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Discovery of tetrahydro-β-carbolines as inhibitors of the mitotic kinesin KSP

Fei Liu [a,b], Li-Qin Yu [a,b], Cheng Jiang [a,b], Lei Yang [c], Wu-Tong Wu [d], Qi-Dong You [a,b,*]

[a] Jiangsu Key Laboratory of Carcinogenesis and Intervention, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b] Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[c] Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[d] School of Life Science and Technology, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China

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ABSTRACT

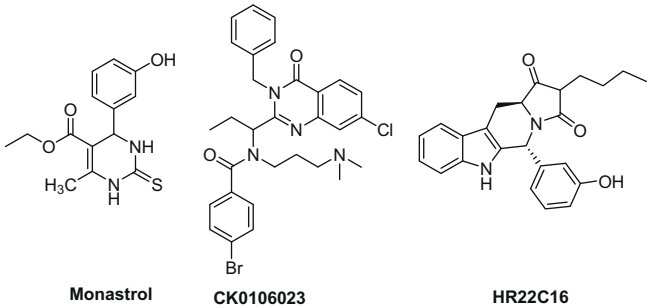
Inhibitors of kinesin spindle protein (KSP) are a promising class of anticancer agents that cause mitotic arrest in cells from a failure to form functional bipolar mitotic spindles. Here, we report the synthesis and biological evaluation of a novel series of tetrahydro-β-carboline analogs based on the structure of the known KSP inhibitor HR22C16. Preferred compounds **11b**, **12a** and **19b** were identified as potent inhibitors in a KSP ATPase assay with good anti-proliferative activity in A549 cells.

1. Introduction

Antimitotic agents, such as vinca alkaloids, taxol and epothilones, are a major class of cytotoxic drugs in the treatment of cancer. By interfering with the tubulins' polymerization and depolymerization, antimitotics inhibit mitotic spindle and arrest dividing cells in metaphase.[1,2] However, the use of these agents is associated with undesired side effects, such as neurotoxicity, related to the central role tubulin plays in cellular transport processes.[3] The mechanism-related toxicities and acquired resistance have stimulated considerable interest in developing antimitotics which do not act on microtubule directly.

Members of the kinesin superfamily play important roles in cargo transport, spindle and chromosome movement, and regulation of microtubule dynamics.[4] KSP (kinesin spindle protein, also known as Hs Eg5) is a plus-end-directed motor of the BimC kinesin subfamily which is responsible for the formation of the bipolar spindle.[5] KSP plays an important role in the early stage of mitosis and mediates centrosome separation. Inhibition of KSP leads to a stable mitotic block with monoastral microtubule arrays.[6] The function of KSP provides a novel route for the manipulation of the cell cycle and the induction of apoptosis. Therefore, KSP inhibitors have become attractive and promising anti-proliferative agents for cancer chemotherapy.[7,8]

In 1999, Mayer et al. discovered monastrol, the first small molecule able to inhibit the mitotic kinesin Eg5 (KSP) using a phenotype-based assay.[9] Subsequently, a series of second-generation of monastrol analogs with improved cellular potency and solubility was developed. Moreover, several inhibitors that exhibit great chemical diversity have also been reported including CK0106023, dihydropyrazoles, tetrahydroquinolines, HR22C16, tetrahydroisoquinolines, and many more (Fig. 1).[10,11] HR22C16 possesses a tetrahydro-β-carboline based structure and several laboratories have reported efforts around it.[12,13] Recently, a co-crystal structure of an analog of HR22C16 in the KSP motor domain has confirmed that this series of inhibitors binds in the allosteric loop L5 binding pocket.[14] The X-ray crystal structure also suggests that the alkyl



Monastrol CK0106023 HR22C16

Figure 1. The KSP inhibitors monastrol, CK0106023 and HR22C16.

* Corresponding author.
E-mail address: youqidong@gmail.com (Q.-D. You).

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side chain along with the hydantoin ring point towards the solvent exposed areas of the binding pocket, and what's more, this HR22C16 analog forms a hydrogen bond to the KSP backbone through its phenol. During the course of preparing this paper, the X-ray structure of a terahydro-β-carboline analogs bound to Eg5 in an allosteric site of Eg5 (PDB code: 3K3B) was reported,[15] which depicted that there was a strong hydrogen bond between the phenolic –OH and the backbone carbonyl of Glu118, as well as a hydrogen bond between the carboline –NH and amide carbonyl of Glu116. A series of *N*-acyl-tetrahydro-β-carbolines were studied afterward and SAR was also discussed in due course.

In the present paper, guided by the results of the co-crystal structure of HR22C16 analog, we describe our effort on designing more potent terahydro-β-carboline analogs with the speculation that retaining tetrahydro-β-carboline core with a pendant 3-hydroxyphenyl, removal of hydantoin ring of HR22C16 and varying the modifications at the N2, C3, C4 and C6/8 positions could lead to potent KSP inhibitors (Fig. 2). Alternate to the pendent 1-(3-hydroxyphenyl) group was also investigated, giving rise to 1-(3-indolyl)-2-acyl tetrahydro-β-carbolines in which the 3-indolyl ring substituent was supposed to act in a similar fashion as the 3-hydroxyphenyl moiety. Following above ideas, four structural series of compounds were synthesized and tested for there ability to inhibit KSP measured by the MT-activated ATPase activity and A549 cell proliferation.

2. Chemistry

Pictet–Spengler cyclization was one of the most powerful methods to synthesize the tetrahydro-β-carboline ring system. Utilizing diverse 3-indolylethylamines and benzaldehyde as substrates, the core scaffold of tetrahydro-β-carbolines was constructed favorably. The following compounds were all synthesized as racemic mixtures.

The synthetic pathways used to prepare 2,3-dicarbonyl tetrahydro-β-carboline derivatives are shown in Schemes 1. Commercially available tryptophan underwent Pictet–Spengler cyclization with 3-hydroxy benzaldehyde to give 3-carboxylic acid intermediate, which was further esterified to 3-ethyl carboxylate **5**. Treatment of compound **5** with diverse acyl chloride led to the bis-acylated compounds which underwent selective single hydrolysis when treated with $NaHCO_3$ to generate the desired compound **6a–d**. Compounds **6b** and **6c** were further hydrolyzed to 3-carboxylic acid **7a** and **7b** (Scheme 1).

3-Amide tetrahydro-β-carboline analogs, **8a** and **8b**, were prepared from 3-ethyl carboxylate intermediate **5** under amination

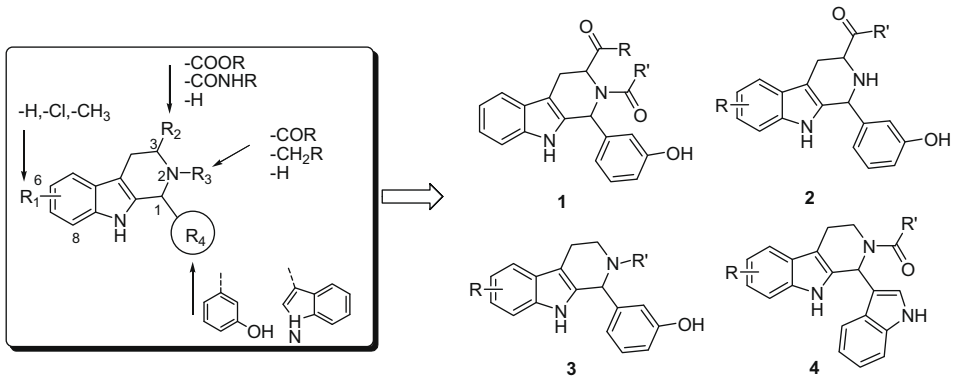
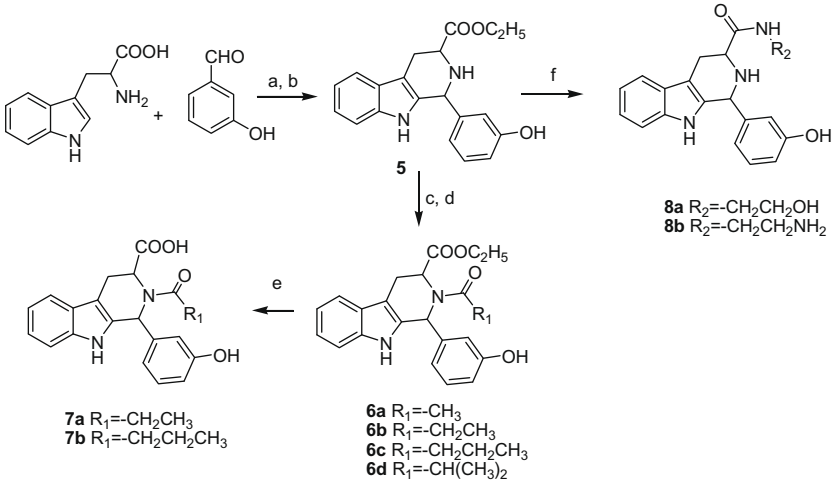


Figure 2. Design of novel series of tetrahydro-β-carboline analogs: series 1—*N*2,*C*3-dicarbonyl-1-(3-hydroxylphenyl) tetrahydro-β-carboline analogs; series 2—*C*3-acyl-1-(3-hydroxylphenyl) tetrahydro-β-carboline analogs; series 3—N2-substituted-1-(3-hydroxylphenyl) tetrahydro-β-carboline analogs; series 4—*N*2-acyl-1-(3-indolyl) tetrahydro-β-carboline analogs.



Scheme 1. Synthesis of 2,3-dicarbonyl and 3-amide tetrahydro-β-carboline derivatives. Reagents and condition: (a) acetic acid, 95 °C; (b) $SOCl_2$, C_2H_5OH; (c) TEA, THF, acyl chloride; (d) $NaHCO_3$; (e) 10% NaOH, then HCl; (f) 2-aminoethanol or 1,2-diaminethane.

condition using ethanolamine and 1,2-diaminethane, respectively (Scheme 1).

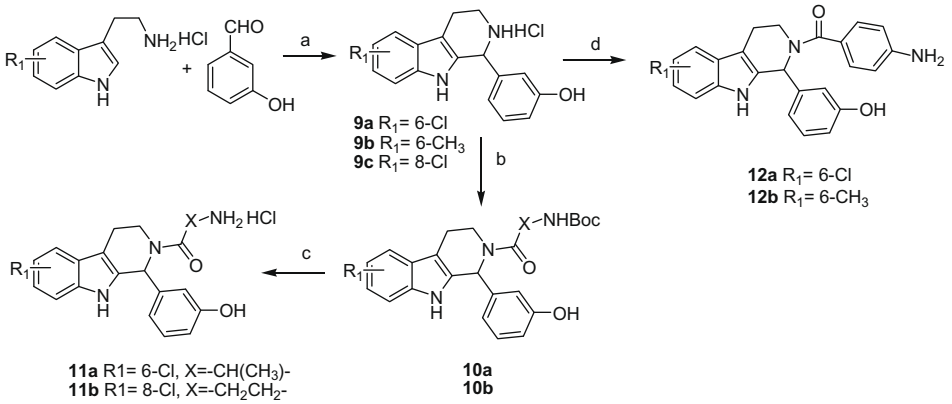
In order to obtain 3-unsubstituted tetrahydro-β-carboline analogs, tryptamine and substituted tryptamine were first prepared according the procedure reported by our group.[16] Utilizing the Pictet–Spengler cyclization conditions described in Scheme 1, the tetrahydro-β-carboline core **9** were prepared from substituted tryptamine and 3-hydroxy benzaldehyde. *N*2-Aminoalkylacyl compounds **11a–b** were obtained via standard amide coupling reaction with Boc protected amino acid followed by Boc deprotection. *p*-Aminobenzoic acid condensed directly with **9** providing desired *p*-Aminobenzoyl **12a–b** (Scheme 2).

2-Alkyl compounds **13a–e** were synthesized utilizing Leuckart reaction conditions (Scheme 3). In order to generate the homologated 2-aminoalkyl analogs **17**, C6 substituted tryptamine was cyclized with 3-hydroxy-protected benzaldehyde to generate intermediate **14**, followed by standard coupling, hydrazinolysis and deprotection, to furnish 2-aminoalkyl analogs **17a–f**. Since dehalogenated byproduct was observed when 6-chrolo-3′OBn tetrahydro-b-carbolines was subjected to the deprotection condition of Pt–C/$HCOONH_4$, isopropyl is a good alternation for benzyl group at this manner, which was favorably deprotected with $AlCl_3$.

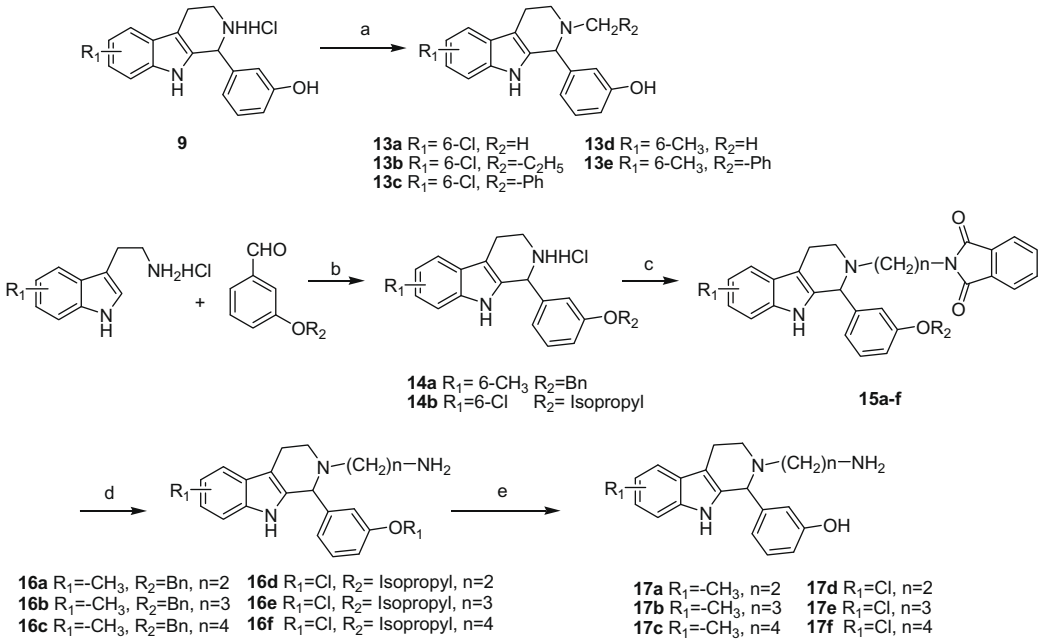
Different from one step Pictet–Spengler cyclization, the building of 1-(3-indolyl) tetrahydro-β-carboline core **18** involves two steps: a condensation reaction between tryptamine and 1H-indole-3-carbaldehyde to generate the corresponding imine, and then an intramolecular cyclization catalyzed by TFA to finish the cyclized intermediate **18** (Scheme 4). Further treatment of **18** with diverse acyl chloride provided desired compounds **19a–f**. Conversion of **18** to the final 2-(β-aminopropionyl) **19g** was achieved by coupling with *N*-Boc β-alanine, followed by Boc cleavage with HCl.

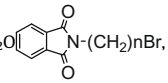
3. Result and discussions

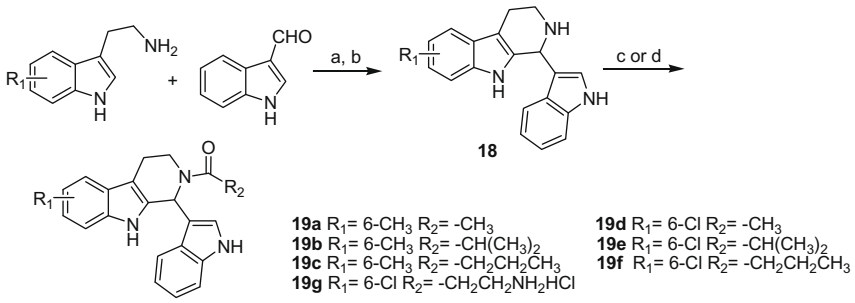
All synthesized tetrahydro-β-carbolines were tested for their inhibitory activities against KSP determined by measuring the



Scheme 2. Synthesis of *N*2-acyl tetrahydro-β-carboline derivatives. Reagents and condition: (a) acetic acid, reflux, then HCl; (b) PyBOP, TEA, THF, $BocNHCH(CH_3)COOH$ or $BocNHCH_2CH_2COOH$; (c) HCl, ethyl acetate; (d) PyBOP, TEA, THF, 4-aminobenzoic acid.



Scheme 3. Synthesis of *N*2-alkly tetrahydro-β-carboline derivatives. Reagents and conditions: (a) R_2CHO, K_2CO_3, HCOOH, DMF; (b) CH_3COOH, 95 °C, then HCl; (c) KOH, DMF, reflux; (d) CH_3CH_2OH, NH_2–NH_2·H_2O,  , reflux; (e) 10% Pd–C, $HCOONH_4$ or $AlCl_3$,CH_2Cl_2.



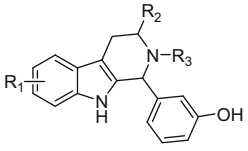
Scheme 4. Synthesis of 1-(3-indolyl) tetrahydro-β-carboline derivatives. Reagents and conditions: (a) toluene, reflux; (b) TFA; (c) R_2COCl, TEA, THF; (d) PyBOP, TEA, $BocNHCH_2CH_2COOH$, THF, then HCl, EtOAc.

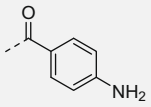
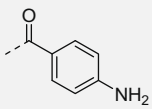
MT-activated ATPase activity. The anti-proliferative cytotoxic activity was also evaluated by using A549 tumor cell line. CK0106023 was synthesized and used as positive control.[17]

The initial series tested were the 2,3-dicarbonyl-1-(3-hydroxylphenyl) tetrahydro-β-carboline analogs (**6 and 7**) represented in Table 1. There appears to be an optimal chain length of the substitutes at the N2 while an ethyl carboxylate group was substituted at C3 position. The maximal activity of the propionyl **6b** is reduced neither as the length increases to the butyryl **6c** nor shorten to acetyl analog **6a**. Additional branching did not improve activity, as seen with isopropyl analog **6d**. The hydrolysis of 3-ethyl carboxylate into 3-carboxylic acid analogs leads to a twofold (**7a** vs **6b**) and 15-fold (**7b** vs **6c**) loss of KSP activity. 3-(*N*-(2-Aminoethyl)) amide **8b** exhibited sub-micromolar KSP activity (65 nM). As the amino group of **8b** changed to hydroxyl (**8a**), fourfold of decrease in activity was observed, indicating a strict special restriction close to the solvent exposed region of enzyme pockets. However, all of these compounds were relatively ineffective at inhibiting cell proliferation in the A549 assay. The poor cellular activity of these compounds prompted the research for alterative substitutions.

The next investigation of tetrahydro-β-carboline core was focused on C6/8 substitution along with N2 acylation (Table 2).

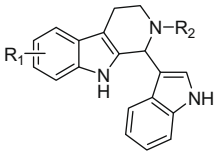
Table 1
Activities of 1-(3-hydroxyphenyl) tetrahydro-β-carboline compounds



Compound	R^1	R^2	R^3	KSP[a] IC_{50} (μM)	A549[b] IC_{50} (μM)
6a	H	$-COOC_2H_5$	$-COCH_3$	0.724	>10
6b	H	$-COOC_2H_5$	$-COCH_2CH_3$	0.075	>10
6c	H	$-COOC_2H_5$	$-COCH_2CH_2CH_3$	0.224	>10
6d	H	$-COOC_2H_5$	$-COCH(CH_3)_2$	0.380	>10
7a	H	–COOH	$-COCH_2CH_3$	0.118	>10
7b	H	–COOH	$-COCH_2CH_2CH_3$	3.453	>10
8a	H	$-CONHCH_2CH_2OH$	H	0.290	>10
8b	H	$-CONHCH_2CH_2NH_2$	H	0.065	>10
11a	6-Cl	H	$-COCH(CH_3)NH_2HCl$	0.483	5.65
11b	8-Cl	H	$-COCH_2CH_2NH_2HCl$	0.040	0.94
12a	6-Cl	H		0.076	6.66
12b	$6-CH_3$	H		0.050	7.93
13a	6-Cl	H	$-CH_3$	0.664	1.38
13b	6-Cl	H	$-CH_2CH_2CH_3$	0.169	1.55
13c	6-Cl	H	$-CH_2Ph$	0.710	5.70
13d	$6-CH_3$	H	$-CH_3$	0.462	8.73
13e	$6-CH_3$	H	$-CH_2Ph$	12.378	6.86
17a	$6-CH_3$	H	$-CH_2CH_2NH_2HCl$	0.237	7.79
17b	$6-CH_3$	H	$-CH_2CH_2CH_2NH_2HCl$	0.083	6.86
17c	$6-CH_3$	H	$-CH_2CH_2CH_2CH_2NH_2HCl$	0.454	9.19
17d	6-Cl	H	$-CH_2CH_2NH_2HCl$	0.007	8.70
17e	6-Cl	H	$-CH_2CH_2CH_2NH_2HCl$	0.204	6.73
17f	6-Cl	H	$-CH_2CH_2CH_2CH_2NH_2HCl$	0.347	9.06
CK0106023	—	—	—	0.049	7.02

[a] The inhibitory activities against KSP were determined by measuring the MT-activated ATPase activity.
[b] Data represent mean values of at least three separate experiments.

Table 2
Activities of 1-(3-indolyl) tetrahydro-β-carboline compounds



Compound	R^1	R^2 IC_{50} (μM)	KSP[a] IC_{50} (μM)	A549[b] IC_{50} (μM)
19a	6-CH_3	$-COCH_3$	0.051	>10
19b	6-CH_3	$-COCH_2CH_2CH_3$	0.216	>10
19c	6-CH_3	$-COCH(CH_3)_2$	<0.001	>10
19d	6-Cl	–COCH3	0.337	9.80
19e	6-Cl	$-COCH_2CH_2CH_3$	1.703	6.83
19f	6-Cl	$-COCH(CH_3)_2$	0.163	9.11
19g	6-Cl	$-COCH_2CH_2NH_2HCl$	0.043	1.79
CK0106023	—	—	0.049	7.02

[a] The inhibitory activities against KSP were determined by measuring the MT-activated ATPase activity.
[b] Data represent mean values of at least three separate experiments.

Within the two 2-aminopropionyl derivatives, optimal KSP and cellular activity (IC_{50} = 40 and 930 nM, respectively) is observed with the β-amino analog **11b**, relative to the corresponding α-amino analog **11a**. Both 2-(*p*-aminobenzoyl) analogs **12a** and **12b** are highly potent KSP inhibitors with good cellular activity. The improved A549 activity and sub-micromolar KSP potency seen with **11** and **12** prompted analysis of the *N*2-alkyl tetrahydro-β-carbolines.

To further investigate the enzymatic and cellular inhibitory activity of 2,6-substituted KSP inhibitors, *N*2-alkyl tetrahydro-β-carbolines were synthesized. The initial analogs tested, compounds 13a–**e** exhibit moderate KSP and cellular activity. Appending a phenyl group at 2-methyl gave a little increase (cf. **13c** with **13a**) or significantly reduced (cf. **1e** with **1d**) of KSP inhibiting capacity. Interestingly the 6-methyl-2-benzyl **13e** although showing weak enzyme inhibition displayed moderate micromolar anti-proliferative activity.

Incorporation of an amino group at the terminal region of hydrophobic chain was proved to be preferred in the solvent exposed region, exemplified by **8b** and **11b**. As seen with the 2-aminoalkyl analogs **17a–f**, a trend of an optimal length of chain between the terminal amino and tetrahydro-β-carboline core was observed. As the aminoalkyl chain of 6-methly analogs is extended form the aminoethyl **17a**, aminopropyl **17b**, to aminobutyl **17c**, KSP activity is maximal with an optimal three carbon atom linker **17b**. However, when it referred to 2-alkyl-6-chloro tetrahydro-β-carbolines, aminoethyl **17d** with two carbon atom chain is the most potent (APTase IC_{50} = 7 nM). In general, the cellular activities tested were proximately identical and displayed no obvious dependence on KSP potency.

Above all, among the most potent compounds with sub-micromolar KSP activity, 3-(*N*-(2-aminoethyl)) amide **8b**, for example, possesses similar potency compared to 8-chloro-2-(β-aminopropionyl) **11b**, 6-chloro-2-(*p*-aminobenzoyl) **12a** and 6-methyl-2-aminopropyl **17b** (65 vs 40, 76 and 83 nM, respectively), suggesting the length of chain between the terminal amino and tetrahydro-β-carboline core is an important driver of potency and not necessarily the functionality contained in the linker or the substitution position of N2 or C3. As seen in the first three structural series, there is a preference for a 2- to 4-atom length of chain between the terminal amino group and the tetrahydro-β-carboline core, which were diverged between different structural series. Moreover, the substitution at C3 position seems to be detrimental to cytotoxicity.

Table 3
Cellular IC_{50} (μM) of selected compounds

Compound	A549	AGS	HepG2	HT-29	PC-3
11b	0.94	1.31	7.47	5.75	1.28
12a	6.66	6.07	8.52	6.30	7.30
12b	7.93	8.86	>10	7.47	>10
13a	1.38	0.75	>10	7.40	>10
16d	8.70	8.39	>10	7.44	>10
19f	6.83	7.06	7.64	5.86	7.29
19g	1.79	6.16	>10	6.28	1.55
CK0106023	7.02	5.10	>10	3.33	>10

1-(3-Indolyl)-2-acyl-6-methyl tetrahydro-β-carbolines (Table 2), **19a–c**, exhibited KSP enzymatic inhibition, but lacked potent cellular activity. However, optimal KSP activity (IC_{50} < 1 nM) is observed with the 2-isobutyryl **19c**, relative to the corresponding 2-acetyl **19a** and 2-butyryl **19b**, suggesting branched 3-atom chain is optimal. The KSP activity trend is similar for the 6-chloro analogs; however, they are 7- to 160-fold less potent than the corresponding 6-methyl analogs. Interestingly, their loss in KSP potency leads to an increase in cellular activity. With an amino group at the terminal of 2-propionyl, 1-(3-indolyl)-6-chloro tetrahydro-β-carboline **19g** displayed ~40- and ~4-fold of improvement in KSP and cellular inhibitory activity, respectively, compared with 2-isobutyryl **19e**. The 3-hydroxyl along with the pendant phenyl is alternative with 3-indolyl, suggesting the latter group offer the same function as the former.

The most promising compounds representing different structural series were selected for further evaluation for their anti-proliferative cytotoxic activity against other human tumor cell lines, such as AGS (stomach), HepG2 (liver), HT-29 (colon) and PC-3 (prostate) (Table 3). The majority of the compounds displayed excellent anti-proliferative profiles, showing that removal of hydantoin ring of HR22C16 could maintain biological effects of the molecules. In general, these compounds showed lower anti-proliferative activities against HepG2 and PC-3 than against A549, AGS and HT-29 cells.

4. Conclusion

We have identified a series of novel tetrahydro-β-carboline KSP inhibitors that are characterized by high potency in KSP and cellular proliferation assays. Four structural series of tetrahydro-β-carbolines were synthesized and the most active compounds were 8-chloro-2-(β-aminopropionyl) **11b**, 6-chloro-2-(*p*-aminobenzoyl) **12a**, 1-(3-indolyl)-2-(β-aminopropionyl) **19g**. Substitution of the tetrahydro-β-carboline core at the C6- or C8-positions was required for good cellular potency. The KSP potency maintained by incorporating *C*1-(3-indolyl) groups in stead of the phenol, coupled with introduction of acyl moiety at C2 position. Moreover, all the four structural series described herein were found to exhibit the similar SAR with regard to the length of the side chain. Modification the terminal of side chain with an amino group led to an increase in KSP inhibitory activity. Further studies of these agents and related analogs will be reported in due course.

5. Experimental

5.1. General

Melting points were determined on a Mel-TEMP II melting point apparatus and are uncorrected. Infrared (IR) spectra (KBr) were recorded on a Nicolet Impact 410 instrument (KBr pellet). ^1H NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. MS spectra were recorded on a Shimadzu GC–MS 2010 (EI) or a Mariner Mass Spec-

trum (ESI), or a LC/MSD TOF HR-MS Spectrum. All compounds were routinely checked by TLC and ^{1}H NMR. TLCs and preparative thin-layer chromatography were performed on silica gel GF/UV 254, and the chromatograms were performed on silica gel (200–300 mesh) visualized under UV light at 254 and 365 nm. All solvents were reagent grade and, when necessary, were purified and dried by standards methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results are within (0.40% of the theoretical values).

5.1.1. Ethyl 1-(3-hydroxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (5)

A mixture of tryptophan (1.02 g, 5.0 mmol) and 3-hydroxybenzaldehyde (0.67 g, 5.5 mmol) was dissolved in glacial acetic acid (15 mL) under N_2 protection. After stirring at 95 °C for 2 h, the mixture was cooled to room temperature. The resulted precipitate was filtered and dissolved in ethanol (50 mL). Thionyl chloride (0.57 mL, 8.0 mmol) was added slowly over a period of 10 min. After stirring at 80 °C for 2 h, the solvent was concentrated and the pH of the mixture was adjusted to 8 with saturate $NaHCO_3$ aqueous solution. Afterward, the solution was extracted with acetic ether (3× 20 mL). The organic phase was concentrated and purified by column chromatography on silica gel by eluting with a mixture of petroleum ether/ethyl acetate (1:1) to give the desired product (1.14 g) as off-white solid in 67.8% yield; mp 198–200 °C.

5.1.2. Ethyl 1-(3-hydroxyphenyl)-2-acetyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (6a)

Acetyl chloride (0.14 mL, 2 mmol) in THF (5 mL) was added to a mixture of compound **5** (0.34 g, 1 mmol) in THF (10 mL) over a period of 20 min. After stirring at room temperature for 2 h, the mixture was concentrated and added saturate $NaHCO_3$ aqueous solution (20 mL) and acetone (20 mL). After stirring for additional 3 h, the acetone was evaporated and the water layer was extracted with acetic ether (3× 30 mL). Removal of the solvent in vacuo afforded the crude product which was purified by column chromatography on silica gel by eluting with a mixture of petroleum ether/ethyl acetate (2:1) to give the desired product (0.21 g, 54.5%) as an off-white solid; mp 245 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.78 (m, 3H, –OCH$_2$*CH*$_3$), 2.25 (s, 3H, –COCH$_3$), 2.98 (m, 3H, CH, –O*CH*$_2$CH$_3$), 3.44 (m, 2H, CH$_2$), 3.68 (m, 1H, CH), 5.17 (d, 1H, *CH*-Ar, J = 6.6), 6.55–7.54 (m, 8H, Ar-H), 7.25 (s, 1H, OH), 10.89 (s, 1H, NH). IR (cm^{-1}): 3357, 1705, 1646, 1585, 1236. EI-MS (*m/z*): 378 (M^+). Anal. Calcd for ($C_{22}H_{22}N_2O_4$·0.3H_2O): C, 68.84; H, 5.93; N, 7.30. Found: C, 68.71; H, 5.96; N, 6.96.

5.1.3. Ethyl 1-(3-hydroxyphenyl)-2-propionyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (6b)

Compound **6b** (0.21 g) was synthesized from **5** (0.34 g, 1 mmol) and propionyl chloride (0.17 mL, 2 mmol) according to the procedure used to synthesize **6a** as an off-white solid in 49.2% yield. Mp: 240 °C (dec). IR (cm^{-1}): 3400, 2946, 1704, 1653, 1218. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.73 (m, 3H, –COCH$_2$*CH*$_3$), 1.13 (m, 3H, –OCH$_2$*CH*$_3$), 2.62 (m, 2H, –CO*CH*$_2$CH$_3$), 2.95 (m, 2H, –O*CH*$_2$CH$_3$), 3.31 (m, 1H, CH$_2$), 3.44 (m, 1H, CH$_2$), 3.62 (m, 1H, CH), 5.23 (d, 1H, *CH*-Ar, J = 6.6), 6.56–7.53 (m, 8H, Ar-H), 9.24 (s, 1H, OH), 10.89 (s, 1H, NH). EI-MS (*m/z*): 392 (M^+). Anal. Calcd for ($C_{23}H_{24}N_2O_4$): C, 70.39; H, 6.16; N, 7.14. Found: C, 69.94; H, 6.31; N, 6.76.

5.1.4. Ethyl 1-(3-hydroxyphenyl)-2-butyryl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (6c)

Compound **6c** (0.24 g) was synthesized from **5** (0.34 g, 1 mmol) and butyryl chloride (0.21 mL, 2 mmol) according to the procedure used to synthesize **6a** as an off-white solid in 58.0% yield. Mp: 140–142 °C. IR (cm^{-1}): 3396, 2966, 1743, 1619, 1455. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.73 (m, 3H, –OCH$_2$*CH*$_3$), 0.93 (m, 3H, –CH$_2$CH$_2$*CH*$_3$), 1.62 (m, 2H, –CH$_2$*CH*$_2$CH$_3$), 2.54 (m, 2H, –*CH*$_2$CH$_2$CH$_3$), 2.98 (m, 2H, –O*CH*$_2$CH$_3$), 3.44 (d, 1H, CH$_2$, J = 16.8), 3.60 (m, 1H, CH$_2$), 3.85 (m, 1H, CH), 5.25 (d, 1H, *CH*-Ar, J = 6.6), 6.55–7.53 (m, 8H, Ar-H), 9.24 (s, 1H, OH), 10.89 (s, 1H, NH). EI-MS (*m/z*): 406 (M^+). Anal. Calcd for ($C_{24}H_{26}N_2O_4$): C, 70.92; H, 6.45; N, 6.89. Found: C, 70.60; H, 6.49; N, 6.88.

5.1.5. Ethyl 1-(3-hydroxyphenyl)-2-isobutyryl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (6d)

Compound **6d** (0.21 g) was synthesized from **5** (0.34 g, 1 mmol) and isobutyryl chloride (0.21 mL, 2 mmol) according to the procedure used to synthesize **6a** as an off-white solid in 51.5% yield. Mp: 129–131 °C. IR (cm^{-1}): 3394, 2930, 1721, 1632, 1457. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.80 (m, 9H, –OCH$_2$*CH*$_3$, –CH(*CH*$_3$)$_2$), 2.68 (m, 2H, –O*CH*$_2$CH$_3$), 2.91 (m, 1H, CH$_2$), 3.21 (m, 1H, CH$_2$), 3.35 (m, 1H, –*CH*(CH$_3$)$_2$), 3.70 (m, 1H, CH), 5.13 (d, 1H, *CH*-Ar, J = 6.6), 6.32–7.30 (m, 8H, Ar-H), 9.02 (s, 1H, OH), 10.68 (s, 1H, NH). EI-MS (*m/z*): 406 (M^+). Anal. Calcd for ($C_{24}H_{26}N_2O_4$): C, 70.92; H, 6.45; N, 6.89. Found: C, 70.61; H, 6.64; N, 6.99.

5.1.6. 1-(3-Hydroxyphenyl)-2-propionyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (7a)

Compound **6b** (0.20 g, 0.53 mmol) was dissolved in a mixture of ethanol (20 mL) and 10% NaOH aqueous solution (20 mL) and the solution was allowed to stir at room temperature for 1 h. The ethanol was then removed in vacuo. Afterward, the remaining residue was acidified to pH ~4 with 10% HCl and extracted with acetic ether (3× 30 mL). The organic phase was dried and evaporated to dryness to furnish crude product which was purified by column chromatography on silica gel by eluting with a mixture of dichloromethane/methanol (20:1) to give the desired product **7a** (0.12 g, 63.0%) as a white solid. Mp: 143–147 °C. IR (cm^{-1}): 3340, 1720, 1622, 1454, 1272. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.81 (m, 3H, –COCH$_2$*CH*$_3$), 2.08 (m, 1H, –CO*CH*$_2$CH$_3$), 3.27 (m, 2H, CH$_2$), 5.20 (m, 1H, CH), 5.99 (d, 1H, *CH*-Ar, J = 35.7), 6.50–7.45 (m, 8H, Ar-H), 9.40 (d, 1H, OH, J = 50), 10.93 (d, 1H, NH, J = 18.6), 12.50 (s, 1H, –COOH). EI-MS (*m/z*): 364 (M^+). Anal. Calcd for ($C_{21}H_{20}N_2O_4$·H_2O): C, 65.96; H, 5.80; N, 7.33. Found: C, 66.42; H, 6.02; N, 6.73.

5.1.7. 1-(3-Hydroxyphenyl)-2-butyryl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (7b)

Compound **7b** (0.11 g) was synthesized from **6c** (0.20 g, 0.51 mmol) according to the procedure used to synthesize **7a** as an off-white solid in 58.0% yield. Mp: 196–198 °C. IR (cm^{-1}): 3420, 2966, 1719, 1618, 1454. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.77 (m, 3H, –COCH$_2$CH$_2$*CH*$_3$), 1.45 (m, 2H, –COCH$_2$*CH*$_2$CH$_3$) δ 2.17 (m, 2H, –CO*CH*$_2$CH$_2$CH$_3$), 3.34 (m, 2H, CH$_2$), 5.18 (m, 1H, CH), 6.00 (d, 1H, *CH*-Ar, J = 38.4), 6.49–7.45 (m, 8H, Ar-H), 9.32 (d, 1H, OH, J = 50), 10.92 (d, 1H, NH, J = 20.4), 12.50 (s, 1H, –COOH). EI-MS (*m/z*): 378 (M^+). Anal. Calcd for ($C_{22}H_{22}N_2O_4$·0.5H_2O): C, 68.20; H, 5.98; N, 7.23. Found: C, 68.36; H, 6.17; N, 6.74.

5.1.8. 1-(3-Hydroxyphenyl)-3-carboxy-1,2,3,4-tetrahydro-β-carboline-3-(*N*-(2-hydroxyethyl)) formamide (8a)

A mixture of **5** (0.5 g, 1.6 mmol) and 2-aminoethanol (5 mL) was stirred at 50 °C for 2 h. The surplus ethanolamine was then evaporated. The resulting residue was purified by column chromatography on silica gel by eluting with a mixture of dichloromethane/methanol (20:1) to yield **8a** as a white solid (0.43 g, 78.9%). Mp: 253 °C dec. IR (cm^{-1}): 3395, 3263, 1644, 1585, 1265. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.44 (m, 1H, NH), 2.72 (m, 1H, OH), 3.02 (d, 1H, CH$_2$), 3.19 (m, 2H, NHC*H*$_2$CH$_2$OH), 3.32 (m, 1H, CH$_2$), 3.45 (m, 2H, NHCH$_2$*CH*$_2$OH), 3.58 (m, 1H, CH), 5.09 (d, 1H, *CH*-Ar, J = 4.2), 6.72–7.80 (m, 9H, Ar-H, –OH), 9.31 (s, 1H, NH), 10.32 (s, 1H, NH).

EI-MS (*m/z*): 351 (M^+). Anal. Calcd for ($C_{20}H_{21}N_3O_3 \cdot 0.5H_2O$): C, 66.65; H, 6.65; N, 11.66. Found: C, 67.08; H, 6.18; N, 12.08.

5.1.9. 1-(3-Hydroxyphenyl)-3-carboxy-1,2,3,4-tetrahydro-β-carboline-3-(*N*-(2-aminoethyl)) formamide (8b)

Compound **8b** (0.40 g) was synthesized from **5** (0.5 g, 1.6 mmol), 1,2-diaminethane (5 mL) according to the procedure used to synthesize **8b** as a white solid in 73.5% yield. Mp: 185–197 °C. IR (cm^{-1}): 3400, 2946, 2676, 1708, 1648. ^1H NMR (300 MHz, DMSO-d_6): δ 0.93 (m, 2H, $-NH_2$), 1.23 (m, 1H, NH), 2.43 (m, 4H, NH$CH_2CH_2NH_2$), 2.57–3.12 (m, 3H, CH_2, CH), 5.08 (s, 1H, *CH*-Ar), 6.74–8.00 (m, 10H, Ar-H, –OH, CONH), 10.33 (s, 1H, NH). EI-MS (*m/z*): 350 (M^+). Anal. Calcd for ($C_{20}H_{22}N_4O_2$): C, 68.55; H, 6.33; N, 15.99. Found: C, 68.58; N, 6.18; H, 16.08.

5.1.10. 1-(3-Hydroxyphenyl)-6-chloro-1,2,3,4-tetrahydro-β-carboline hydrochloride (9a)

A mixture of 5-chlorotryptamine hydrochloride (2.31 g, 10 mmol) and 3-hydroxybenzaldehyde (1.34 g, 11 mmol) was dissolved in glacial acetic acid (25 mL) under N_2 protection. After stirring at 95 °C for 2 h, the mixture was cooled to room temperature. The desired compound **9a** (2.85 g, 85.1%) was abstained by filtration as a white solid. Mp: 262–264 °C dec.

5.1.11. 1-(3-Hydroxyphenyl)-6-methyl-1,2,3,4-tetrahydro-β-carboline hydrochloride (9b)

Compound **9b** (2.58 g) was synthesized from 5-methyltryptamine hydrochloride (2.12 g, 10 mmol), 3-hydroxybenzaldehyde (1.34 g, 11 mmol) according to the procedure used to synthesize **9a** as a white solid in 82.0% yield. Mp: 285 °C dec.

5.1.12. 1-(3-Hydroxyphenyl)-8-chloro-1,2,3,4-tetrahydro-β-carboline hydrochloride (9c)

Compound **9c** (2.84 g) was synthesized from 7-chlorotryptamine hydrochloride (2.31 g, 10 mmol), 3-hydroxybenzaldehyde (1.34 g, 11 mmol) according to the procedure used to synthesize **9a** as a white solid in 84.7% yield. Mp: 282 °C dec.

5.1.13. 1-(3-Hydroxyphenyl)-2-(α-(*tert*-butoxycarbonylamino)propionyl)-6-chloro-2,3,4,9-tetrahydrocarboline (10a)

A mixture of compound **9a** (0.67 g, 2 mmol), *N*-Boc β-alanine (0.42 g, 2.2 mmol), PyBOP (1.14 g, 2.2 mmol), TEA (0.5 mL) in THF (30 mL) were stirred at room temperature for 2 h. Then the mixture was filtered under vacuum. The filtrate was concentrated and purified by column chromatography on silica gel by eluting with a mixture of ethyl acetate/petroleum (1:3) to yield **10a** as a white solid (0.65 g, 69.3%). Mp: 160–162 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 1.33 (m, 12H, $-C(CH_3)_3$, $-CH_3$), δ 2.85 (m, 2H, CH_2), δ 3.23 (m, 1H, CH_2), δ 3.85 (m, 1H, CH_2), 4.63 (m, 1H, –CO*CH*(CH_3)NH–), 5.57 (d, 1H, *CH*-Ar, J = 7.8), δ 6.68–7.36 (m, 8H, Ar-H, –OH), δ 7.47 (s, 1H, –COCH(CH_3)*NH*–), δ 8.33 (s, 1H, NH). EI-MS (*m/z*): 469 (M^+).

5.1.14. 1-(3-Hydroxyphenyl)-2-(α-aminopropionyl)-6-chloro-1,2,3,4-tetrahydro-β-carboline hydrochloride (11a)

Compound **10a** (0.47 g, 1 mmol) was dissolved in ethyl acetate (20 mL), treated with HCl gas flow and stirred at room temperature for 2 h. The resulting mixture was then filtered under vacuum to obtain the pure product **11a** (0.22 g, 54.1%) as a white solid. Mp: 280 °C dec. IR (cm^{-1}): 3323, 2974, 1625, 1480, 1272. ^1H NMR (300 MHz, DMSO-d_6): δ 2.86 (m, 3H, $-CH_3$), 3.06 (m, 2H, CH_2), 3.22 (m, 2H, CH_2), 3.96 (m, 1H, CH), 6.63 (s, 1H, *CH*-Ar), 6.66–7.45 (m, 7H, Ar-H), 7.93 (s, 2H, NH_2), 9.42 (s, 1H, OH), 11.26 (s, 1H, NH). EI-MS (*m/z*): 369 (M^+). Anal. Calcd for ($C_{20}H_{20}ClN_3O_2 \cdot HCl \cdot 0.5H_2O$): C, 57.84; H, 5.34; N, 10.12. Found: C, 57.98; H, 5.18: N, 9.92.

5.1.15. 1-(3-Hydroxyphenyl)-2-(β-(*tert*-butoxycarbonylamino)propionyl)-8-chloro-2,3,4,9-tetrahydrocarboline (10b)

Compound **10b** (0.68 g) was synthesized from **9c** (0.67 g, 2 mmol), *N*-Boc-alanine (0.42 g, 2.2 mmol) according to the procedure used to synthesize **10a** as a white solid in 72.7% yield. Mp 230–232 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.35 (s, 9H, $-C(CH_3)_3$), 2.61 (m, 2H, –COCH_2CH_2NH–), 2.82 (m, 2H, CH_2), 3.20 (m, 2H, CH_2), 4.00 (m, 2H, $-CH_2$), 6.60 (s, 1H, *CH*-Ar), 6.63–7.44 (m, 8H, Ar-H, –*NH*–), 7.47 (s, 1H, –OH), 8.33 (s, 1H, NH). EI-MS (*m/z*): 469 (M^+).

5.1.16. 1-(3-Hydroxyphenyl)-2-(β-aminopropionyl)-8-chloro-1,2,3,4-tetrahydro-β-carboline hydrochloride (11b)

Compound **11b** (0.27 g) was synthesized from **10a** (0.47 g, 2.2 mmol) according to the procedure used to synthesize **11a** as a white solid in 67.0% yield. Mp: 235 °C dec. IR (cm^{-1}): 3384, 3229, 1650, 1446, 1277. ^1H NMR (300 MHz, DMSO-d_6): δ 2.95 (m, 7H, –CO$CH_2CH_2NH_2$, CH_2, CH_2), 3.92 (m, 1H, CH_2), 6.62 (s, 1H, *CH*-Ar), 6.64–7.49 (m, 7H, Ar-H), 8.01 (s, 2H, NH_2), 9.41 (s, 1H, OH), 11.39 (s, 1H, NH). EI-MS (*m/z*): 369 (M^+). Anal. Calcd for ($C_{20}H_{20}ClN_3O_2 \cdot HCl \cdot 2.5H_2O$): C, 53.22; H, 5.81; N, 9.31. Found: C, 53.38; H, 5.56; N, 9.43.

5.1.17. 1-(3-Hydroxyphenyl)-2-(*p*-aminobenzoyl)-6-chloro-1,2,3,4-tetrahydro-β-carboline (12a)

Compound **12a** (0.52 g) was synthesized from **9a** (0.67 g, 2 mmol), PyBOP (1.14 g, 2.2 mmol), 4-aminobenzoic acid (0.30 g, 2.2 mmol) according to the procedure used to synthesize **10a** as a white solid in 62.0% yield. Mp: 220–222 °C. IR (cm^{-1}): 3365, 2606, 1623, 1441, 1268. ^1H NMR (300 MHz, DMSO-d_6): δ 2.83 (m, 2H, CH_2), 3.28 (m, 2H, CH_2), 3.95 (m, 2H, NH_2), 5.53 (s, 1H, *CH*-Ar), δ 6.56–7.51 (m, 11H, Ar-H), 9.41 (s, 1H, OH), 11.24 (s, 1H, NH). EI-MS (*m/z*): 417 (M^+). Anal. Calcd for ($C_{24}H_{20}ClN_3O_2 \cdot 0.5H_2O$): C, 67.52; H, 4.96; N, 9.84. Found: C, 67.71; H, 4.95: N, 9.99.

5.1.18. 1-(3-Hydroxyphenyl)-2-(*p*-aminobenzoyl)-6-methyl-1,2,3,4-tetrahydro-β-carboline (12b)

Compound **12b** (0.46 g) was synthesized from **9b** (0.63 g, 2 mmol), PyBOP (1.14 g, 2.2 mmol), 4-aminobenzoic acid (0.30 g, 2.2 mmol) according to the procedure used to synthesize **10a** as a white solid in 58.5% yield. Mp: 136–139 °C. IR (cm^{-1}): 3380, 2921, 1728, 1608, 1458. ^1H NMR (300 MHz, DMSO-d_6): δ 2.43 (s, 3H, $-CH_3$), 2.76 (m, 1H, CH_2), 2.76 (m, 1H, CH_2), 2.88 (m, 1H, CH_2), 3.38 (m, 1H, CH_2), 3.95 (s, 2H, NH_2), 5.57 (s, 1H, *CH*-Ar), 6.55–7.33 (m, 11H, Ar-H), 9.44 (s, 1H, OH), 10.91 (s, 1H, NH). EI-MS (*m/z*): 397 (M^+). Anal. Calcd for ($C_{25}H_{23}N_3O_2 \cdot H_2O$): C, 72.27; H, 6.06; N, 10.11. Found: C, 72.77; H, 6.32; N, 10.17.

5.1.19. 6-Chloro-1-(3-hydroxyphenyl)-2-methyl-1,2,3,4-tetrahydro-β-carboline (13a)

A mixture of compound **9a** (0.67 g, 2.0 mmol), K_2CO_3 (0.2 g, 2.0 mmol), formaldehyde (37%, 0.65 mL, 4.0 mmol), formic acid (0.37 g, 4.0 mmol) in DMF (10 mL) was stirred at 150 °C for 3 h. The mixture was then poured into water (30 mL) and filtered to obtain white solid which was purified by column chromatography on silica gel by eluting with a mixture of ethyl acetate/petroleum (1:1) to yield **13a** as an amber solid (0.38 g, 61.5%). Mp: 252–256 °C. IR (cm^{-1}): 3293, 2961, 1581, 1261, 1097. ^1H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H, $-CH_3$), 2.65 (m, 1H, CH_2), 2.78 (m, 1H, CH_2), 2.85 (m, 1H, CH_2), 3.14 (m, 1H, CH_2), 4.33 (s, 1H, *CH*-Ar), 6.74–7.50 (m, 7H, Ar-H), 9.35 (s, 1H, –OH), 10.40 (s, 1H, NH). EI-MS (*m/z*): 312 (M^+). Anal. Calcd for ($C_{18}H_{17}ClN_2O$): C, 69.12; H, 5.48; N, 8.96. Found: C, 69.19; H, 5.68; N, 8.70.

5.1.20. 6-Chloro-1-(3-hydroxyphenyl)-2-propyl-1,2,3,4-tetrahydro-β-carboline (13b)

Compound **13b** (0.43 g) was synthesized from **9a** (0.67 g, 2.0 mmol), propionaldehyde (0.23 g, 4.0 mmol) according to the procedure used to synthesize **13a** as a white solid in 63.4% yield. Mp: 99–101 °C. IR (cm^{-1}): 3415, 2959, 1598, 1454, 1304. ^1H NMR (300 MHz, DMSO-d_6): δ 0.85 (m, 3H, $-CH_2CH_2CH_3$), 1.47 (m, 2H, $-CH_2CH_2CH_3$), 2.43 (m, 2H, $-CH_2CH_2CH_3$), 2.73 (m, 1H, CH_2), 2.89 (m, 2H, CH_2), 3.12 (m, 1H, CH_2), 4.54 (s, 1H, *CH*-Ar), 6.65–7.43 (m, 7H, Ar-H), 9.27 (s, 1H, OH), 10.44 (s, 1H, NH). EI-MS (*m/z*): 340 (M^+). Anal. Calcd for ($C_{20}H_{21}ClN_2O$): C, 70.48; H, 6.21; N, 8.22. Found: C, 70.12; H, 6.68; N, 8.00.

5.1.21. 6-Chloro-1-(3-hydroxyphenyl)-2-benzyl-1,2,3,4-tetrahydro-β-carboline (13c)

Compound **13c** (0.59 g) was synthesized from **9a** (0.67 g, 2.0 mmol) and benzaldehyde (0.42 g, 4.0 mmol) according to the procedure used to synthesize **13a** as a white solid in 75.5% yield. Mp: 148–150 °C. IR (cm^{-1}): 3410, 3299, 1653, 1452, 1284. ^1H NMR (300 MHz, $CDCl_3$): δ 2.67 (m, 2H, CH_2), 2.9 (m, 1H, CH_2), 3.24 (m, 1H, CH_2), 3.39 (d, 1H, Ph-CH_2, J = 133), 3.92 (d, 1H, Ph-CH_2, J = 127), 4.61 (s, 1H, *CH*-Ar), 5.45 (s, 1H, –OH), 6.83–7.45 (m, 12H, Ar-H), 8.00 (s, 1H, NH). EI-MS (*m/z*): 388 (M^+). Anal. Calcd for ($C_{24}H_{21}ClN_2O$): C, 74.12; H, 5.44; N, 7.20. Found: C, 74.32; H, 5.40; N, 7.02.

5.1.22. 6-Methyl-1-(3-hydroxyphenyl)-2-methyl-1,2,3,4-tetrahydro-β-carboline (13d)

Compound **13d** (0.42 g) was synthesized from **9b** (0.63 g, 2.0 mmol), formaldehyde (37%, 0.65 mL, 4.0 mmol) according to the procedure used to synthesize **13a** as a white solid in 72.0% yield. Mp: 217–219 °C. IR (cm^{-1}): 3331, 2937, 1601, 1451, 1254. ^1H NMR (300 MHz, DMSO-d_6): δ 2.21 (s, 3H, $-NCH_3$), 2.35 (s, 3H, Ar-CH_3), 2.60 (m, 1H, CH_2), 2.78 (m, 1H, CH_2), 2.85 (m, 1H, CH_2), 3.08 (m, 1H, CH_2), 4.25 (s, 1H, *CH*-Ar), 6.67–7.18 (m, 7H, Ar-H), 9.27 (s, 1H, –OH), 9.96 (s, 1H, NH). EI-MS (*m/z*): 292 (M^+). Anal. Calcd for ($C_{19}H_{20}N_2O$): C, 78.05; H, 6.89; N, 9.58. Found: C, 78.37; H, 6.65; N, 9.49.

5.1.23. 6-Methyl-1-(3-hydroxyphenyl)-2-benzyl-1,2,3,4-tetrahydro-β-carboline (13e)

Compound **13e** (0.57 g) was synthesized from **9b** (0.63 g, 2.0 mmol), benzaldehyde (0.42 g, 4.0 mmol) according to the procedure used to synthesize **13a** as a white solid in 77.4% yield. Mp: 144–145 °C. IR (cm^{-1}): 3324, 1653, 1599, 1460, 1386. ^1H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, $-CH_3$), 2.79 (m, 3H, CH_2, CH_2), 3.21 (m, 1H, CH_2), 3.38 (d, 1H, Ph-CH_2, J = 150), 3.92 (d, 1H, Ph-CH_2, J = 150), 4.59 (s, 1H, *CH*-Ar), 5.80 (s, 1H, –OH), 6.81–7.34 (m, 12H, Ar-H), 7.99 (s, 1H, NH). EI-MS (*m/z*): 368 (M^+). Anal. Calcd for ($C_{25}H_{24}N_2O$): C, 81.49; H, 6.57; N, 7.60. Found: C, 81.56; H, 6.83; N, 7.62.

5.1.24. 6-Methyl-1-(3-benzyloxyphenyl)-1,2,3,4-tetrahydro-β-carboline hydrochloride (14a)

Compound **14a** (3.1 g) was synthesized from 5-methyltryptamine hydrochloride (2.1 g, 10 mmol), 3-(benzyloxy)benzaldehyde (2.1 g, 10 mmol) according to the procedure used to synthesize **9a** as a white solid in 76.5% yield. Mp: 260–262 °C.

5.1.25. 6-Chloro-1-(3-isopropyloxyphenyl)-1,2,3,4-tetrahydro-β-carboline hydrochloride (14b)

Compound **14b** (0.28 g) was synthesized from 3-isopropoxybenzaldehyde (3.28 g, 20 mmol), 5-methyltryptamine hydrochloride (4.6 g, 20 mmol) according to the procedure used to synthesize **9a** as a white solid in 85.5% yield. Mp: 264–265 °C.

5.1.26. 6-Methyl-1-(3-benzyloxyphenyl)-2-(2-phthalimide-ethyl)-1,2,3,4-tetrahydro-β-carboline (15a)

A mixture of **14a** (1.98 g, 4.9 mmol), *N*-(2-bromoethyl)phthalimide (1.37 g, 5.4 mmol), K_2CO_3 (0.68 g, 4.9 mmol) in DMF (30 mL) was stirred at 150 °C for 10 h under the protection of N_2. The mixture was then poured into water (30 mL) and filtered to obtain yellow solid which was purified by column chromatography on silica gel by eluting with a mixture of ethyl acetate/petroleum (1:3) to yield **15a** as a white solid (1.12 g, 41.9%). Mp: 183–186 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 2.34 (s, 3H, ArCH_3), 2.56 (m, 2H, CH_2), 2.72 (m, 2H, CH_2), 3.55 (m, 2H, CH_2), 3.90 (m, 2H, CH_2), 4.54 (s, 1H, *CH*-Ar), 4.86 (s, 2H, CH_2–O), 6.53–8.40 (m, 16H, Ar-H), 11.38 (s, 1H, NH). EI-MS (*m/z*): 541 (M^+).

5.1.27. 6-Methyl-1-(3-benzyloxyphenyl)-2-(3-phthalimide-propyl)-1,2,3,4-tetrahydro-β-carboline (15b)

Compound **15b** (0.92 g) was synthesized from **14a** (1.5 g, 3.7 mmol), *N*-(2-bromopropyl)phthalimide (1.07 g, 4 mmol) according to the procedure used to synthesize **15a** as a white solid in 45.0% yield. Mp: 103–105 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.24 (m, 2H, CH_2), 2.19 (s, 3H, ArCH_3), 2.65 (m, 2H, CH_2), 2.85 (m, 2H, CH_2), 3.71 (m, 1H, CH_2), 3.87 (m, 2H, CH_2), 4.11 (m, 1H, CH_2), 5.05 (s, 1H, *CH*-Ar), 5.19 (s, 2H, CH_2–O), 6.89–7.83 (m, 16H, Ar-H), 10.98 (s, 1H, NH). EI-MS (*m/z*): 555 (M^+).

5.1.28. 6-Methyl-1-(3-benzyloxyphenyl)-2-(4-phthalimide-butyl)-1,2,3,4-tetrahydro-β-carboline (15c)

Compound **15c** (0.79 g) was synthesized from **14a** (1.5 g, 3.7 mmol), *N*-(4-bromobutyl)phthalimide (1.13 g, 4 mmol) according to the procedure used to synthesize **14a** as a white solid in 37.5% yield. Mp: 133–136 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.20 (m, 4H, CH_2CH_2), 2.39 (s, 3H, ArCH_3), 2.72 (m, 2H, CH_2), 2.83 (m, 2H, CH_2), 3.87 (m, 4H, 2× CH_2), 5.02 (s, 1H, *CH*-Ar), 5.19 (s, 2H, CH_2–O), 7.02–7.834 (m, 16H, Ar-H), 10.97 (s, 1H, NH). EI-MS (*m/z*): 569 (M^+).

5.1.29. 6-Chloro-1-(3-isopropyloxyphenyl)-2-(2-phthalimide-ethyl)-1,2,3,4-tetrahydro-β-carboline (15d)

Compound **15d** (0.70 g) was synthesized from **14b** (1.5 g, 4 mmol), *N*-(2-bromoethyl)phthalimide (1.02 g, 4 mmol) according to the procedure used to synthesize **15a** as a white solid in 34.0% yield. Mp: 172–173 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.32 (m, 6H, $-CH(CH_3)_2$), 2.49 (m, 4H, CH_2, $-NCH_2CH_2N-$), 3.30 (m, 2H, CH_2), 3.63 (m, 2H, $-NCH_2CH_2N-$), 4.75 (m, 1H, $-CH(CH_3)_2$), 4.95 (s, 1H, *CH*-Ar,), 7.06–8.48 (m, 11H, Ar-H), 11.63 (s, 1H, NH). EI-MS (*m/z*): 513 (M^+).

5.1.30. 6-Chloro-1-(3-isopropyloxyphenyl)-2-(3-phthalimide-propyl)-1,2,3,4-tetrahydro-β-carboline (15e)

Compound **15e** (0.71 g) was synthesized from **14b** (1.5 g, 4 mmol), *N*-(2-bromopropyl)phthalimide (1.07 g, 4 mmol) according to the procedure used to synthesize **15a** as a white solid in 42.6% yield. Mp: 190–192 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 0.82 (m, 6H, $-CH(CH_3)_2$), 1.81 (m, 2H, $-NCH_2CH_2CH_2N-$), 2.69 (m, 4H, CH_2, $-NCH_2CH_2CH_2N-$), 3.08 (m, 2H, CH_2), 3.63 (m, 2H, $-NCH_2CH_2CH_2N-$), 4.53 (m, 1H, $-CH(CH_3)_2$), 4.65 (s, 1H, *CH*-Ar), 6.73–7.84 (m, 11H, Ar-H), 10.53 (s, 1H, NH). EI-MS (*m/z*): 527 (M^+).

5.1.31. 6-Chloro-1-(3-isopropyloxyphenyl)-2-(4-phthalimide-butyl)-1,2,3,4-tetrahydro-β-carboline (15f)

Compound **15f** (0.81 g) was synthesized from **14b** (1.5 g, 4 mmol), *N*-(4-bromobutyl)phthalimide (1.13 g, 4 mmol) according to the procedure used to synthesize **15a** as a white solid in 37.5% yield. Mp: 150–152 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.19 (m, 6H, $-CH(CH_3)_2$), 1.55 (m, 4H, $-NCH_2CH_2CH_2CH_2N-$), 2.65 (m, 4H, CH_2, $-NCH_2CH_2CH_2CH_2N-$), 3.12 (m, 2H, CH_2), 3.52 (m,

2H, –NCH$_2$CH$_2$CH$_2$CH$_2$N–), 4.49 (m, 1H, –CH(CH$_3$)$_2$), 4.59 (s, 1H, CH-Ar,), 6.76–7.88 (m, 11H, Ar-H), 10.45 (s, 1H, NH). EI-MS (*m/z*): 541 (M^+).

5.1.32. 6-Methyl-1-(3-benzyloxyphenyl)-2-(2-aminoethyl)-1,2,3,4-tetrahydro-β-carboline (16a)

To a solution of **15a** (1.0 g, 1.8 mmol) in alcohol (50 mL) was added NH$_2$–NH$_2$·H$_2$O (0.5 mL). The solution was stirred at 80 °C for 3 h, then cooled to room temperature and filtered under vacuum. The filtrate was added sodium hydroxide aqueous (10%, 2 mL). The resulting mixture was evaporated to dryness and purified by column chromatography on silica gel by eluting with a mixture of dichloromethane/methanol (20:1) to yield **16a** as a white solid (0.55 g, 72.4%). Mp: 166–170 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.20 (m, 2H, –NH$_2$), 2.22 (s, 3H, ArCH$_3$), 2.70 (m, 4H, –CH$_2$CH$_2$NH$_2$), 3.15 (m, 4H, 2× CH$_2$), 5.05 (s, 1H, CH-Ar), 5.25 (s, 2H, ArCH$_2$-O), 6.81–8.50 (m, 12H, Ar-H), 10.12 (s, 1H, NH). EI-MS (*m/z*): 411 (M^+).

5.1.33. 6-Methyl-1-(3-benzyloxyphenyl)-2-(3-aminopropyl)-1,2,3,4-tetrahydro-β-carboline (16b)

Compound **16b** (0.48 g) was synthesized from **15b** (1.0 g, 1.8 mmol)), NH$_2$–NH$_2$·H$_2$O (0.5 mL) according to the procedure used to synthesize **16a** as a white solid in 62.7% yield. Mp: 89–91 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.54 (m, 2H, –CH$_2$CH$_2$CH$_2$NH$_2$), 1.60 (m, 2H, –NH$_2$), 2.35 (s, 3H, ArCH$_3$), 2.72 (m, 4H, CH$_2$CH$_2$CH$_2$NH$_2$), 3.17 (m, 4H, 2CH$_2$), 4.48 (s, 1H, CH-Ar), 5.05 (s, 2H, ArCH$_2$-O), 6.80–7.43 (m, 12H, Ar-H), 10.07 (s, 1H, NH). EI-MS (*m/z*): 425 (M^+).

5.1.34. 6-methyl-1-(3-benzyloxyphenyl)-2-(4-aminobutyl)-1,2,3,4-tetrahydro-β-carboline (16c)

Compound **16c** (0.50 g) was synthesized from **15c** (1 g, 1.8 mmol), NH$_2$–NH$_2$·H$_2$O (0.5 mL) according to the procedure used to synthesize **16a** as a white solid in 64.8% yield. Mp: 108–110 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.18 (m, 2H, –NH$_2$), 2.36 (m, 7H, Ar-CH$_3$, N–CH$_2$CH$_2$CH$_2$CH$_2$NH$_2$), 2.47 (m, 6H, N–CH$_2$CH$_2$CH$_2$CH$_2$NH$_2$, CH$_2$), 2.90 (m, 2H, CH$_2$), 5.19 (s, 1H, CH-Ar), 5.25 (s, 2H, ArCH$_2$-O), 6.81–8.48 (m, 12H, Ar-H), 10.14 (s, 1H, NH). EI-MS (*m/z*): 439 (M^+).

5.1.35. 6-Chloro-1-(3-isopropyloxyphenyl)-2-(2-aminoethyl)-1,2,3,4-tetrahydro-β-carboline (16d)

Compound **16d** (0.61 g) was synthesized from **15d** (1 g, 1.95 mmol), NH$_2$–NH$_2$·H$_2$O (0.5 mL) according to the procedure used to synthesize **16a** as a white solid in 82.0% yield. Mp: 171–173 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.94 (m, 6H, –CH(CH$_3$)$_2$), 1.83 (s, 2H, NH$_2$), 2.72 (m, 6H, 2× CH$_2$, –NCH$_2$CH$_2$NH$_2$), 3.12 (m, 2H, –NCH$_2$CH$_2$NH$_2$), 4.54 (m, 1H, –CH(CH$_3$)$_2$), 4.65 (s, 1H, CH-Ar), 6.77–7.45 (m, 7H, Ar-H), 10.52 (s, 1H, NH). EI-MS (*m/z*): 383 (M^+).

5.1.36. 6-Chloro-1-(3-isopropyloxyphenyl)-2-(3-aminopropyl)-1,2,3,4-tetrahydro-β-carboline (16e)

Compound **16e** (0.61 g) was synthesized from **15e** (1 g, 1.90 mmol), NH$_2$–NH$_2$·H$_2$O (0.5 mL) according to the procedure used to synthesize **16a** as a white solid in 71.0% yield. Mp: 190–192 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.04 (m, 6H, –CH(CH$_3$)$_2$), 1.56 (m, 2H, –NCH$_2$CH$_2$CH$_2$N–), 1.83 (s, 2H, NH$_2$), 2.55 (m, 4H, CH$_2$, –NCH$_2$CH$_2$CH$_2$N–), 2.74 (m, 2H, CH$_2$), 3.12 (m, 2H, –NCH$_2$CH$_2$CH$_2$N–), 4.54 (m, 1H, –CH(CH$_3$)$_2$), 4.59 (s, 1H, CH-Ar), 6.78–7.44 (m, 7H, Ar-H), 10.49 (s, 1H, NH). EI-MS (*m/z*): 397 (M^+).

5.1.37. 6-Chloro-1-(3-isopropyloxyphenyl)-2-(4-aminobutyl)-1,2,3,4-tetrahydro-β-carboline (16f)

Compound **16f** (0.50 g) was synthesized from **15f** (1 g, 1.85 mmol), NH$_2$–NH$_2$.H$_2$O (0.5 mL) according to the procedure used to synthesize **16a** as a white solid in 65.5% yield. Mp: 148–150 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25 (m, 6H, –CH(CH$_3$)$_2$), 1.33 (m, 4H, –NCH$_2$CH$_2$CH$_2$CH$_2$N–), 1.51 (m, 2H, NH$_2$), 2.43 (m, 2H, CH$_2$), 2.62 (m, 2H, CH$_2$), 2.73 (m, 2H, CH$_2$), 3.11 (m, 2H, CH$_2$), 4.55 (m, 1H, –CH(CH$_3$)$_2$), 4.60 (s, 1H, CH-Ar), 6.78–7.44 (m, 7H, Ar-H), 10.48 (s, 1H, NH). EI-MS (*m/z*): 411 (M^+).

5.1.38. 6-Methyl-1-(3-hydroxyphenyl)-2-(2-aminoethyl)-1,2,3,4-tetrahydro-β-carboline (17a)

To a solution of compound **16a** (0.2 g, 3.7 × 10^{-4} mol) in methanol (50 mL) was added Pd–C (10%, 0.05 g), HCOONH$_4$ (0.2 g). The mixture was stirred at room temperature for 3 h, then the Pd–C was filtered and the solvent was concentrated to dryness and partitioned between water (20 mL) and acetyl acetate (20 mL). The aqueous layer was extracted with acetyl acetate (3× 30 mL). The combined organic phase was evaporated and purified by column chromatography on silica gel by eluting with a mixture of dichloromethane: methanol (20:1) to yield **17a** as a white solid (0.14 g, 84.0%). Mp: 115–117 °C. IR (cm^{-1}): 3410, 2926, 1595, 1459, 1308. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.74 (m, 2H, NH$_2$), 2.27 (s, 3H, Ar-CH$_3$), 2.66 (m, 6H, N–CH$_2$CH$_2$NH$_2$, CH$_2$), 3.17 (m, 2H, CH$_2$), 4.57 (d, 1H, CH-Ar, *J* = 14.4), 6.64–7.18 (m, 8H, Ar-H, –OH), 10.11 (s, 1H, –NH). EI-MS (*m/z*): 321 (M^+). Anal. Calcd for (C$_{20}$H$_{23}$N$_3$O·0.5H$_2$O): C, 72.69; H, 7.32; N, 12.72. Found: C, 72.41; H, 7.68; N, 12.56.

5.1.39. 6-Methyl-1-(3-hydroxyphenyl)-2-(3-aminopropyl)-1,2,3,4-tetrahydro-β-carboline (17b)

Compound **17b** (0.27 g) was synthesized from **16b** (0.4 g, 0.94 mmol) according to the procedure used to synthesize **17a** as a white solid in 87.0% yield. Mp: 101–103 °C. IR (cm^{-1}): 3408, 2931, 1597, 1460, 1313. ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.04 (m, 2H, N–CH$_2$CH$_2$CH$_2$NH$_2$), 1.40 (m, 2H, NH$_2$), 2.41 (s, 3H, Ar-CH$_3$), 2.65 (m, 4H, N–CH$_2$CH$_2$CH$_2$NH$_2$), 2.90 (m, 2H, CH$_2$), 3.36 (m, 2H, CH$_2$), 4.34 (s, 1H, CH-Ar), 6.73–7.25 (m, 8H, Ar-H, OH). EI-MS (*m/z*): 335 (M^+). Anal. Calcd for (C$_{21}$H$_{25}$N$_3$O.0.75H$_2$O): C, 72.28; H, 7.65; N, 12.04. Found: C, 72.16; H, 7.87; N, 11.99.

5.1.40. 6-Methyl-1-(3-hydroxyphenyl)-2-(4-aminobutyl)-1,2,3,4-tetrahydro-β-carboline (17c)

Compound **17c** (0.28 g) was synthesized from **16c** (0.4 g, 0.91 mmol) according to the procedure used to synthesize **17a** as a white solid in 89.2% yield. Mp: 200 °C dec. IR (cm^{-1}): 3410, 2941, 1598, 1452, 1278. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.63 (m, 2H, NH$_2$), 2.30 (m, 7H, Ar-CH$_3$, N–CH$_2$CH$_2$CH$_2$CH$_2$NH$_2$), 2.47 (m, 6H, N–CH$_2$CH$_2$CH$_2$CH$_2$NH$_2$, CH$_2$), 2.90 (m, 2H, CH$_2$), 4.41 (s, 1H, CH-Ar), 6.46–7.24 (m, 7H, Ar-H), 7.25 (s, 1H, –OH), 10.32 (s, 1H, NH). EI-MS (*m/z*): 349 (M^+). Anal. Calcd for (C$_{22}$H$_{27}$N$_3$O·0.75H$_2$O): C, 72.80; H, 7.91; N, 11.57. Found: C, 72.57; H, 7.83; N, 12.17.

5.1.41. 6-Chloro-1-(3-hydroxyphenyl)-2-(2-aminoethyl)-1,2,3,4-tetrahydro-β-carboline (17d)

To solution of **17d** (0.4 g, 1.04 mmol) in CH$_2$Cl$_2$ cooled in an ice bath (50 mL) was added AlCl$_3$ (0.2 g). After stirring at room temperature for 2 h, the solvent was concentrated to dryness and partitioned between saturate NaHCO$_3$ aqueous solution (20 mL) and acetyl acetate (20 mL). The insoluble substance was removed by filtration and the aqueous layer was extracted with acetyl acetate (3× 30 mL). The combined organic phase was evaporated and purified by column chromatography on silica gel by eluting with a mixture of dichloromethane/methanol (20:1) to yield **17d** as a white solid (0.26 g, 70.5%). Mp: 125–128 °C. IR (cm^{-1}): 3413, 1603, 1453, 1313, 799. ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.72 (m, 2H, NH$_2$), 2.21 (m, 1H, CH$_2$), 2.73 (m, 4H, N–CH$_2$CH$_2$NH$_2$), 3.17 (m, 2H, CH$_2$), 4.42 (s, 1H, CH-Ar), 6.69–7.60 (m, 7H, Ar-H), 7.61 (s, 1H,

–OH). EI-MS (*m/z*): 341 (M^+). Anal. Calcd for ($C_{19}H_{20}ClN_3O\cdot0.5H_2O$): C, 65.04; H, 6.03; N, 11.98. Found: C, 65.23; H, 6.05; N, 12.13.

5.1.42. 6-Chloro-1-(3-hydroxyphenyl)-2-(3-aminopropyl)-1,2,3,4-tetrahydro-β-carboline (17e)

Compound **17e** (0.50 g) was synthesized from **16e** (0.4 g, 1.00 mmol) according to the procedure used to synthesize **17d** as a white solid in 68.0% yield. Mp: 113–115 °C. IR (cm^{-1}): 3417, 1650, 1605, 1465, 1267. ^1H NMR (300 MHz, $CDCl_3$): δ 1.05 (m, 2H, N–$CH_2CH_2CH_2NH_2$), 1.45 (s, 2H, NH_2), 2.55 (m, 6H, N–$CH_2CH_2CH_2NH_2$, CH_2), 3.39 (m, 2H, CH_2), 4.39 (s, 1H, *CH*-Ar), 6.71–7.40 (m, 8H, Ar-H, OH). EI-MS (*m/z*): 355 (M^+). Anal. Calcd for ($C_{20}H_{22}ClN_3O\cdot0.77H_2O$): C, 64.97; H, 6.42; N, 11.36. Found: C, 64.88; H, 6.90; N, 10.87.

5.1.43. 6-Chloro-1-(3-hydroxyphenyl)-2-(3-aminobutyl)-1,2,3,4-tetrahydro-β-carboline (17f)

Compound **17f** (0.21 g) was synthesized from **16f** (0.4 g, 0.97 mmol) according to the procedure used to synthesize **16d** as a white solid in 57.5% yield. Mp: 180–182 °C. IR (cm^{-1}): 3288, 2936, 1600, 1452, 1286. ^1H NMR (300 MHz, DMSO-d_6): δ 1.19 (m, 4H, –$NCH_2CH_2CH_2CH_2N$–), 1.44 (m, 2H, NH_2), 2.55 (m, 6H, –$NCH_2CH_2CH_2CH_2NH_2$, CH_2), 3.63 (m, 2H, CH_2), 4.55 (s, 1H, *CH*-Ar), 6.66–7.44 (m, 8H, Ar-H, –OH), 10.48 (s, 1H, NH). EI-MS (*m/z*): 369 (M^+). Anal. Calcd for ($C_{21}H_{24}ClN_3O\cdot0.5H_2O$): C, 66.57; H, 6.65; N, 11.09. Found: C, 66.62; H, 6.87; N, 11.48.

5.1.44. 6-Methyl-1-(indol-3-yl)-1,2,3,4-tetrahydro-β-carboline (18a)

A mixture of 5-methyltryptamine (2.21 g, 10 mmol), 1H-indole-3-carbaldehyde (1.45 g, 10.00 mmol) in toluene (40 mL) was stirred at 130 °C for 3 h. Then evaporate most of the toluene and the resulting residue was filtered under vacuum to obtain a light yellow solid. To a solution of the solid in $CHCl_3$ (20 mL), CF_3COOH (8 mL) in $CHCl_3$ (10 mL) was added by dropwise. After stirring at room temperature for 24 h, the mixture was poured into water (100 mL). The pH of the aqueous layer was adjusted to 9 by adding ammonia water. Afterward, the mixture was extracted with ethyl acetate (3× 100 mL) and dried over Na_2SO_4. Removal of the solvent in vacuo afforded the crude product which was purified by column chromatography on silica gel by eluting with a mixture of dichloromethane/methanol (20:1) to give the desired product **18a** as an amber solid (1.47 g, 48.7%). Mp: 164–166 °C.

5.1.45. 6-Chloro-1-(indol-3-yl)-1,2,3,4-tetrahydro-β-carboline (18b)

Compound **18b** (1.35 g) was synthesized from 5-chlorotryptamine (1.8 g, 9.25 mmol) and 1H-indole-3-carbaldehyde (1.34 g, 9.25 mmol) according to the procedure used to synthesize **18a** as a white solid in 52.5% yield. Mp: 135–137 °C.

5.1.46. 6-Methyl-1-(indol-3-yl)-2-acetyl-1,2,3,4-tetrahydro-β-carboline (19a)

To a solution of **18a** (0.30 g, 1.0 mmol), TEA (0.2 mL) in THF (20 mL) was added acetyl chloride (0.11 mL, 1.5 mmol) over a period of 20 min. The mixture was stirred at room temperature for 2 h, and then was purified by column chromatography on silica gel by eluting with a mixture of petroleum ether/ethyl acetate (3:1) to yield title compound **19a** as a white solid (0.26 g, 77.0%). Mp: 171–173 °C. IR (cm^{-1}): 3409, 3260, 1622, 1442, 753. ^1H NMR (300 MHz, DMSO-d_6): δ 2.13 (s, 3H, –$COCH_3$), 2.38 (s, 3H, Ar-CH_3), 2.75 (m, 1H, CH_2), 2.84 (m, 1H, CH_2), 3.37 (m, 1H, CH_2), 3.90 (m, 1H, CH_2), 6.77 (s, 1H, *CH*-Ar), 6.78–8.30 (m, 8H, Ar-H), 10.75 (s, 1H, –NH), 10.96 (s, 1H, NH). EI-MS (*m/z*): 343 (M^+). Anal. Calcd for ($C_{22}H_{21}N_3O$): C, 76.94; H, 6.16; N, 12.24. Found: C, 76.89; H, 6.34; N, 12.50.

5.1.47. 6-Methyl-1-(indol-3-yl)-2-butyryl-1,2,3,4-tetrahydro-β-carboline (19b)

Compound **19b** (0.18 g) was synthesized from **18a** (0.30 g, 1.0 mmol), butyryl chloride (0.16 mL, 1.5 mmol) according to the procedure used to synthesize **19a** as a white solid in 48.5% yield. Mp: 175–178 °C. IR (cm^{-1}): 3416, 3267, 2921, 1620, 1466. ^1H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, 3H, –$CH_2CH_2CH_3$), 1.58 (m, 2H, –$CH_2CH_2CH_3$), 2.39 (m, 5H, –$CH_2CH_2CH_3$, Ar-CH_3), 2.78 (m, 2H, CH_2), 3.32 (m, 1H, CH_2), 3.96 (m, 1H, CH_2), 6.78 (s, 1H, *CH*-Ar), 6.79–7.60 (m, 8H, Ar-H), 10.76 (s, 1H, –NH), 10.96 (s, 1H, NH). EI-MS (*m/z*): 371 (M^+). Anal. Calcd for ($C_{24}H_{25}N_3O$): C, 77.60; H, 6.78; N, 11.31. Found: C, 77.24; H, 6.77; N, 11.32.

5.1.48. 6-Methyl-1-(indol-3-yl)-2-isobutyryl-1,2,3,4-tetrahydro-β-carboline (19c)

Compound **19c** (0.24 g) was synthesized from **18a** (0.30 g, 1.0 mmol), isobutyryl chloride (0.16 mL, 1.5 mmol) according to the procedure used to synthesize **19a** as a white solid in 64.7% yield. Mp: 186–189 °C. IR (cm^{-1}): 3414, 3236, 1611, 1469, 745. ^1H NMR (300 MHz, DMSO-d_6): δ 1.04 (m, 6H, –$CH(CH_3)_2$), 2.39 (s, 3H, Ar-CH_3), 2.80 (s, 2H, CH_2), 2.96 (m, 1H, –$CH(CH_3)_2$), 3.42 (m, 1H, CH_2), 4.03 (m, 1H, CH_2), 6.77 (s, 1H, *CH*-Ar), 6.78–7.55 (m, 8H, Ar-H), 10.78 (s, 1H, –NH), 10.95 (s, 1H, NH). EI-MS (*m/z*): 371 (M^+). Anal. Calcd for ($C_{24}H_{25}N_3O$): C, 77.60; H, 6.78; N, 11.31. Found: C, 77.19; H, 6.80; N, 11.15.

5.1.49. 6-Chloro-1-(indol-3-yl)-2-acetyl-1,2,3,4-tetrahydro-β-carboline (19d)

Compound **19d** (0.19 g) was synthesized from **18b** (0.32 g, 1.0 mmol), acetyl chloride (0.11 mL, 1.5 mmol) according to the procedure used to synthesize **19a** as an amber solid in 52.8% yield. Mp: 203–205 °C. IR (cm^{-1}): 3421, 2676, 1625, 1475, 1036. ^1H NMR (300 MHz, DMSO-d_6): δ 2.13 (s, 3H, –$COCH_3$), 2.82 (m, 1H, CH_2), 3.37 (m, 1H, CH_2), 3.91 (m, 1H, CH_2), 3.49 (m, 1H, CH_2), 6.80 (s, 1H, *CH*-Ar), 6.95–7.60 (m, 8H, Ar-H), 10.99 (s, 1H, –NH), 11.15 (s, 1H, NH). EI-MS (*m/z*): 363 (M^+). Anal. Calcd for ($C_{21}H_{18}ClN_3O$): C, 69.32; H, 4.99; N, 11.55. Found: C, 69.28; H, 5.18; N, 11.61.

5.1.50. 6-Chloro-1-(indol-3-yl)-2-butyryl-1,2,3,4-tetrahydro-β-carboline (19e)

Compound **19e** (0.26 g) was synthesized from **18b** (0.32 g, 1.0 mmol), butyryl chloride (0.16 mL, 1.5 mmol) according to the procedure used to synthesize **19a** as a white solid in 67.0% yield. Mp: 248–249 °C. IR (cm^{-1}): 3426, 3245, 1620, 1445, 1208. ^1H NMR (300 MHz, DMSO-d_6): δ 0.89 (m, 3H, –$CH_2CH_2CH_3$), 1.58 (m, 2H, –$CH_2CH_2CH_3$), 2.39 (m, 2H, –$CH_2CH_2CH_3$), 2.82 (m, 2H, CH_2), 3.32 (m, 1H, CH_2), 3.96 (m, 1H, CH_2), 6.80 (s, 1H, *CH*-Ar), 6.95–7.60 (m, 8H, Ar-H), 10.99 (s, 1H, –NH), 11.16 (s, 1H, NH). EI-MS (*m/z*): 391 (M^+). Anal. Calcd for ($C_{23}H_{22}ClN_3O$): C, 70.49; H, 5.66; N, 10.72. Found: C, 70.24; H, 5.69; N, 10.61.

5.1.51. 6-Chloro-1-(indol-3-yl)-2-isobutyryl-1,2,3,4-tetrahydro-β-carboline (19f)

Compound **19f** (0.23 g) was synthesized from **18b** (0.32 g, 1.0 mmol), isobutyryl chloride (0.16 mL, 1.5 mmol) according to the procedure used to synthesize **19a** as a white solid in 60.1% yield. Mp: 205 °C dec. IR (cm^{-1}): 3306, 2976, 1587, 1438, 1228. ^1H NMR (300 MHz, DMSO-d_6): δ 1.04 (m, 6H, –$CH(CH_3)_2$), 2.82 (d, 2H, CH_2, J = 5.52), 2.96 (m, 1H, –$CH(CH_3)_2$), 3.34 (m, 1H, CH_2), 4.04 (m, 1H, CH_2), 6.80 (s, 1H, *CH*-Ar), 6.94–7.55 (m, 8H, Ar-H), 10.99 (s, 1H, –NH), 11.18 (s, 1H, NH). EI-MS (*m/z*): 391 (M^+). Anal.

Calcd for ($C_{23}H_{22}ClN_3O$): C, 70.49; H, 5.66; N, 10.72. Found: C, 70.22; H, 5.70; N, 10.65.

5.1.52. 6-Chloro-1-(indol-3-yl)-2-(β-aminopropionyl)-1,2,3,4-tetrahydro-β-carbolinehydrochloride (19g)

Compound **19g** was synthesized according to the procedure used to synthesize **10a** and then followed by **11a** as a white solid in 55.6% overall yield. Mp: 222–224 °C. IR (cm^{-1}): 3410, 1651, 1600, 1452, 1289. ^1H NMR (300 MHz, DMSO-d_6): δ 2.95 (m, 4H, –CO$CH_2CH_2NH_2$), 3.67 (m, 3H, CH_2, CH_2), 4.10 (m, 1H, CH_2), 4.25 (s, 1H, *CH*-Ar), 6.20 (m, 2H, NH_2), 6.67–8.54 (m, 8H, Ar-H), 10.65 (s, 1H, NH), 11.26 (s, 1H, NH). EI-MS (*m/z*): 406 (M^+). Anal. Calcd for ($C_{23}H_{24}Cl_2N_4O{\cdot}0.5H_2O$): C, 61.07; H, 5.57; N, 12.38. Found: C, 61.26; H, 5.83; N, 12.32.

5.2. Bioactivity test

5.2.1. Preparation of Eg5

Coding regions were PCR amplified from a template (obtained in our lab) containing full-length human Eg5. The primers used were, forward 5′-TATAGG GCG AAT TCC GCC ATG GCG TCG CAG CCA-3′ and reverse 5′-ACG GGC TGC AGC AAG CTC GAG TTT TAAACG TTC TAT-3′. The region encoding residues 2-386 was subcloned into pET28a (NOVAGEN). Protein expression in *Escherichia coli* cells was induced with 0.5 mM IPTG. Cells were harvested after 20 h of growth at 20 °C and then disrupted by sonication. The soluble lysate was clarified by centrifugation and applied to a SP-Sepharose column (Amersham Pharmacia Biotech) in a buffer A (20 mM Na–PIPES, pH 6.3; 20 mM NaCl; 1 mM $MgCl_2$; 1 mM Na–EGTA). Protein was eluted with a linear gradient of 20–1000 mM NaCl. Eg5 was identified by SDS–PAGE, and then applied to Mono-Q columns (Amersham Pharmacia Biotech) in a buffer B (20 mM Tris–HCl, pH 8.8; 1 mM $MgCl_2$; 1 mM Na–EGTA). A gradient from 0 to 1000 mM NaCl was used to elute Eg5) Fractions were analyzed by SDS–PAGE. The most concentrated fraction was dialyzed against ATPase buffer (20 mM Na–PIPES, pH 7.5; 1 mM $MgCl_2$; 1 mM Na–EGTA) and then aliquoted, frozen in liquid nitrogen, stored at −80 °C.

5.2.2. ATPase activity assay

All experiments were done at room temperature. The reagents were added to wells of a 96-well clear plate and the final reaction of the assay contained 20 mM PIPES, pH 7.5, 5.0 mM $MgCl_2$, 1 mM EGTA, 10 mM paclitaxel, 0.6 mM tubulin (MT), 0.5 mM ATP, 2% DMSO containing inhibitors in a reaction volume of 100 mL Reactions were started by adding ATP. The plates were incubated at 37 °C for 30 min. Following incubation the malachite-green based reagents were added to detect the release of inorganic phosphate. The plates were incubated for an additional 5 min at the room temperature, and then 10 mL of 34% sodium citrate was added. The absorbance at 610 nm was determined using Multiskan Spectrum Microplate Spectrophotometer (Thermo Electron Corporation). The controls without Eg5 or MTs are the background and should be subtracted from all values. The controls with MTs but without Eg5 give the nucleotide hydrolysis by MTs and should be subtracted from corresponding values with Eg5 and the same concentration of MTs. The data were analyzed using Microsoft Excel to obtain the IC_{50} of the test compounds.

5.2.3. SRB proliferation assays

Sulforhodamine B, trichloroacetic acid, trizma base, and acetic acid were purchased from Sigma Chemical Co. (St. Louis, USA). Lung cancer A549 was cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum at 37 °C in humidified air under 5% CO_2. A pilot-screening operation was initiated in which the panel lines were inoculated onto a series of standard 96-well plates on day 0, in the majority of cases at 20,000 cells/well, and then preincubated in the absence of drug for 24 h. Test agents were then added in 10-fold dilutions starting from the highest soluble concentration, and incubated for a further 48 h. Following this, the cells were fixed in situ, washed, and dried. SRB was added, followed by further washing and drying of the stained, adherent cell mass. The inhibition of cell proliferation was assessed by measuring changes in total optical density after a culture of each cell line that was subjected to 48 h of drug treatment. The results were obtained in one independent experiment run in triplicate.

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