

Figure 2. Synthesis of β-carboline analogs **40**–**44**.

Table 2
Cytotoxicity of compounds against human tumor cell lines[a,b]

Compound	KB	KB-VIN	A549	DU145
27	>40	22.56	>40	>40
28	20.6	9.29	26.6	13.8
31	15.9	14.5	19.0	13.3
Paclitaxel	0.00487	>1.0	0.00558	0.00555

[a] Mean GI_{50} (μM), from two or more independent tests.
[b] Compounds **1**, **2**, **4**–**8**, **11**, **13**–**22**, **24**, **25**, and **40**–**44** were inactive (GI_{50} >40 μM) against all four cell lines.

of the 1-benzoyl ring exhibited the most significant inhibition of PGE_2 synthesis with inhibitory percentages of 100%, 98%, 98%, and 100%, respectively, at 1 μM. In comparison, the unsubstituted compound **1** and *ortho*-substituted analogs **4** and **7** were not active at this concentration, although they were active at 3 and 10 μM. Moreover, the electron-releasing methoxy and electron-withdrawing nitro substituents in **8** and **11**/**13** did not contribute to PGE_2 inhibition. Based on these results, we concluded that *m*- or *p*-substituted weak electron-donating or -withdrawing groups in the 1-benzoyl ring could improve the inhibitory activity on PGE_2 production. In addition, the 3-methyl ester derivative **44** also showed a high inhibition percentage of 98% at 1 μM. Similarly, among the 1-benzoyl substituted β-carboline derivatives **14**–**26**, only the *m*-methyl-, *p*-bromo-, and *m*-bromo-benzoyl substituted compounds **16**, **18**, and **19** exhibited high inhibition (97%, 98%, and 97%, respectively) of PGE_2 synthesis in LPS/IFN-γ-stimulated cells at 1 μM. The (9*H*-β-carbolin-1-yl)-phenyl-methanol derivatives **27**, **28**, **31**, and **34** did not show significant inhibition at any tested concentration, suggesting that the 1-carbonyl functionality played an important role in the inhibitory activity of the derivatives on PGE_2 production.

The inhibitory effects of the synthetic analogs on the generation of NO were further evaluated in LPS/IFN-γ stimulated RAW 264.7

Table 3
Effects of synthetic compounds on LPS/IFN-β-induced PGE_2 production in RAW 264.7 macrophages

Basal LPS/IFN-γ	0–12.9 pg/mL 113–259 pg/mL		
	% of inhibition	% of inhibition	% of inhibition
Concentration	1 μM	3 μM	10 μM
1	0	12	90
2	100	100	—
3	98	100	—
4	0	82	100
5	98	100	—
6	100	100	—
7	0	78	96
8	28	98	—
11	0	92	100
13	0	97	100
14	0	39	82
15	25	99	100
16	97	100	—
17	0	44	82
18	98	100	—
19	97	98	—
20	0	37	89
21	0	95	95
22	0	71	98
24	0	100	100
25	0	95	96
27	0	0	0
28	15	29	—
31	0	21	34
34	0	0	19
40	0	0	0
41	0	0	59
42	0	0	0
43	0	95	95
44	98	100	—
Aminoguanidine	8	21	42

'—': Not determined.

Table 4
Effects of synthetic compounds on LPS/IFN-γ-induced NO production in RAW 264.7 macrophages

Compound	EC_{50} (μM)	CC_{50} (μM) cytotoxic concn	Therapeutic index (T.I.) (CC_{50}/EC_{50})
1	3.65	564	155
2	0.50	17.7	35
3	0.47	16.4	34
4	2.58	62300	>2000
5	0.53	10.7	20
6	0.57	29.4	51
7	6.96	524	75
8	0.23	43.1	187
11	2.94	15.0	5
13	2.88	12.8	4
14	2.67	131	49
15	1.26	52.8	42
16	0.20	23.7	120
17	2.12	51500	>2000
18	0.30	149	492
19	1.16	51.4	44
20	6.97	53.8	8
21	4.59	83.4	18
22	2.96	16.3	6
24	0.66	3.86	6
25	1.06	21.6	20
27	0.31	574	186
28	4.94	NT	>10[10]
31	0.74	70.1	95
34	2.08	1590	764
40	0.25	NT	>10[10]
41	6.65	NT	>10[10]
42	NA	0.28	ND
43	0.92	19.8	21
44	0.51	1.95	4
Aminoguanidine (*i*NOS inhibitor)	22.6	358	16

NA: not active; ND: not determined; NT: not toxic.

macrophages, according to the method reported in literature,[26] and the data are summarized in Table 4. Among the tested compounds, the 1-benzoyl-3-carboxy-β-carbolines **2**, **3**, **5**, **6**, **8**, **40**, **43**, and **44**, 1-benzoyl-β-carbolines **16**, **18**, and **24**, and (9*H*-β-carbolin-1-yl)-phenyl-methanol derivatives **27** and **31** showed significant NO production inhibitory activity with EC_{50} values of <1 μM. The β-carboline **16** with an *m*-methylbenzoyl group displayed the strongest NO inhibition activity with an EC_{50} value of 0.20 μM, which was two-order better than that of the *i*NOS inhibitor aminoguanidine. Although the corresponding *o*-methylbenzoyl compound **17** was less potent (EC_{50}: 2.12 μM) than **16**, it had a higher therapeutic index due to low cytotoxicity. Overall, these 1-methylbenzoyl-β-carbolines may have potential to be developed as new *i*NOS inhibitors. While the 1,3-disubstituted β-carbolines **43** and **44** with 3-amide and -ester substitutions still possessed significant NO inhibition activity, they also showed some increased cytotoxicity, which resulted in lower therapeutic indexes. Thus, 3-amide and -ester substituted compounds may not merit development as new anti-inflammatory lead compounds.

Following the initial screening, the mechanisms of action of the selected synthetic compounds **6** and **16** on *i*NOS and COX2 expression in LPS/IFN-γ-stimulated RAW264.7 macrophages were then investigated using western blot analysis. The results are displayed in Figure 3. These two compounds significantly reduced the protein expression of *i*NOS and COX2 at 18 h after LPS/IFN-γ stimulation, which suggested that the synthetic β-carboline analogs inhibited NO and PGE_2 production at the translational level or the upstream pathway of transcription.

In addition, the β-carboline derivatives were also assayed for inhibition of $O_2^{\cdot-}$ generation and elastase release by human neutrophils in response to *N*-formyl-L-methionyl-phenylalanine (FMLP)/cytochalasin B. The data are displayed in Table 5. The 1-benzoyl-3-carboxy-β-carboline derivatives **1**, **2**, **4**–**8**, **11**, and **13** inhibited $O_2^{\cdot-}$ generation and elastase release in FMLP/cytochalasin B-activated human neutrophils in a concentration-dependent manner. Compound **6** demonstrated the most significant inhibition towards $O_2^{\cdot-}$ generation and compound **5** towards elastase release with IC_{50} values of 2.05 ± 0.21 and 1.99 ± 0.10 μM, respectively, compared with the reference compound LY294002[27,28] (IC_{50} 1.12 ± 0.20 and 1.92 ± 0.22 μM towards $O_2^{\cdot-}$ generation and elastase release, respectively). In contrast, the decarboxylated 1-benzoyl-β-carboline analogs **14–25** were inactive, except for **22** (elastase) and **25** ($O_2^{\cdot-}$). The (9*H*-β-carbolin-1-yl)-phenyl-methanol derivatives **27** and **31** also significantly inhibited $O_2^{\cdot-}$ generation, but were inactive or less

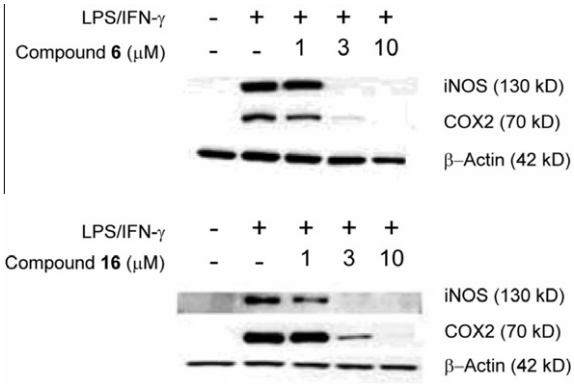

Figure 3. Western blot analysis of synthetic compounds **6** and **16** on *i*NOS and COX2 expressions in LPS/IFNγ-induced RAW 264.7 macrophages.

Table 5
Effects of synthetic compounds on superoxide anion generation and elastase release by human neutrophils in response to FMLP/cytochalasin B

Compound	IC_{50}[a] (μM)	
	Superoxide anion generation	Elastase release
1	17.7 ± 0.89	13.4 ± 0.54
2	6.27 ± 1.00	3.13 ± 1.24
4	5.36 ± 0.62	14.83 ± 1.01
5	2.28 ± 0.33	1.99 ± 0.10
6	2.05 ± 0.21	2.78 ± 0.15
7	5.33 ± 0.53	3.07 ± 1.71
8	16.1 ± 0.95	10.4 ± 1.11
11	6.65 ± 1.62	6.21 ± 0.82
13	17.6 ± 0.50	13.2 ± 1.22
14	>30	>30
15	>30	>30
16	>30	>30
17	>30	>30
18	>30	>30
19	>30	>30
20	>30	>30
21	>30	>30
22	>30	13.2 ± 1.22[b]
24	>30	>30
25	16.6 ± 1.70	—
27	7.88 ± 0.33	>30
28	>30	>30
31	8.84 ± 0.82	14.17 ± 2.00
40	>30	>30
41	5.03 ± 1.33	>30
42	9.40 ± 4.81	>30
43	14.5 ± 0.71	5.82 ± 0.58
44	>30	>30
Indomethacin	38.3 ± 5.38	32.0 ± 6.49
LY294002[c]	1.12 ± 0.20	1.92 ± 0.22

Results are presented as mean ± SEM (n = 3).
[a] Concentration necessary for 50% inhibition.
[b] Compound **22** induced elastase release in the pretreatment of cytochalasin B.
[c] LY294002, a phosphatidylinositol-3-kinase inhibitor, was used as a positive control for superoxide anion generation and elastase release.

active against elastase release. In addition, the β-carboline analogs **41**–**43** were generally effective against O_2^- generation, but less active against elastase release. Compounds **40** and **44** were inactive in both anti-inflammatory assays.

Generally speaking, the formation of O_2^- in neutrophils can be inhibited by modulating cellular signaling pathways, but also by direct radical scavenging. However, at a concentration of 250 μM, the tested synthetic compounds (**1–13**) did not significantly scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals in cell-free systems, indicating that the inhibition of O_2^- generation by β-carboline derivatives **1–13** is mediated by modulating cellular signaling pathways (data not shown). FMLP is known to activate phospholipase C and phosphatidylinositol-3-kinase (PI3K), and it also activates MAPKs. Activation of these signal transduction pathways is known to cause various physiological responses.[29] Upon activation of PI3K, protein kinase B (AKT) is recruited to the plasma membrane, where it undergoes phosphorylation and activation. Activation of *c-Jun N*-terminal kinase (JNK) and PI3K/AKT has been shown to contribute to human neutrophil functions, such as respiratory burst and degranulation.[30–32] Therefore, the effects of **6** on phosphorylation of JNK and AKT in FMLP-stimulated human neutrophils were further determined by western blot analysis, and the results are displayed in Figure 4. FMLP-induced phosphorylations of JNK and AKT were clearly inhibited by **6**, suggesting that **6** suppressed human neutrophil functions by inhibiting the activation of JNK and AKT signaling pathways.

4. Conclusions

In summary, we designed and synthesized new 1-substituted and 1,3-disubstituted β-carbolines, which were evaluated for cytotoxicity and anti-inflammatory activity. The results demonstrated that, among the synthetic β-carbolines tested, most of the synthetic derivatives exhibited significant NO inhibition activity, and were more effective than the *i*NOS inhibitor aminoguanidine. The most active compound **16** displayed the strongest NO inhibition activity with an EC_{50} value of 0.20 μM. 1-Benzoyl-3-carboxy derivatives **1–13** also inhibited O_2^- generation and elastase release in FMLP/cytochalasin B-activated human neutrophils, compared with the reference compound LY294002. In addition, the synthetic compounds showed low cytotoxicity. Therefore, this group of β-carbolines has the potential to be developed as new anti-inflammatory drugs.

5. Experimental

5.1. General analytical and spectroscopic methods

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. ^{1}H and ^{13}C NMR spectra were obtained on the Bruker Avance-300 NMR spectrometer, with tetramethylsilane (TMS) as internal standard and the chemical shifts were reported in δ values (ppm). 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. Elemental analyses were performed on an Elementar Vario EL III analyzer. The identities of the reported compounds have been unambiguously proven by their spectroscopic data, and the purity assessed by analytical HPLC performed on a Shimadzu LC-10AT^{VP} series pumping system equipped with a Shimadzu SPD-6AV UV–vis spectrophotometric detector at 254 nm, and a Luna C-18 packed column (4.6 × 250 mm, 5 μm) with an isocratic elution of mixing solvent of methanol and water (60:40). The purity of all compounds, as determined by HPLC, was better than 98.0%.

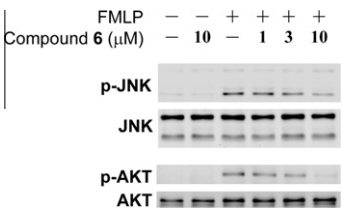

Figure 4. Western blot analysis of synthetic compound **6** on JNK and AKT phosphorylations in FMLP-induced human neutrophils.

5.2. General synthetic procedure

To a stirred suspension of L-tryptophan (0.174 g, 0.854 mmol, 1.3 equiv) and *p*-toluenesulfonic acid monohydrate (0.125 g, 0.657 mmol, 1.0 equiv), phenylglyoxal monohydrate (0.1 g, 0.657 mmol, 1.0 equiv) was added. The resulting solution was stirred at 50 °C for 2 h, and the phenylglyoxal was shown 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 EtOAc to afford **1**, **14**, and **27**. Compounds **2**–**13**, **15–26, 28–39**, and **40–44** were prepared with similar procedures.

5.2.1. 1-Benzoyl-9*H*-β-carboline-3-carboxylic acid (1)

Yellow powder (EtOAc–MeOH), mp 281–283 °C. ^{1}H NMR and ^{13}C NMR: see Ref. 25.

5.2.2. 1-(4-Methylbenzoyl)-9*H*-β-carboline-3-carboxylic acid (2)

Yellow powder (EtOAc–MeOH), mp 259–260 °C. ^{1}H NMR and ^{13}C NMR: see Ref. 25.

5.2.3. 1-(3-Methylbenzoyl)-9*H*-β-carboline-3-carboxylic acid (3)

Yellow powder (EtOAc–MeOH), mp 280–282 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.40 (3H, s, CH_3), 7.28 (1H, t, J = 7.5 Hz, H-6), 7.42 (2H, m, H-4′ and -5′), 7.56 (1H, t, J = 7.5 Hz, H-7), 7.76 (1H, d, J = 8.1 Hz, H-8), 8.20 (1H, br s, H-2′), 8.30 (1H, m, H-6′), 8.31 (1H, d, J = 7.8 Hz, H-5), 8.93 (1H, s, H-4), 11.90 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 21.4, 113.2, 119.0, 120.4, 121.1, 122.0, 128.2, 128.9, 129.2, 130.9, 131.8, 133.3, 134.9, 136.0, 137.5, 137.8, 142.2, 166.0, 199.7. FABMS m/*z* 331 $[M+H]^+$ (12), 316 (14), 287 (18), 167 (16), 154 (100), 136 (79). HRFABMS *m/z* 331.1081 $[M+H]^+$ (calcd for $C_{20}H_{15}N_2O_3$, 331.1083).

5.2.4. 1-(2-Methylbenzoyl)-9*H*-β-carboline-3-carboxylic acid (4)

Yellow powder (EtOAc–MeOH), mp 286–287 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.37 (3H, s, CH_3), 7.31 (1H, t, J = 7.5 Hz, H-6), 7.37 (2H, m, H-3′ and -5′), 7.47 (1H, t, J = 7.5 Hz, H-7), 7.65 (2H, m, H-4′ and -6′), 7.86 (1H, d, J = 8.2 Hz, H-8), 8.47 (1H, d, J = 7.8 Hz, H-5), 9.14 (1H, s, H-4), 12.45 (1H, br s, D_2O exchangeable, NH), 12.88 (1H, br s, D_2O exchangeable, CO_2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 20.4, 113.6, 120.6, 121.0, 121.2, 122.4, 125.2, 129.6, 130.7, 130.7, 130.9, 131.8, 135.8, 136.6, 136.9, 137.2, 138.2, 142.4, 166.5, 198.0. EIMS *m/z* 330 $[M]^+$ (35), 302 (91), 283 (16), 258 (100), 257 (50), 256 (95), 255 (70), 166 (15), 140 (21), 91 (62). HREIMS *m/z* 330.1002 $[M]^+$ (calcd for $C_{20}H_{14}N_2O_3$, 330.1004).

5.2.5. 1-(4-Bromobenzoyl)-9*H*-β-carboline-3-carboxylic acid (5)

Yellow powder (EtOAc–MeOH), mp 290–291 °C. ^{1}H NMR and ^{13}C NMR: see Ref. 25.

5.2.6. 1-(3-Bromobenzoyl)-9*H*-β-carboline-3-carboxylic acid (6)

Yellow powder (EtOAc–MeOH), mp 282–283 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.37 (1H, t, J = 7.4 Hz, H-6), 7.57 (1H, t,

J = 7.8 Hz, H-5′), 7.66 (1H, t, J = 7.4 Hz, H-7), 7.86 (1H, d, J = 8.5 Hz, H-8), 7.89 (1H, br d, J = 9.4 Hz, H-4′), 8.37 (1H, d, J = 7.7 Hz, H-6′), 8.48 (1H, d, J = 7.8 Hz, H-5), 8.68 (1H, br s, H-2′), 9.18 (1H, s, H-4), 12.43 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.5, 120.6, 121.0, 121.2, 121.5, 122.5, 129.6, 130.2, 130.5, 131.9, 134.1, 135.2, 135.4, 136.5, 136.9, 139.1, 142.3, 166.5, 191.0. EIMS m/z 396 $[M+2]^+$ (95), 394 $[M]^+$ (94), 349 (53), 322 (51), 315 (52), 271 (81), 241 (100). HREIMS m/z 393.9949 $[M]^+$ (calcd for $C_{19}H_{11}N_2O_3Br$, 393.9953).

5.2.7. 1-(2-Bromobenzoyl)-9*H*-β-carboline-3-carboxylic acid (7)

Yellow powder (EtOAc–MeOH), mp 281–283 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 7.39 (1H, t, J = 7.5 Hz, H-6), 7.47–7.57 (2H, m, H-5′ and -7), 7.67 (2H, m, H-3′ and -4′), 7.75 (1H, d, J = 6.5 Hz, H-6′), 7.87 (1H, d, J = 8.0 Hz, H-8), 8.48 (1H, d, J = 8.1 Hz, H-5), 9.15 (1H, s, H-4), 12.54 (1H, br s, D_2O exchangeable, NH), 12.90 (1H, br s, D_2O exchangeable, CO_2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.6, 119.6, 120.6, 121.4, 121.5, 122.6, 127.3, 129.8, 130.7, 131.8, 131.9, 132.8, 134.6, 136.5, 137.2, 140.4, 142.5, 166.3, 196.4; EIMS m/z 394 $[M]^+$ (1), 393 (2), 315 (100), 271 (16), 242 (24). HREIMS m/z 393.9951 $[M]^+$ (calcd for $C_{19}H_{11}N_2O_3Br$, 393.9953).

5.2.8. 1-(4-Methoxybenzoyl)-9*H*-β-carboline-3-carboxylic acid (8)

Yellow powder (EtOAc–MeOH), mp 264–266 °C. ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.9. 1-(3-Methoxybenzoyl)-9*H*-β-carboline-3-carboxylic acid (9)

Yellow powder (EtOAc–MeOH), mp 282–284 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 3.87 (3H, s, OCH_3), 7.26 (1H, dd, J = 8.1, 2.6 Hz, H-4′), 7.37 (1H, t, J = 7.6 Hz, H-6), 7.51 (1H, t, J = 8.0 Hz, H-5′), 7.65 (1H, t, J = 7.4 Hz, H-7), 7.85 (1H, d, J = 8.1 Hz, H-8), 7.94 (1H, d, J = 7.6 Hz, H-6′), 8.27 (1H, br s, H-2′), 8.48 (1H, d, J = 8.4 Hz, H-5), 9.17 (1H, s, H-4), 12.41 (1H, br s, D_2O exchangeable, NH), 13.03 (1H, br s, D_2O exchangeable, CO_2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 55.4, 113.5, 116.7, 119.3, 120.7, 120.7, 121.2, 122.4, 123.5, 129.4, 129.5, 131.7, 135.8, 136.3, 136.9, 138.2, 142.2, 158.9, 166.6, 191.9. FABMS m/z 347 $[M+H]^+$ (7), 313 (7), 191 (10), 154 (26). HRFABMS m/z 347.1031 $[M+H]^+$ (calcd for $C_{20}H_{15}N_2O_4$, 347.1032).

5.2.10. 1-(4-Nitrobenzoyl)-9*H*-β-carboline-3-carboxylic acid (11)

Yellow powder (EtOAc–MeOH), mp 279–280 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 7.38 (1H, t, J = 7.5 Hz, H-6), 7.66 (1H, t, J = 7.5 Hz, H-7), 7.87 (1H, d, J = 8.3 Hz, H-8), 8.39 (2H, d, J = 8.7 Hz, H-2′ and -6′), 8.49 (1H, d, J = 8.2 Hz, H-5), 8.53 (2H, d, J = 8.7 Hz, H-3′ and -5′), 9.20 (1H, s, H-4), 12.49 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.7, 120.7, 121.4, 121.5, 122.6, 123.2, 129.8, 132.1, 132.7, 134.9, 136.8, 137.0, 142.5, 142.7, 149.7, 166.6, 192.0. EIMS m/z 361 $[M]^+$ (33), 331 (14), 316 (22), 287 (16), 270 (22), 214 (19), 194 (19), 184 (14), 168 (18), 167 (18), 140 (18), 120 (20), 105 (100), 77 (62). HREIMS m/z 361.0699 $[M]^+$ (calcd for $C_{19}H_{11}N_3O_5$, 361.0699).

5.2.11. 1-(2-Nitrobenzoyl)-9*H*-β-carboline-3-carboxylic acid (13)

Yellow powder (EtOAc–MeOH), mp 280–281 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 7.39 (1H, t, J = 7.4 Hz, H-6), 7.68 (1H, t, J = 7.7 Hz, H-7), 7.84 (1H, d, J = 7.5 Hz, H-6′), 7.87 (1H, d, J = 6.7 Hz, H-8), 7.88 (1H, t, J = 8.0 Hz, H-5′), 7.98 (1H, t, J = 7.4 Hz, H-4′), 8.25 (1H, d, J = 8.3 Hz, H-3′), 8.47 (1H, d, J = 7.8 Hz, H-5), 9.11 (1H, s, H-4), 12.55 (1H, br s, D_2O exchangeable, NH), 12.81 (1H, br s, D_2O exchangeable, CO_2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.7, 120.5, 121.4, 121.5, 122.6, 123.8, 129.9, 130.2, 131.8, 132.0, 134.2, 134.8, 134.8, 135.7, 137.0, 142.6, 148.3, 166.1, 194.2. FABMS m/z 362 $[M+H]^+$ (2), 307 (20), 289 (11). HRFABMS m/z 361.0699 $[M]^+$ (calcd for $C_{19}H_{11}N_3O_5$, 361.0699).

5.2.12. (9*H*-β-Carbolin-1-yl)-phenyl-methanone (14)

Yellow needles (EtOAc), mp 135–138 °C. ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.13. (9*H*-β-Carbolin-1-yl)-*p*-tolyl-methanone (15)

Yellow needles (EtOAc), mp 158–161 °C. ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.14. (9*H*-β-Carbolin-1-yl)-*m*-tolyl-methanone (16)

Yellow needles (EtOAc), mp 142–144 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.41 (3H, s, CH_3), 7.31 (1H, t, J = 7.5 Hz, H-6), 7.44 (2H, m, H-4′ and -5′), 7.60 (1H, t, J = 7.5 Hz, H-7), 7.81 (1H, d, J = 8.2 Hz, H-8), 7.96 (2H, m, H-2′ and -6′), 8.32 (1H, d, J = 7.8 Hz, H-5), 8.44 (1H, d, J = 4.9 Hz, H-4), 8.52 (1H, d, J = 4.9 Hz, H-3), 12.04 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 21.2, 113.1, 119.0, 120.2, 120.4, 122.0, 128.0, 128.3, 129.1, 131.1, 131.1, 133.0, 135.9, 136.6, 137.3, 137.3, 137.8, 141.8, 194.3. EIMS m/z 286 $[M]^+$ (100), 285 (98), 271 (27), 258 (68), 257 (86), 243 (12), 167 (15), 140 (32). HREIMS m/z 286.1108 $[M]^+$ (calcd for $C_{19}H_{14}N_2O$, 286.1106).

5.2.15. (9*H*-β-Carbolin-1-yl)-*o*-tolyl-methanone (17)

Yellow needles (EtOAc), mp 150–151 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.27 (3H, s, CH_3), 7.28–7.35 (3H, m, H-6, -3′ and -5′), 7.43 (1H, t, J = 7.4 Hz, H-4′), 7.49 (1H, d, J = 7.5 Hz, H-6′), 7.62 (1H, d, J = 7.5 Hz, H-7), 7.83 (1H, d, J = 8.2 Hz, H-8), 8.32 (1H, d, J = 7.8 Hz, H-5), 8.43 (2H, s, H-3 and -4), 12.15 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 19.9, 113.3, 119.4, 120.2, 120.5, 122.1, 125.1, 129.2, 129.3, 130.0, 130.5, 131.3, 135.5, 136.0, 136.4, 137.8, 139.4, 142.0, 199.1. EIMS m/z 286 $[M]^+$ (79), 285 (100), 271 (29), 258 (60), 257 (61), 243 (14), 167 (9), 140 (18), 119 (29), 91 (48). HREIMS m/z 286.1109 $[M]^+$ (calcd for $C_{19}H_{14}N_2O$, 286.1106).

5.2.16. (4-Bromophenyl)-(9*H*-β-carbolin-1-yl)-methanone (18)

Yellow needles (EtOAc), mp 194–196 °C. ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.17. (3-Bromophenyl)-(9*H*-β-carbolin-1-yl)-methanone (19)

Yellow needles (EtOAc), mp 148–150 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 7.32 (1H, t, J = 7.5 Hz, H-6), 7.55 (1H, t, J = 7.8 Hz, H-5′), 7.61 (1H, t, J = 7.6 Hz, H-7), 7.82 (1H, d, J = 8.3 Hz, H-4′), 7.86 (1H, d, J = 8.2 Hz, H-8), 8.16 (1H, d, J = 7.9 Hz, H-6′), 8.33 (1H, d, J = 7.2 Hz, H-5), 8.34 (1H, d, J = 1.4 Hz, H-2′), 8.48 (1H, d, J = 4.9 Hz, H-4), 8.55 (1H, d, J = 4.9 Hz, H-3), 12.10 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.2, 119.5, 120.2, 120.5, 121.2, 122.1, 129.2, 129.8, 130.4, 131.4, 133.3, 134.9, 135.9, 136.1, 137.5, 139.8, 141.9, 192.5. EIMS m/z 352 $[M+2]^+$ (87), 351 $[M+1]^+$ (94), 350 $[M]^+$ (88), 349 (79), 324 (56), 323 (53), 322 (57), 321 (43), 271 (56), 243 (100), 242 (53). HREIMS m/z 350.0058 $[M]^+$ (calcd for $C_{18}H_{11}N_2OBr$, 350.0055).

5.2.18. (2-Bromophenyl)-(9*H*-β-carbolin-1-yl)-methanone (20)

Yellow plate crystals (EtOAc), mp 209–211 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 7.31 (1H, t, J = 7.5 Hz, H-6), 7.41–7.55 (3H, m, H-3′, -4′, and -5′), 7.61 (1H, t, J = 7.4 Hz, H-7), 7.69 (1H, d, J = 7.8 Hz, H-6′), 7.82 (1H, d, J = 8.2 Hz, H-8), 8.29 (1H, d, J = 7.8 Hz, H-5), 8.40 (2H, br s, H-3 and -4), 12.17 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.3, 119.1, 119.9, 120.1, 120.8, 122.1, 127.5, 129.5, 129.6, 131.4, 131.5, 132.5, 135.3, 135.5, 138.1, 141.5, 142.1, 197.1. EIMS m/z 352 $[M+2]^+$ (13), 351 $[M+1]^+$ (7), 350 $[M]^+$ (13), 324 (7), 323 (7), 322

(8), 321 (6), 271 (100), 243 (63), 242 (48), 136 (49). HREIMS *m/z* 350.0056 $[M]^+$ (calcd for $C_{18}H_{11}N_2OBr$, 350.0055).

5.2.19. (9*H*-β-Carbolin-1-yl)-(4-methoxyphenyl)-methanone (21)

Yellow needles (EtOAc), mp 185–187 °C. ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.20. (9*H*-β-Carbolin-1-yl)-(3-methoxyphenyl)-methanone (22)

Yellow plate crystals (EtOAc), mp 192–194 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 3.93 (3H, s, OCH_3), 7.20 (1H, dd, J = 7.2, 1.9 Hz, H-2′), 7.29 (1H, t, J = 7.5 Hz, H-6), 7.45 (1H, t, J = 8.0 Hz, H-5′), 7.58 (1H, t, J = 7.5 Hz, H-7), 7.68 (1H, d, J = 1.9 Hz, H-2′), 7.73 (1H, d, J = 7.5 Hz, H-6′), 7.79 (1H, d, J = 8.2 Hz, H-8), 8.27 (1H, d, J = 7.8 Hz, H-5), 8.39 (1H, d, J = 4.8 Hz, H-4), 8.49 (1H, d, J = 4.8 Hz, H-3), 12.01 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 55.7, 113.3, 116.3, 118.2, 119.3, 120.5, 120.7, 122.2, 123.7, 129.5, 129.6, 131.5, 136.2, 136.7, 137.6, 139.1, 142.0, 159.1, 194.0. EIMS *m/z* 302 $[M]^+$ (99), 301 (100), 287 (31), 274 (48), 273 (82), 271 (25), 259 (21), 258 (21), 244 (29), 243 (26), 230 (16). HREIMS *m/z* 302.1052 $[M]^+$ (calcd for $C_{19}H_{14}N_2O_2$, 302.1055).

5.2.21. (9*H*-β-Carbolin-1-yl)-(4-nitrophenyl)-methanone (24)

Yellow needles (EtOAc), mp 216–217 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 7.34 (1H, t, J = 7.6 Hz, H-6), 7.63 (1H, t, J = 7.5 Hz, H-7), 7.84 (1H, d, J = 8.2 Hz, H-8), 8.33 (2H, d, J = 8.7 Hz, H-2′ and -6′), 8.34 (1H, m, H-5), 8.40 (2H, d, J = 8.7 Hz, H-3′ and -5′), 8.50 (1H, d, J = 4.9 Hz, H-4), 8.54 (1H, d, J = 4.9 Hz, H-3), 12.18 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.3, 119.8, 120.2, 120.7, 122.1, 123.1, 129.4, 131.6, 132.0, 135.5, 136.1, 137.7, 142.0, 143.4, 149.3, 193.2. EIMS *m/z* 317 $[M]^+$ (100), 289 (46), 270 (39), 243 (62), 167 (17), 140 (23). HREIMS *m/z* 317.0800 $[M]^+$ (calcd for $C_{18}H_{11}N_3O_3$, 317.0800).

5.2.22. (9*H*-β-Carbolin-1-yl)-(3-nitrophenyl)-methanone (25)

Yellow plate crystals (EtOAc), mp 215–217 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 7.34 (1H, t, J = 7.4 Hz, H-6), 7.63 (1H, t, J = 7.6 Hz, H-7), 7.84 (1H, d, J = 7.7 Hz, H-8), 7.88 (1H, t, J = 8.0 Hz, H-5′), 8.35 (1H, d, J = 7.8 Hz, H-5), 8.50 (1H, dd, J = 9.0, 1.3 Hz, H-6′), 8.51 (1H, d, J = 4.5 Hz, H-4), 8.56 (1H, d, J = 4.5 Hz, H-3), 8.61 (1H, d, J = 7.6 Hz, H-4′), 9.01 (1H, br s, H-2′), 12.17 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.4, 119.8, 120.3, 120.7, 122.2, 125.7, 126.7, 129.4, 130.0, 131.7, 135.6, 136.3, 137.2, 137.7, 139.0, 142.1, 147.6, 191.8. FABMS *m/z* 341 $[M+Na]^+$ (34), 318 $[M+H]^+$ (66). HRFABMS *m/z* 318.0878 $[M+H]^+$ (calcd for $C_{18}H_{12}N_3O_3$, 318.0879).

5.2.23. (9*H*-β-Carbolin-1-yl)-phenyl-methanol (27)

Yellow powder (EtOAc), mp 141–144 °C. ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.24. (9*H*-β-Carbolin-1-yl)-*p*-tolyl-methanol (28)

Yellow powder (EtOAc), mp 184–185 °C. ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.25. (4-Bromophenyl)-(9*H*-β-carbolin-1-yl)-methanol (31)

Yellow powder (EtOAc), mp 150–151 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 6.14 (1H, d, J = 4.1 Hz, H-14), 6.62 (1H, d, J = 4.1 Hz, OH-14), 7.20 (1H, dd, J = 7.6, 7.5 Hz, H-6), 7.47 (2H, d, J = 8.5 Hz, H-2′ and -6′), 7.51 (1H, dd, J = 8.0, 7.5 Hz, H-7), 7.55 (2H, d, J = 8.5 Hz, H-3′ and -5′), 7.71 (1H, d, J = 8.0 Hz, H-8), 7.99 (1H, d, J = 5.3 Hz, H-4), 8.17 (1H, d, J = 7.6 Hz, H-5), 8.23 (1H, d, J = 5.3 Hz, H-3), 11.27 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 75.2, 112.7, 114.0, 119.4, 120.3, 120.6, 121.6, 128.2, 128.7, 129.0, 131.1, 132.4, 137.1, 140.9, 143.3, 147.1. EIMS *m/z* 354 $[M+2]^+$ (34), 352 $[M]^+$ (35), 335 (24), 333 (22), 255 (100), 197 (15), 168 (30). 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, 3.17; N, 7.93. Found: C, 61.12; H, 3.79; N, 7.75.

5.2.26. (9*H*-β-Carbolin-1-yl)-(4-methoxyphenyl)-methanol (34)

Yellow powder (EtOAc), mp 145–147 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 3.66 (3H, s, OCH_3), 6.08 (1H, d, J = 3.9 Hz, H-14), 6.41 (1H, d, J = 3.9 Hz, OH-14), 6.82 (2H, d, J = 8.6 Hz, H-3′ and -5′), 7.19 (1H, dd, J = 7.6, 7.4 Hz, H-6), 7.48 (2H, d, J = 8.5 Hz, H-2′ and -6′), 7.49 (1H, dd, J = 7.8, 7.4 Hz, H-7), 7.71 (1H, d, J = 7.8 Hz, H-8), 7.96 (1H, d, J = 5.2 Hz, H-4), 8.17 (1H, d, J = 7.6 Hz, H-5), 8.21 (1H, d, J = 5.2 Hz, H-3), 11.22 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 55.2, 75.6, 112.7, 113.6, 113.7, 119.3, 120.6, 121.5, 127.7, 128.1, 128.8, 132.3, 136.0, 137.0, 140.8, 148.0, 158.5. EIMS *m/z* 304 $[M]^+$ (100), 285 (98), 271 (76), 255 (72), 242 (30), 197 (12), 168 (46), 140 (30), 121 (60). HREIMS *m/z* 304.1215 $[M]^+$ (calcd for $C_{19}H_{16}N_2O_2$, 304.1212).

5.2.27. 1-Acetyl-9*H*-β-carboline-3-carboxylic acid (40)

Yellow powder (EtOAc–MeOH), mp 292 °C (dec). ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.28. 1-(9*H*-β-Carbolin-1-yl)-ethanone (41)

Yellow needles (EtOAc), mp 207–209 °C. ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.29. 1-(9*H*-β-Carbolin-1-yl)-ethanol (42)

Yellow powder (EtOAc), mp 168–170 °C. ^1H NMR and ^{13}C NMR: see Ref. 25.

5.2.30. 1-Acetyl-9*H*-β-carboline-3-carboxylic acid amide (43)

Yellow powder (EtOAc–MeOH), mp 275–277 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.89 (3H, s, CH_3), 7.33 (1H, dd, J = 7.8, 7.5 Hz, H-6), 7.61 (1H, dd, J = 8.1, 7.5 Hz, H-7), 7.64 (1H, br s, D_2O exchangeable, NH), 7.83 (1H, d, J = 8.2 Hz, H-8), 8.20 (1H, br s, D_2O exchangeable, NH), 8.42 (1H, d, J = 7.8 Hz, H-5), 9.10 (1H, s, H-4), 12.14 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 26.2, 113.5, 118.2, 120.5, 121.0, 122.4, 129.4, 132.1, 134.1, 135.0, 139.1, 142.5, 166.5, 201.4. EIMS *m/z* 253 $[M]^+$ (100), 236 (18), 208 (26), 194 (14), 182 (8). HREIMS *m/z* 253.0849 $[M]^+$ (calcd for $C_{14}H_{11}N_3O_2$, 253.0851).

5.2.31. 1-(4-Methoxybenzoyl)-9*H*-β-carboline-3-carboxylic acid methyl ester (44)

Yellow powder (EtOAc–MeOH), mp 181–183 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 3.89 (3H, s, OCH_3-4′), 3.95 (3H, s, OCH_3-15), 7.13 (2H, d, J = 8.9 Hz, H-3′ and -5′), 7.36 (1H, dd, J = 7.6, 7.5 Hz, H-6), 7.64 (1H, dd, J = 7.9, 7.6 Hz, H-7), 7.82 (1H, d, J = 7.9 Hz, H-8), 8.48 (1H, d, J = 7.5 Hz, H-5), 8.51 (2H, d, J = 8.9 Hz, H-2′ and -6′), 9.15 (1H, s, H-4), 12.37 (1H, br s, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 52.5, 55.8, 113.5, 113.8, 120.5, 120.7, 121.1, 122.4, 129.4, 129.5, 131.4, 134.0, 135.1, 136.8, 142.2, 163.4, 165.6, 190.5. EIMS *m/z* 360 $[M]^+$ (100), 359 (71), 345 (15), 331 (32), 299 (28), 274 (22), 229 (16), 135 (51). HREIMS *m/z* 360.1110 $[M]^+$ (calcd for $C_{21}H_{16}N_2O_4$, 360.1110). Anal. Calcd for $C_{21}H_{16}N_2O_4$: C, 69.99; H, 4.48; N, 7.77. Found: C, 69.82; H, 4.48; N, 7.74.

5.3. Chemicals and antibodies

Prior to use, the synthetic compounds were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions. LPS (*Escherichia coli* serotype 0111:B4), FMLP, cytochalasin B, ferricytochrome *c*, penicillin, streptomycin, sulfanilamide, naphthylenediamine, EDTA, Triton X-100, phenylmethylsulfonyl fluoride, Tris–HCl, leupeptin, aprotinin, and NP-40 were obtained from Sigma (St.

Louis, MO, USA). IFN-γ was obtained from Genzyme (Cambridge, MA). Dulbecco's Modified Eagle's Medium (DMEM) and Hank's balanced salt solution (HBSS) were purchased from Gibco (Grand Island, NY).

5.4. Cell growth inhibition assay

All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates with compounds added from DMSO diluted stock. The plates were incubated for an additional 72 h after attachment and drug addition, and the assay was terminated by 10% trichloroacetic acid. Then, 0.4% sulforhodamine B dye in 1% HOAc was added to stain the cells for 10 min. Unbound dye was removed by repeated washing with 1% HOAc, and the plates were air-dried. Bound stain was subsequently solved with 10 mM Trizma base and the absorbance read at 515 nm. Growth inhibition of 50% (GI_{50}) was calculated as the drug concentration that caused a 50% reduction in the net protein increase in control cells during the drug incubation. The mean GI_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent determinations. Variation between replicates was no more than 5% of the mean. The following human tumor cell lines were used in the assay: A549 (non-small cell lung cancer), DU145 (prostate cancer), KB (nasopharyngeal carcinoma), and KB-VIN (vincristine-resistant KB subline). All cell lines were obtained from the Lineberger Cancer Center (UNC-CH) or from ATCC (Rockville, MD). Cells propagated in RPMI-1640 supplemented with 10% FBS, penicillin (100 IU/mL), streptomycin (1 μg/mL), and amphotericin B (0.25 μg/mL), and were cultured at 37 °C in a humidified atmosphere of 95% air/5% CO_2.

5.5. Cell culture

RAW 264.7 macrophages (ATCC TIB-71) were cultured in DMEM containing 10% heat-inactivated fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin and grown at 37 °C with 5% CO_2 in fully humidified air. Cells used for experiments were between passages 9 and 20.

5.6. Measurement of PGE_2

Assessment of PGE_2 synthesis was performed using a commercially available PGE_2 competitive enzyme immunoassay kit (Cayman, MI). Supernatant from the culture medium and the standards were put into different wells on the goat anti-mouse IgG-coated microtiter plate provided in the kit. Mouse anti-PGE_2 antibody and peroxidase-conjugated PGE_2 were added to each well, and the plate was incubated at room temperature with shaking for 1 h. The wells were drained and washed, and 3,3′,5,5′-tetramethylbenzidine/hydrogen peroxide solution was then added. The plate was incubated at room temperature with shaking, and the reaction was stopped after 30 min through the addition of H_2SO_4. The absorbance of the content of each well was then measured at a wavelength of 450 nm.

5.7. Nitrite assay

LPS (1 μg/mL) plus IFN-γ (50 U/mL) was added to fresh culture medium for 18 h to induce nitrite formation, an indicator of NO synthesis. Nitrite concentration in the absence or presence of the synthetic compounds was measured by adding Griess reagent (1% sulfanilamide and 0.1% naphthylenediamine in 5% phosphoric acid). The optical density at 550 nm (OD_{550}) was measured with a microplate reader.

5.8. Cell viability

Cell respiration, an indicator of cell viability, was determined by the mitochondrial-dependent reduction of 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) to formazan. The extent of the reduction of MTT to formazan within cells was quantified by measuring the optical density at 550 nm.

5.9. *i*NOS and COX2 expression

After incubation, cells were lysed in ice-cold RIPA buffer (25 mM Tris–HCl, pH 7.2; 0.1% SDS; 0.1% Triton X-100; 1% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM sodium orthovanadate; 1 mM phenylmethanesulfonyl fluoride; 10 μg/mL aprotinin, and 5 μg/mL leupeptin). The protein concentration was determined using Bio-Rad protein assay kit and separated by 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). After transferring onto nitrocellulose membrane, blot was first incubated with antibodies for *i*NOS and COX2 (Chemicon, Temecula, CA), respectively, and then incubated with horseradish peroxidase (HRP)-conjugated anti-IgG. Immunoreactive bands were visualized by an enhanced chemiluminescence system (Amersham Biosciences Buckinghamshire, UK). Thereafter, the same membrane was stripped and re-probed with mouse anti-α-tubulin antibody.

5.10. Preparation of human neutrophils

Blood was taken from healthy human donors (20–30 years old) by venipuncture, using a protocol approved by the institutional review board at Chang Gung Memorial Hospital. Neutrophils were isolated with a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes. Purified neutrophils that contained >98% viable cells, as determined by the trypan blue exclusion method, were re-suspended in a calcium (Ca^{2+})-free HBSS buffer at pH 7.4, and were maintained at 4 °C before use.

5.11. Measurement of $O_2^{\cdot-}$ generation

The $O_2^{\cdot-}$ generation assay was based on the SOD-inhibitable reduction of ferricytochrome *c*.[33] In brief, after supplementation with 0.5 mg/mL ferricytochrome c and 1 mM Ca^{2+}, neutrophils (6×10^5 cells/mL) were equilibrated at 37 °C for 2 min and incubated with drugs or an equal volume of vehicle (0.1% DMSO) for 5 min. Cells were activated with 100 nM FMLP during the preincubation of 1 μg/mL cytochalasin B (FMLP/cytochalasin B) for 3 min. Changes in the absorbance with a reduction in ferricytochrome c at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan).

5.12. Measurement of elastase release

Degranulation of azurophilic granules was determined by elastase release as described previously.[30] Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-pnitroanilide as the elastase substrate. Briefly, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-p-nitroanilide (100 μM), neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with drugs or an equal volume of vehicle (0.1% DMSO) for 5 min. Cells were activated by 100 nM FMLP and 0.5 μg/mL cytochalasin B, and changes in absorbance at 405 nm were continuously monitored to assay elastase release. The results were expressed as the percent of elastase release in the FMLP/cytochalasin B-activated, drug-free control system.

5.13. JNK and AKT phosphorylation

Neutrophils were incubated with drugs for 5 min at 37 °C before being stimulated with FMLP (0.1 μM) for 0.5 min. The reaction was stopped by placing the mixture on ice, and cells were centrifuged at 4 °C. After removing the supernatants, the pellets were lysed in 150 μL buffer [50 mM HEPES (pH 7.4), 100 mM NaCl, 1 mM EDTA, 2 mM Na_3VO_4, 10 mM *p*-nitrophenyl phosphate, 5% β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1% dilution of Sigma protease inhibitor cocktails, and 1% Triton X-100]. Samples were centrifuged at 14,000 *g* for 20 min at 4 °C to yield whole-cell lysates. Proteins derived from whole-cell lysates were separated by SDS–PAGE using 12% polyacrylamide gels and blotted onto nitrocellulose membranes. Immunoblotting was performed using the indicated antibodies and HRP-conjugated secondary anti-rabbit antibodies (Cell Signaling Technology, Beverly, MA, USA). The immunoreactive bands were visualized by an ECL system (Amersham Biosciences).

5.14. Statistical analysis

Results were expressed as mean ± SE. Nitrite production is indicated as absolute concentrations in μM. Computation of 50% inhibitory concentration (IC_{50}) was computer-assisted (PHARM/PCS v.4.2). Statistical comparisons were made between groups using Student's *t* test. Values of *P* less than 0.05 were considered to be statistically significant.

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