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Novel tetrahydro-β-carboline-1-carboxylic acids as inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2)

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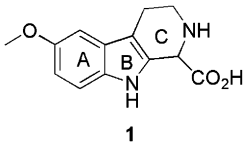
Abstract—A structure–activity relationship study was conducted on a series of tetrahydro-β-carboline-1-carboxylic acid analogs in order to identify the key functionality responsible for activity against the mitogen-activated protein kinase-activated protein kinase 2 enzyme (MK-2). The compounds were further evaluated for their ability to inhibit TNFα production in U937 cells and in vivo. These compounds represent a novel structural class of compounds capable of inhibiting MK-2 with remarkable selectivity.

The proinflammatory cytokine TNF-α was originally described as a product of monocytes that induced tumor lysis[1] and since then has been implicated in several inflammatory diseases in humans.[2] The development of anti-TNFα biological therapies has been described and been shown to be effective in diseases such as rheumatoid arthritis and psoriasis.[3] More recently, many other biological targets have been identified for the inhibition of TNF-α production. The most notable of the targets is the kinase p38 MAPK.[4,5] Several publications have demonstrated the effect of p38 inhibitors in in vitro and in vivo models, as well as demonstrating efficacy in rheumatoid arthritis (RA) patients in a clinical study.[6]

The mitogen-activated protein kinase-activated protein kinase 2 (MK-2) has only recently received a significant degree of interest for its potential as a target for treatment of RA.[7] MK-2 is a direct substrate of the p38α/β kinases and has been linked to TNF-α, IL-6, and IFN-γ expression levels.[8] A genetic knockout of the MK-2 enzyme in mice has been described[8a] and shown to be resistant to disease in models of arthritis.[7b] For example, treatment of MK-2 knockout mice with LPS resulted in only a small increase of serum TNFα (10–20%) of the wild type control.[8a] Additionally, the MK-2 knockout mice were observed to be resistant to collagen induced arthritis (CIA),[7b] a model well established for the study of (RA). Furthermore, while MK-2 knockout mice are healthy and have a normal phenotype, genetic knockout of the p38 gene is embryonic lethal, suggesting an improved safety profile for MK-2 inhibition relative to p38. These observations taken together lend strong support for MK-2 as an attractive target for treatment of TNF-α mediated diseases and rheumatoid arthritis (RA).

Efforts from our laboratories have recently been reported for a series of aminocyanopyridines and a series of pyrrolopyridines as inhibitors of MK-2.[9] Through our compound screening effort, we also identified commercially available β-carboline derivative **1** as a modest inhibitor of MK-2 (Fig. 1).[10] The compound exhibited



1

MK-2 IC_{50} = 2.5 μM

Kinase Selectivity (IC_{50})
MK-3, MSK1, JNK2, IKK2 > 200 μM
p38α, ERK2 > 100 μM
PRAK = 19 μM

Figure 1. Structure of MK-2 inhibitor and in vitro biological activity.

Keywords: MAPKAP kinase 2; MK-2; TNF-α; Inflammation; β-Carboline-1; Rheumatoid arthritis.
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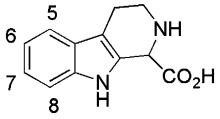
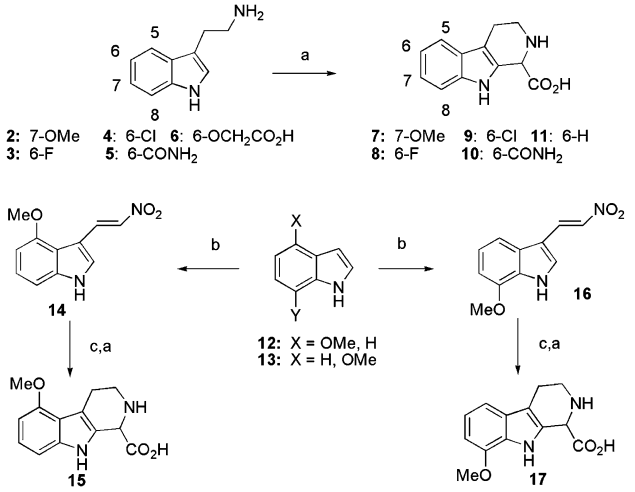
an MK-2 IC_{50} value[11] of 2.5 μM and was identified to be an ATP competitive inhibitor. This structure was of particular interest to us given its low molecular weight and unusually good selectivity versus a number of related kinases[12] (see Fig. 1). In order to elucidate the essential features required for the activity observed and to further improve the potency of compound **1**, an investigation of the structure–activity relationship between **1** and the MK-2 enzyme was initiated. It is important to note that no X-ray crystal structure of the MK-2 enzyme existed at the time of this work.[13] In this report, we describe the synthesis and key structure–activity relationships identified during the investigation of this novel series of tetrahydro-β-caroline-1-carboxylic acids as selective inhibitors of MK-2. It is also important to note that SAR for this class of β-carboline carboxylic acids was conducted on the racemates as the α-carbon was observed to be readily epimerizable.

Starting with the A-ring, an investigation of the role of the methoxy group was conducted (see Table 1). The syntheses of these analogs are presented in Schemes 1 and 2.[14] The position of the methoxy group was found to be critical as the 6-position was optimal by more than ten-fold (**1**, **7**, **15**, **17**). Addition of a second group adjacent to the 6-methoxy group was not tolerated (**32**, **35**), presumably due to twisting of the methoxy group out of the plane. Substitution of the 6-methoxy for other functionalities was subsequently investigated (**8**–**10**, **20**, **23**, **29**). Hydroxy analog **20** and methyl ester **29** proved to be well tolerated, while other variations, including deletion, resulted in a 10- to 100-fold loss in potency against MK-2 (**8**–**11**, **23**, **29**). Extension of the methoxy group to benzyloxy was tolerated to some extent (**19**), but acetophenone derivative **26** was essentially equipotent to methoxy, demonstrating the tolerability for larger groups extending from the A-ring.

Table 1. IC_{50} values for β-carboline derivatives



Compound	Substitution	MK-2 IC_{50} (μM)
1	6-OMe	2.5
15	5-OMe	42
7	7-OMe	>200
17	8-OMe	>200
32	6-OMe, 7-OH	53
35	6-OMe, 5-Me	>200
20	6-OH	4.5
29	6-CO_2Me	6.6
23	6-OAc	170
11	6-H	87
8	6-F	26
9	6-Cl	19
10	6-$CONH_2$	28
19	6-OCH_2Ph	12
26	6-$OCH_2C(O)$Ph	1.1

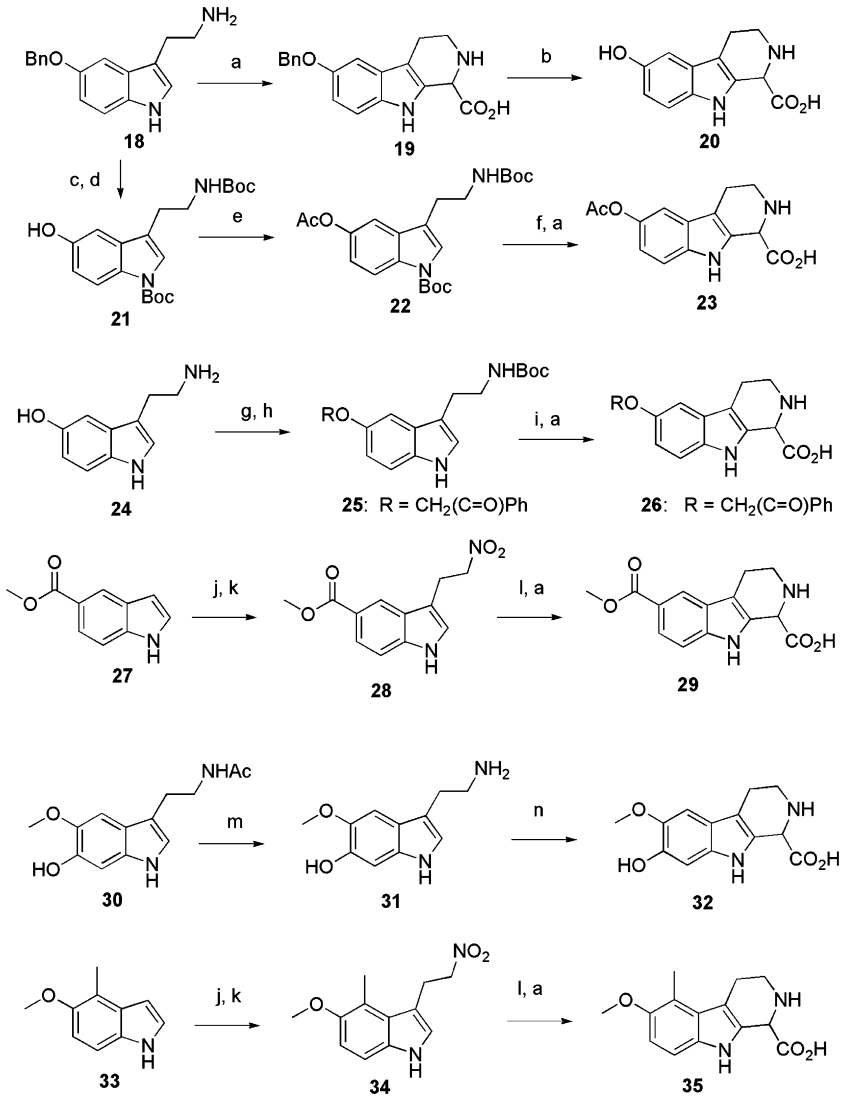


Scheme 1. Synthesis of compounds **2**–**11**, and **15**, **17**. Reagents and conditions: (a) Glyoxylic acid, pH 3–4, H_2O/KOH, rt, 12 h; (b) $Me_2NCH{=}CHNO_2$, TFA, rt; (c) LAH, THF, rt then reflux.

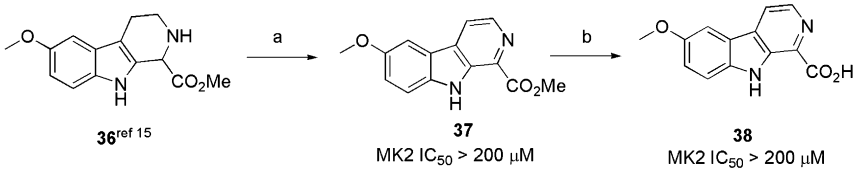
Having established the importance of the 6-methoxy group in the A-ring for potency, we turned our attention to the C-ring. Oxidation of the tetrahydropyridine to pyridine was investigated but proved to be incompatible with MK-2 activity (Scheme 2; **37** and **38**). The lack of potency of compound **38** in particular suggests a requirement of the acid functionality to exist out of plane with respect to the ring system. The lack of potency may also be a result of a reduction in basicity of the nitrogen in the C-ring (secondary amine to pyridine) (Scheme 3).

Examination of the role of the indole and amino acid functionalities was also conducted (Table 2). The synthesis of compounds **36**–**63** is shown in Schemes 4 and 5. The presence of the nitrogen was found to be critical for potency as demonstrated by carbon analog **65** and oxygen analog **61**. While the nitrogen itself was required for activity, the hydrogen was not, as demonstrated by N-alkylation with small alkyl groups such as methyl or ethyl (**40** and **49**). Larger groups such as isopropyl and benzyl, however, resulted in significant losses in potency (**50** and **51**), suggesting a sensitive steric environment.

Modification of the carboxylic acid moiety to other functional groups was also investigated. Conversion of the carboxylic acid to a methyl ester (**36**), primary amide (**43**), primary alcohol (**42**) or amine (**56**) all resulted in a dramatic loss in potency. Functional groups capable of mimicking the acid, such as a hydroxamic acid **44** or tetrazole **58**, did not prove to be suitable replacements for the acid. Addition of a methyl group in the α-position (**54**) or incorporation of a methylene spacer between the C-ring and the carboxylic acid (**55**) was not tolerated. The intolerance for any changes to the carboxylic acid suggests a key ionic interaction between the inhibitor and MK-2 is critical for binding affinity in this series. Methylation of the indole NH also proved to be detrimental to activity (**52**), suggesting a potential interaction



Scheme 2. Synthesis of compounds **20**, **23**, **26**, **29**, **32**, and **35**. Reagents and conditions: (a) Glyoxylic acid, pH 3–4, H_2O/KOH, rt, 12 h; rt; (b) 40 psi H_2, 10% Pd/C, MeOH, rt; (c) K_2CO_3 then Boc_2O, THF, DMAP, rt, 2 h; (d) 40 psi H_2, 10% Pd/C, MeOH, rt; (e) Ac_2O, pyridine, CH_2Cl_2, 0 °C to rt, 12 h; (f) 2.0 N HCl in Et_2O, rt, 3 h; (g) Boc_2O, K_2CO_3, H_2O, rt, 14 h; (h) $PhC(O)CH_2Br$, K_2CO_3, MEK, 80 °C, 14 h; (i) 2.0 N HCl in Et_2O, CH_2Cl_2, rt, 14 h; (j) $Me_2NCH{=}CHNO_2$, TFA, rt; (k) $NaBH_4$, THF:MeOH, rt; (l) 10% Pd/C, NH_4CO_2H, MeOH, reflux, 14, h; (m) 85% $H_2NNH_2^*H_2O$, 75 °C, 14 h; (n) glyoxylic acid, H_2O, pH 4, 80 °C, 30 min.

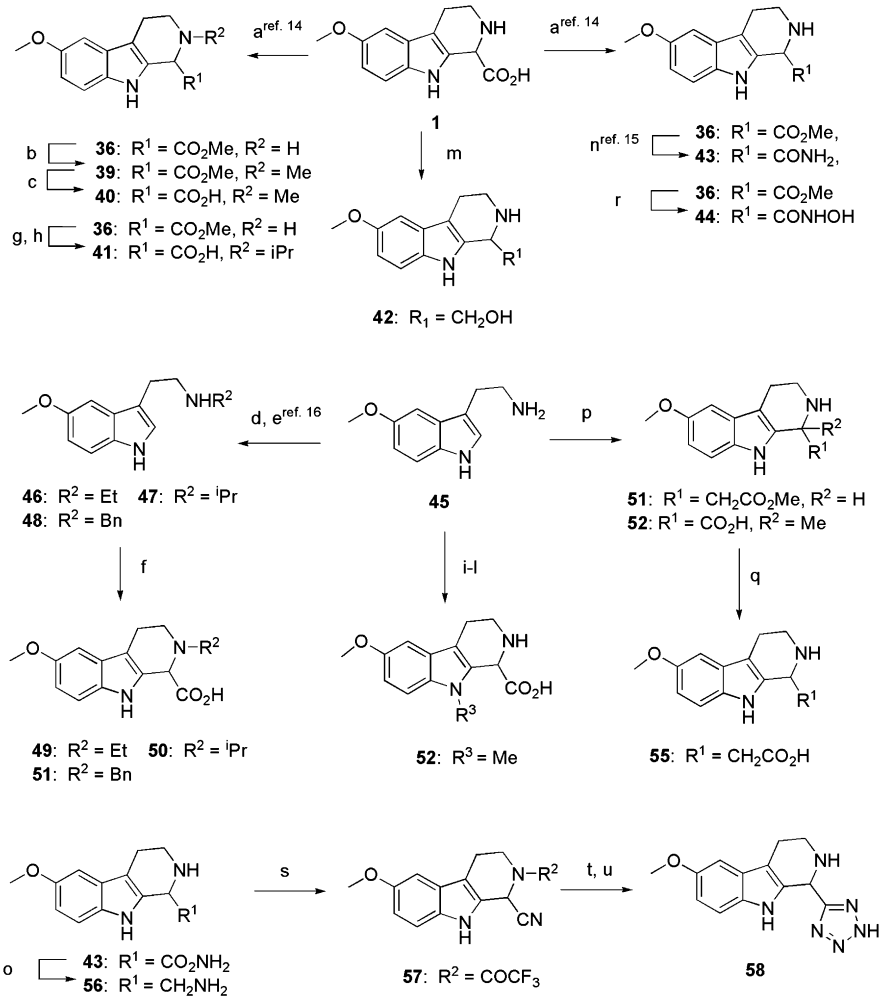


Scheme 3. Synthesis of compounds **37** and **38**. Reagents and conditions: (a) S, *m*-xylene, reflux, 4 h; (b) LiOH*H_2O, THF:H_2O, rt, 4 h.

of the indole N–H with the enzyme, a limited steric environment, or distortion of the orientation of the carboxylic acid.

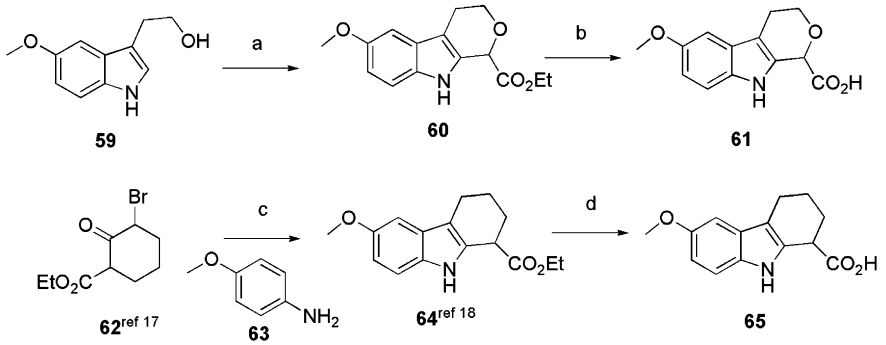
In order to further probe the tolerance of MK-2 to substitution of the β-carboline C-ring, analogs were prepared to probe the 3- and 4-positions (Table 3 and Scheme 6). The enzyme exhibited tolerance of only small substituents such as methyl and ethyl in the 4-position (**70**–**72**), but less tolerant of alkyl substituents, even ethyl, in the 3-position (**88**, **89**). Further exploration of the 3-position demonstrated tolerance for only a select group of small functional groups capable of H-bonding (**76**, **82**, **83**) (Table 4).

Having established the tolerance of 2- and 4-positions in the C-ring for alkylation with small groups such as methyl or ethyl, a series of C-ring bridged analogs were prepared (see Table 5 and Scheme 7). While the three-carbon bridge between N-2 and C-4 was tolerated

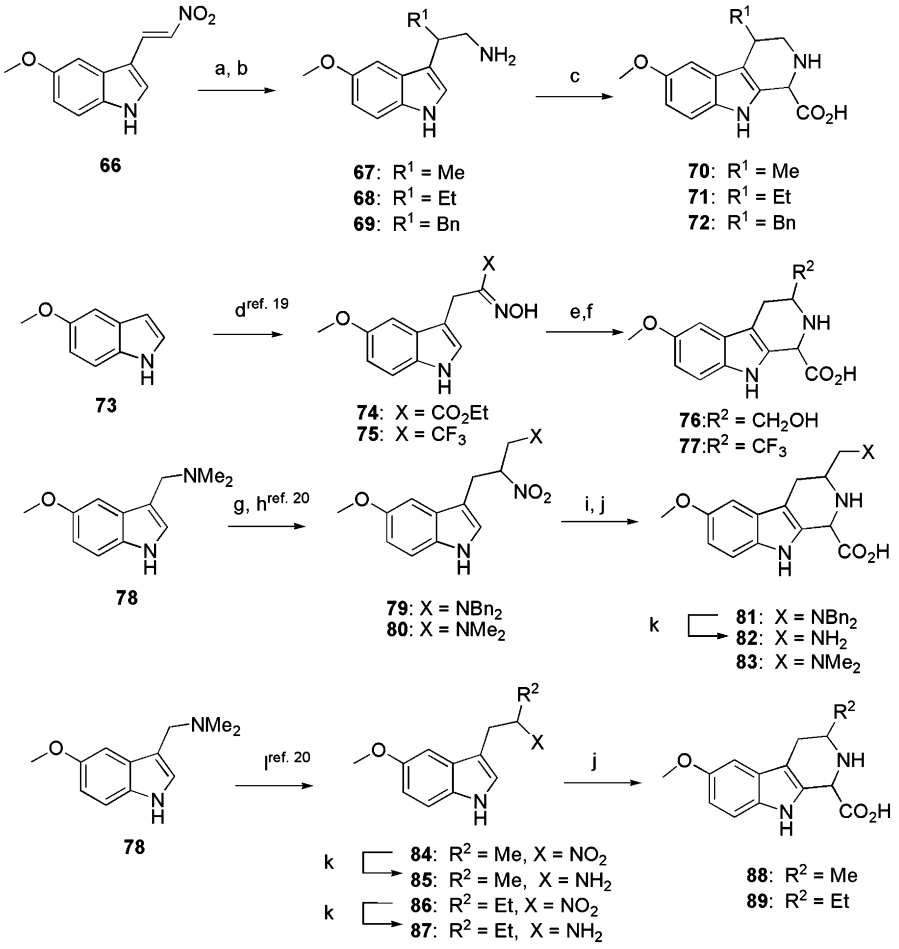


Scheme 4. Synthesis of compounds **36**, **39–44**, **49–52**, **54–56**, and **58**. Reagents and conditions: (a) MeOH/HCl, rt; (b) Na_2CO_3, MeI, THF:H_2O, rt; (c) KOH(aq), THF:MeOH; (d) for **46**: AcCl, Et_3N, CH_2Cl_2, rt; for **47**: acetone, MeOH, rt; for **48**: PhCHO, MeOH, reflux$_4$, MeOH; (e) for **446**: LAH, THF, rt; for **47** and **48**: $NaBH_4$, MeOH, 0 °C; (f) for **49**: glyoxylic acid, TFA, CF_3CN, rt; for **51** and **52**: glyoxylic acid, H_2O, rt; (g) acetone, $Na(OAc)_3BH$, CH_2Cl_2, rt; (h) NaOH, EtOH; (i) Boc_2O, THF, rt; (j) NaH, MeI, DMF, rt; (k) TFA, CH_2Cl_2, anisole, rt; (l) glyoxylic acid, H_2O, rt; (m) LAH, Et_2O, 0 °C to rt; (n) conc. NH_4OH(aq); (o) LAH, THF, rt; (p) for **53**: $(MeO)_2CH_2CO_2Me$, CH_3CN, TFA, 80 °C; for **54**: $Me(C{=}O)CO_2H$, MeOH:H_2O, 50 °C; (q) LiOH, THF:H_2O, rt; (r) 50% NH_2OH(aq), THF:H_2O, K_2CO_3, rt; (s) TFAA, Et_3CN, CH_2Cl_2, 0 °C; (t) Me_3SnN_3, dioxane, reflux, 5 h; (u) K_2CO_3, MeOH/H_2O, rt. See above mentioned references for further information.

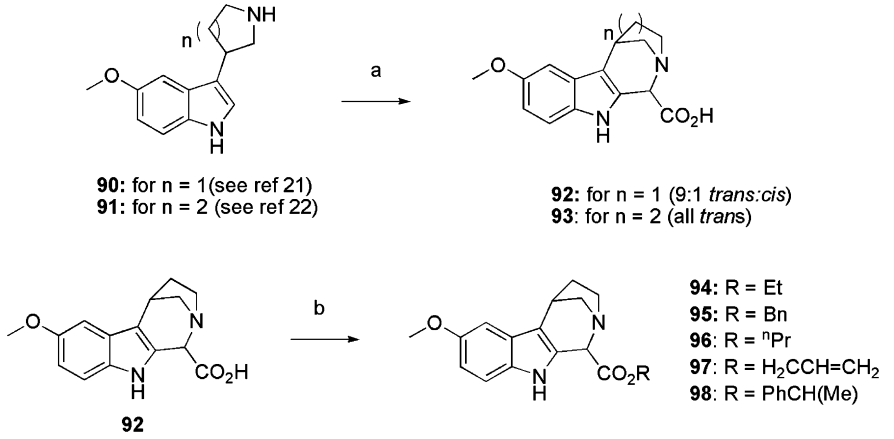
(**92**), the two-carbon bridge resulted in a 6-fold enhancement in potency, representing the first β-carboline we identified to achieve submicromolar potency against MK-2. Furthermore, compound **92** exhibited remarkable selectivity against a number of structurally related kinases (IC_{50}, μM: MSK1 > 200, MNK1 > 200,



Scheme 5. Synthesis of compounds **61** and **65**. Reagents and conditions: (a) $HCOCO_2Et$, pTsOH, rt to 80 °C, 20 h; (b) MeOH:H_2O, KOH, rt, 2 h; (c) **63**, EtOH, reflux, 24 h; (d) LiOH, THF:H_2O:MeOH, 4 h.



Scheme 6. Synthesis of compounds **70**–**72**, **76**, **77**, **82**, **83**, **88**, and **89**. Reagents and conditions: (a) for **67**: MeMgBr (3.0 M in Et_2O), THF, −50 °C, 2 h then 0 °C, 1 h; for **68**: EtMgBr, THF, −78 °C, 3 h; for **69**: $PhCH_2MgCl$ (2.0 M in Et_2O), THF, −50 °C, 2 h then 0 °C, 1 h; (b) 10% Pd/C, HCO_2NH_4, MeOH, 80 °C; (c) glyoxylic acid, H_2O, pH 4–5, rt, 3 h; (d) for **74**: $BrCH_2C(=NOH)CO_2Et$, Na_2CO_3, CH_2Cl_2, rt, 24 h; for **75**: $BrCH_2C(=NOH)CF_3$, Na_2CO_3, ^tBuOMe, rt, 24 h; (e) LAH, THF, −50 °C to rt; (f) glyoxylic acid, H_2O, pH 4–5, rt, 24 h; (g) NaOMe, Me_2SO_4, $MeNO_2$, MeOH, rt, 5 h; (h) for **79**: Bn_2NH, H_2CO, H_2O:MeOH, rt then reflux, 3 h; for **80**: Me_2NH, H_2CO, H_2O, MeOH, rt; (i) for **81**: LAH, THF, 0 °C then reflux; for **83**: HCO_2NH_4, 10% Pd/C, MeOH, reflux; (j) glyoxylic acid, MeOH/H_2O, pH 3–4, 55 °C; (k) HCO_2NH_4, 10% Pd/C, MeOH, reflux; (l) for **84**: $nPrNO_2$, NaOMe, Me_2SO_4, MeOH, rt; for **86**: $EtNO_2$, NaOMe, Me_2SO_4, MeOH, rt.

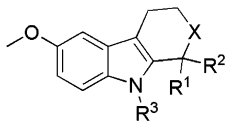


Scheme 7. Synthesis of compounds **92** and **93** and **94**–**98**. Reagents and conditions: (a) For **92**: glyoxylic acid, MeOH:H_2O, 60 °C, 10 h; for **93**: same as **92** except 45 h; (b) ROH, EDAC, DMAP, rt, 5 h.

JNK2 > 200, p38α > 100, IKK2 > 200), even close homologs MK-3 and PRAK (IC_{50}, μM: 90 and 24, respectively).

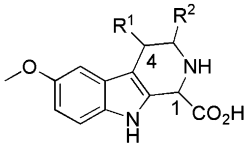
The potency and selectivity of compound **92** prompted further investigation in cellular models, specifically the inhibition of TNFα production in U937 cells.

Table 2. IC_{50} values for β-carboline derivatives



Compound	R^1	R^2	X	R^3	MK-2 IC_{50} (μM)
1	CO_2H	H	NH	H	2.5
65	CO_2H	H	CH_2	H	>200
61	CO_2H	H	O	H	>200
40	CO_2H	H	NMe	H	1.0
49	CO_2H	H	NEt	H	5.8
50	CO_2H	H	N^{i}Pr	H	170
51	CO_2H	H	NBn	H	130
36	CO_2Me	H	NH	H	47
43	$CONH_2$	H	NH	H	>200
42	CH_2OH	H	NH	H	>200
56	CH_2NH_2	H	NH	H	>200
44	CONHOH	H	NH	H	88
58	Tetrazole	H	H	H	130
54	CO_2H	Me	NH	H	>200
55	CH_2CO_2H	H	NH	H	>200
52	CO_2H	H	NH	Me	>200

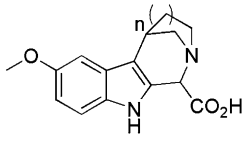
Table 3. IC_{50} values for β-carboline derivatives



Compound	R^1	R^2	MK-2 IC_{50} (μM)
70	Me	H	2.2
71	Et	H	2.2
72	Bn	H	16
88	H	Me	7.1
89	H	Et	64
77	H	CF_3	>200
76	H	CH_2OH	3.0
82	H	CH_2NH_2	5.9
83	H	CH_2NMe_2	>200

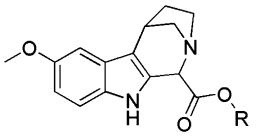
Unfortunately, compound **92** did not possess the desired cellular activity. It was thought that the zwitterionic character of the amino and carboxylic acid moieties was limiting the cellular permeability of this compound.

Table 4. IC_{50} values for β-carboline derivatives **92** and **93**



Compound	*n*	MK-2 IC_{50} (μM)	MK-3 IC_{50} (μM)	PRAK IC_{50} (μM)
92	1	0.29	90	24
93	2	5.1	160	25

Table 5. IC_{50} values for β-carboline derivatives **92**–**97**



Compound	R	MK-2 IC_{50} (μM)	U937 IC_{50} (μM)
92	H	0.29	80.8
94	Et	29.3	5.6
95	Bn	7.09	1.7
96	*n*-Pr	10.1	0.83
97	$H_2C{=}CHCH_2$	3.27	14.4
98	PhCH(Me)	13.0	10.3

Indeed, when ester prodrugs of **92** were synthesized and evaluated in the U937 cell assay, a dramatic enhancement in cell potency was observed, suggesting an intracellular conversion of the ester to the active acid. The most effective compound in the U937 cellular assay was identified to be compound **96**, an *n*-propyl ester (see Table 5). The compound was further evaluated in the rat LPS model (40 mpk, IP) and was shown to inhibit TNF-α production (84%, compound administered 1 h prior to LPS stimulation).

In conclusion, a group of β-carbolines capable of inhibiting MK-2 has been discovered, and the SAR of the series has been described. Key SAR observations include (1) the importance of an ether functionality at the 6-position of the A-ring, (2) the critical sensitivity of the carboxylic acid, and (3) the enhancement in binding affinity observed by bridging the C-ring nitrogen with the 4-position. Compound **92** represents remarkable selectivity for MK-2 over a number of closely related kinases, including MK-3 and PRAK. While the carboxylic acids were inactive in U937 cells, an ester prodrug of **92**, compound **96**, was found to be effective in modulation of TNFα production in both U937 cellular assays and in the rat LPS model.

Acknowledgments

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