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Synthesis and evaluation of the anti-proliferative and NF-κB activities of a library of simplified tylophorine analogs

Micah J. Niphakis [†], Bryant C. Gay [†], Kwon Ho Hong, Nicholas P. Bleeker, Gunda I. Georg [*]

Department of Medicinal Chemistry and the Institute for Therapeutics Discovery & Development, University of Minnesota, 717 Delaware Street SE, Minneapolis, MN 55414, USA

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ABSTRACT

Tylophorine and many related phenanthropiperidine alkaloids are extraordinarily potent anti-proliferative agents. Despite their impressive anti-cancer activity, clinical development of these alkaloids has been hampered by their poor solubility and neurological side effects. Although it has been suggested that developing polar phenanthropiperidines will mitigate these undesired properties, the lack of practical methods for the synthesis of such analogues has limited this effort. Here, we present a concise synthetic approach to *N*-substituted phenanthropiperidines, which enabled a systematic investigation of structure-activity relationships at an underexplored region of the tylophorine scaffold. This work suggests that ring E of tylophorine is essential for the anti-proliferative activity of the 6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline core scaffold.

1. Introduction

Phenanthroindolizidine and phenanthroquinolizidine natural products (hereon referred to as phenanthropiperidines) have been extensively studied for over 50 years due to their extraordinary biological activity.[1–3] The NCI (NCI 60 tumor cell lines) and other research groups have shown that members of this class exhibit extremely potent anti-proliferative activity against normal and multi-drug resistant cancer cell lines and possess anti-inflammatory properties.[1,3–6] These findings have inspired efforts to develop novel chemotherapeutic agents based on the phenanthropiperidine scaffold. Although the molecular target(s) of these natural products have not been unambiguously identified, and may even differ between members of this class of compounds, recent studies have shown that these alkaloids exert their growth inhibitory activity in a manner that is distinct from known anti-cancer agents.[4,7,8]

Tylophorine and its hydroxylated analogue, DCB-3503, have been subjected to the most extensive mechanistic studies (Fig. 1). DCB-3503, for instance, suppresses protein biosynthesis resulting in a down-regulation of multiple pro-oncogenic and pro-survival proteins such as cyclin D1, survivin, β-catenin, p53 and p21.[4,8] DCB-3503 specifically interferes with the elongation phase of translation during protein biosynthesis.[4] When regimented correctly, DCB-3503 has been shown to possess promising therapeutic activity in mouse models of cancer,[4,8] lupus[9] and arthritis[6] without overt signs of toxicity. These intriguing pharmacological properties suggest that the phenanthropiperidines could enhance our understanding of cancer and inflammatory diseases and provide a means to treat them.

Synthetic approaches to the phenanthropiperidines have greatly improved over the past few decades and compound availability is no longer limited by natural abundance.[10–15] Despite these advanced synthetic approaches, a systematic investigation of phenanthropiperidine structure–activity relationships (SAR) has remained challenging, because many of the current synthetic strategies are highly linear, making library synthesis impractical. A better understanding of phenanthropiperidine SARs would prove highly beneficial for the further development of phenanthropiperidine chemotherapeutics. There are at least two important reasons why the parent alkaloids require further development. First, phenanthropiperidines are notoriously insoluble. This is most likely attributable to their high lipophilicity and planarity.[16] Secondly, many members of this class have physicochemical properties conducive to BBB-penetration.[17] This is a concern because the one member of this class to have been studied in humans, tylocrebrine (Fig. 1), exhibited neurological side effects.[18] Although the design of polar analogs may mitigate CNS-exposure and improve solubility, attempts to incorporate polar functional groups in a pharmacologically silent manner have been largely unsuccessful. Indeed, new synthetic approaches that enable the synthesis of informative libraries[19] to systematically identify the essential and nonessential structural features for bioactivity would be invaluable for this endeavor.

* Corresponding author.
E-mail address: georg@umn.edu (G.I. Georg).
† These authors contributed equally to this work.

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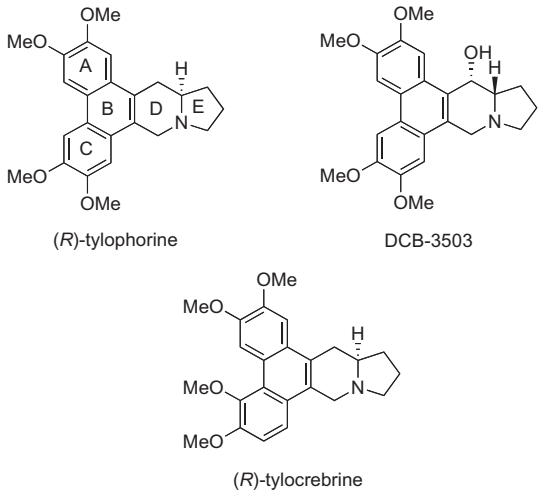


Figure 1. Structures of phenanthroindolizidine alkaloids tylophorine, DCB-3503 and tylocrebrine.

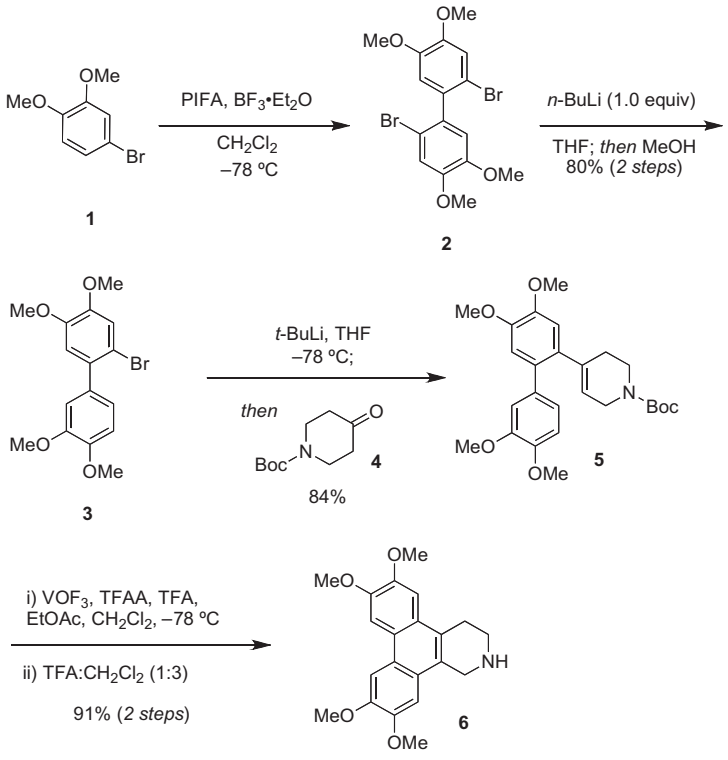
Herein, we report the synthesis and biological evaluation of a library of novel, simplified tylophorine analogues in order to gain a better understanding of phenanthropiperidine SAR. Tylophorine was used as a representative of the phenanthropiperidines, because it is one of the most well-studied members of its class. We chose to focus on the aliphatic portion of the scaffold since SARs in this region are underexplored. Previous studies have shown that disconnections within ring D result in a significant loss of activity or change in their mode of action.[20–22] Moreover, ring E was found to be highly sensitive to heteroatom incorporation.[19] Surprisingly, whether or not ring E is required for activity at all remains elusive.[23] In this regard, we chose to prepare a library of tylophorine analogues lacking ring E, consequently eliminating the sole stereogenic center and dramatically simplifying the synthesis. Importantly, this simplification enables diversification of the secondary amine through reductive alkylation reactions, which can be used to access each library member in a single step from a common intermediate.

2. Results and discussion

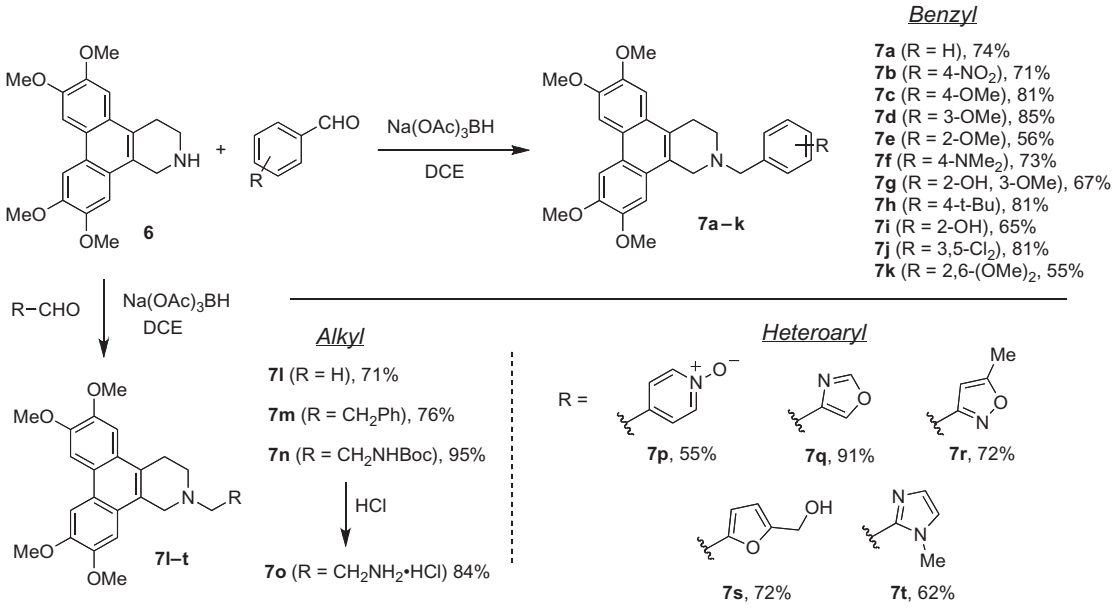
2.1. Synthesis of *N*-substituted phenanthropiperidines

In order to obtain sufficient quantities of the library precursor, we first devised a concise and high-yielding sequence that allowed us to prepare gram quantities of the piperidine **6** (Scheme 1). 4-Bromoveratrole (**1**) was first dimerized using a PIFA-mediated oxidative biaryl coupling reaction.[24] The resultant dibromoveratrole dimer **2** was desymmetrized though a lithium-halogen exchange using a single equivalent of *n*-butyllithium. Notably, these two steps could be carried out on large scale (>5 g) without relying on chromatography for purification. Following another lithium-halogen exchange with aryl bromide **3**, the biaryl moiety was added to piperidinone **4** which spontaneously dehydrated to afford tetrahydropyridine **5**. Using our recently developed aryl-alkene coupling conditions,[13] the phenanthrene system could be constructed in excellent yields. Subsequent deprotection of the Boc group with TFA provided the desired phenanthropiperidine **6**. This sequence provided gram quantities of the key intermediate **6** in 5 steps and 61% overall yield.

Previous SAR studies have shown that amide analogues of the phenanthropiperidines are substantially less active than the naturally occurring amines.[25,26] As such, we chose to prepare a library of *N*-alkylated phenanthropiperidines from intermediate **6**. We decided that the reductive amination reaction would be most practical for this endeavor based on the commercial availability of a diverse collection of aldehydes and the amenability of this reaction for parallel synthesis. It should be noted that phenanthropiperidine



Scheme 1. Synthesis of phenanthropiperidine core **6**.



Scheme 2. Synthesis of *N*-substituted phenanthropiperidine library using reductive amination reactions.

6 is poorly soluble in most organic solvents, limiting the chemistry that can be used to derivatize this intermediate. Fortunately, halogenated solvents such as CH_2Cl_2 and DCE could sufficiently solubilize this amine, albeit at low concentrations (0.02 M), to enable efficient *N*-alkylations with a diverse collection of commercially available aldehydes. In general, these reactions proceeded in excellent yield (55–95%) providing the desired benzyl-(**7a**–**k**), alkyl-(**7l**–**o**) and heteroaryl-(**7p**–**t**) derivatives of tylophorine to aid our understanding of phenanthropiperidine SAR (Scheme 2).

2.2. Anti-proliferative activity of phenanthropiperidines

The anti-proliferative activity of phenanthropiperidines has been investigated in many cell lines.[1] Interestingly, the most well studied alkaloids show little discrimination between these cell lines and even retain their activity in those resistant to the most commonly used anti-cancer drugs. The NCI 60-tumor cell line screen of several members of this class bears witness to the potent and broad-spectrum activity of these alkaloids. Although numerous studies have established SARs for the phenanthrene and piperidine systems, the necessity of the pyrrolidine (ring E) system of tylophorine for activity remains underexplored. In order to assess the potential of these compounds for further development, we first tested the anti-proliferative activity of each compound at 5 μM in MCF-7 cells (Fig. 2).

We were pleased to find that many library members exhibited greater than 50% inhibition of cell growth at 5 μM. Most compounds, however, were significantly less active than tylophorine. Notably, the unsubstituted phenanthropiperidine core **6** and *N*-methyl analogue **7l**, the simplest library members, inhibited cell proliferation by 50% suggesting that the intact indolizidine and quinolizidine moieties found in phenanthropiperidine natural products contribute significantly to their activity. Benzylation of the nitrogen (**7a**) resulted in near complete loss of activity; however, a clear phenyl-substituent effect was observed for these benzyl analogues. 4-Substituted benzyl groups (**7b** and **7c**) displayed low activity, whereas 2-substituted benzyl analogues **7e**, **7g** and **7i** were the most potent compounds in the series. The

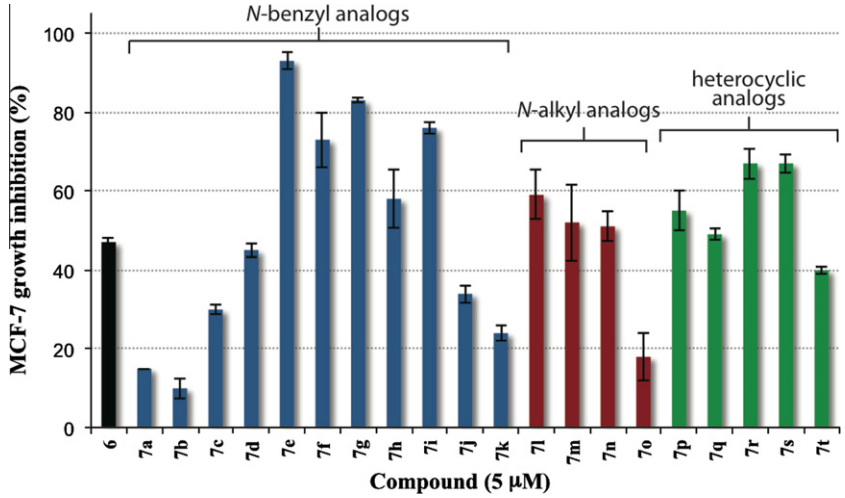


Figure 2. Anti-proliferative activity of *N*-substituted phenanthropiperidines at 5.0 μM in MCF-7 cells.

Table 1
Anti-proliferative and NF-κB activity of compounds **7e** and **7g**

Compound	MCF-7	GI_{50} (μM)[a]		NF-κB inhibition[b] IC_{50} (μM)
		DU-145	A549	
(±)-Tylophorine[c]	0.042 ± 0.001	0.071 ± 0.004	0.045 ± 0.003	0.030 ± 0.01
7e	0.6 ± 0.2	1.5 ± 0.1	1.6 ± 0.1	15 ± 4
7g	3 ± 1	2.1 ± 0.1	2.1 ± 0.1	18.9 ± 0.7

GI_{50} and IC_{50} values are reported as the mean ± SEM from three independent experiments.
[a] MCF-7 = human breast carcinoma; DU-145 = human prostate carcinoma; A549/NF-κB-luc = human lung carcinoma.
[b] NF-κB inhibition was measured by monitoring the luciferase activity from A549/NF-κB-luc cells upon treatment with the indicated compound.
[c] GI_{50} MCF-7: (*R*)-tylophorine: 0.032 μM; (*S*)-tylophorine 0.042 μM.[13]

2-methoxybenzyl analogue **7e** was particularly active showing ~90% growth inhibition at 5 μM. However, incorporation of an additional 2-methoxy substituent significantly reduced activity (**7k**). *N*-Alkyl substituted analogues **7l**–**7n** were equipotent to the unsubstituted analog **6**. Boc-deprotection of **7n** significantly reduced its anti-proliferative activity (**7o**). We also tested several heterocyclic analogues, which we hoped would improve the solubility of these highly lipophilic molecules. Unfortunately, only a slight improvement of activity was observed for the most active heterocyclic compounds, isooxazole **7r** and furan **7s**.

After obtaining these preliminary results, we analyzed the most active compounds in more detail. Two compounds, **7e** and **7g**, were assayed to determine their GI_{50} values against MCF-7, DU-145 and A549 cell proliferation. The most active of these compounds was the 2-OMe analogue **7e**, which displayed a GI_{50} of 600 nM in MCF-7 and 1.5 and 1.6 μM in DU-145 and A549 cells, respectively (Table 1). Despite this respectable anti-proliferative activity, this analogue was greater than 10-fold less potent than the parent compound, tylophorine. Motivated by previous reports that tylophorine inhibits NF-κB-mediated transcription,[8] we examined the effect of **7e** and **7g** on NF-κB activity using A549 cells stably expressing the luciferase reporter gene downstream of NF-κB. Consistent with previous findings, tylophorine potently inhibited NF-κB activity. In contrast, analogues **7e** and **7g** only inhibited this pathway at concentrations of greater than 10 μM. Considering the structurally sensitive nature of the alkaloids' mechanism of action,[19,27] it is tempting to speculate that these simplified tylophorine analogs could also have a different mechanism of action.

3. Conclusion

We have prepared a library of novel tylophorine analogues, which was used to explore the effects of *N*-substitution at the phenanthropiperidine core. The synthetic route developed here enabled a highly efficient synthesis of the phenanthropiperidine scaffold from which each library member could be prepared through reductive alkylation reactions. Although the library members, in general, had reduced activity relative to tylophorine, we noted a significant improvement of anti-proliferative activity for 2-substituted *N*-benzyl analogues, the best of which, **7e**, had a GI_{50} value of 600 nM against MCF-7 cell proliferation. The lack of NF-κB inhibitory activity, however, suggests that these structural analogs may exert their anti-proliferative activity through a mechanism distinct from tylophorine.

Given the synthetic accessibility of these analogues and the suitability of this route for library development, this work opens new avenues to explore the stripped-down phenanthropiperidine core. Importantly, this work has also shed light on the operative pharmacophore and establishes the necessity of ring E for potent anti-proliferative activity of analogues of the 6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline core structure **6**.

4. Materials and methods

4.1. Materials

All commercially available reagents and solvents were used without further purification. Flash column chromatography was carried out on silica gel. TLC was conducted on silica gel 250 micron, F_{254} plates. ^{1}H NMR spectra were recorded on a 400 MHz NMR instrument. Chemical shifts are reported in ppm with TMS or $CHCl_3$ as an internal standard (TMS: 0.0 ppm, $CHCl_3$/7.26). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration and coupling constants (Hz). ^{13}C NMR spectra were recorded on a 100 MHz NMR spectrometer with complete proton decoupling. Chemical shifts are reported in ppm with the solvent as internal standard ($CDCl_3$: 77.2 ppm).

4.2. Synthesis of the phenanthropiperidine core

4.2.1. 2-Bromo-3′,4,4′,5-tetramethoxy-1,1′-biphenyl (3)

Dimerization: 4-Bromoveratrole (20.0 g, 92.1 mmol, 1.00 equiv) was dissolved in CH_2Cl_2 (1000 mL) in a 2 L round-bottomed flask and cooled to −78 °C. To this solution was added dropwise (over 1 h) a pre-mixed solution of PIFA (21.8 g, 50.7 mmol, 0.55 equiv) and $BF_3{\cdot}Et_2O$ (12.7 mL, 101 mmol, 1.10 equiv) in CH_2Cl_2 (500 mL). The reaction mixture was stirred until the starting material was completely consumed (~1 h). The reaction was quenched by adding 10% NaOH (500 mL) and the reaction mixture was vigorously stirred for 1 h. The product was extracted with CH_2Cl_2 (3×). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo and recrystallized from EtOH. The veratrole dimer was obtained as a white crystalline solid (18.8 g, 95%). Analytical data was in agreement with that reported.[24]

Dehalogenation: Veratrole dimer (10.0 g, 23.0 mmol, 1.00 equiv) was dissolved in anhydrous THF (400 mL) and cooled to −78 °C. To this solution was added *n*-BuLi (9.30 mL, 23.0 mmol, 1.00 equiv, 2.5 M in hexanes) dropwise via syringe pump (0.62 mL/min). Upon complete addition of *n*-BuLi the reaction was stirred for another 20 min before 2.0 mL of MeOH was added as a proton source. The quenched reaction mixture was stirred for 5 min at −78 °C and then poured into a separatory funnel with 100 mL of H_2O. The product was extracted with EtOAc (3×). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo and recrystallized from MeOH. The title compound was obtained as an off-white crystalline solid (6.79 g, 84%). Analytical data was in agreement with that reported.[28]

4.2.2. *tert*-Butyl 4-(3′,4,4′,5-tetramethoxy-[1,1′-biphenyl]-2-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (5)

Biaryl monobromide (6.74 g, 19.1 mmol, 1.1 equiv) was dissolved in anhydrous THF and cooled to −78 °C. To this solution was added *t*-BuLi (23.5 mL, 39.9 mmol, 2.3 equiv, 1.7 M in

hexanes) dropwise via a syringe pump. Upon complete addition of *t*-BuLi the reaction was stirred for 30 min before *N*-Boc-piperidone was added (3.46 g, 17.3 mmol, 1.0 equiv) dropwise in 30 mL THF. That solution was allowed to warm to room temperature while stirring slowly. Upon completion (~3 h) of the reaction, silica gel was added, stirred for 1 h, concentrated, loaded directly on to a column, and purified via column chromatography (25% EtOAc/Hex) to yield 6.64 g (84%) of the title compound as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94–6.89 (m, 2H), 6.86 (d, J = 8.8 Hz, 1H), 6.82 (s, 1H), 6.75 (s, 1H), 5.87–5.52 (m, 1H), 4.03–3.94 (m, 2H), 3.91 (s, 3H) 3.89 (s, 3H), 3.89 (s, 3H) 3.84 (s, 3H), 3.29 (s, 2H), 2.00–1.79 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.96, 148.54, 148.10, 148.07, 147.88, 138.73, 134.47, 133.85, 131.96, 123.23, 121.09, 113.35, 112.83, 112.36, 111.02, 79.63, 56.14, 56.08, 56.06, 55.96, 55.94, 29.49, 28.54. IR (neat) 2933, 2836, 1694, 1603, 1505, 1464, 1417, 1248, 1211, 1173, 1151, 1028, 864,764 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{26}H_{34}NO_6^+$: 456.2386, found 456.2884.

4.2.3. *tert*-Butyl 6,7,10,11-tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]-isoquinoline-2(1*H*)-carboxylate (5b)

Biphenyl **5** (6.53 g, 12.7 mmol, 1.0 equiv) was dissolved in anhydrous CH_2Cl_2 (40 mL) and cooled to −78 °C. Two drops of TFAA were then added to this solution. In a separate flask containing VOF_3 (3.45 g, 27.9 mmol, 2.2 equiv) was added anhydrous CH_2Cl_2 (40 mL), anhydrous EtOAc (20 mL), TFA (1.5 mL) and TFAA (2 drops). The VOF_3 solution was then added over 20 min to the biphenyl **5** solution. The reaction mixture was stirred until the starting material had been consumed (~1 h) and quenched with 10% NaOH (50 mL). The biphasic mixture was vigorously stirred for 1 h at room temperature. The aqueous layer was extracted (3×) with CH_2Cl_2. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. Filtration and concentration in vacuo gave the crude product. Purification by flash chromatography (silica gel, 50% EtOAc/hexanes) yielded 6.18 g (95%) of **5b** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 2H), 7.28 (s, 1H), 7.17 (s, 1H), 4.96 (s, 2H), 4.12 (d, J = 3.5 Hz, 6H), 4.03 (d, J = 5.2 Hz, 6H), 3.87 (t, J = 5.7 Hz, 2H), 3.15 (t, J = 5.6 Hz, 2H), 1.54 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.06, 149.02, 148.93, 148.81, 148.76, 125.52, 123.96, 123.71, 123.55, 103.97, 103.53, 103.46, 102.88, 80.11, 56.18, 56.03, 55.98, 28.64, 28.55 IR (neat) 2972, 2834, 1690, 1620, 1514, 1471, 1424, 1248, 1169, 1150, 1047, 1021, 838, 767 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{26}H_{32}NO_6^+$: 454.2230, found 454.2209.

4.2.4. 6,7,10,11-Tetramethoxy-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (6)

Piperidine **5b** (6.01 g, 13.26 mmol, 1.00 equiv) was dissolved in anhydrous CH_2Cl_2 (40 mL). TFA (13 mL) was added to the solution. The reaction mixture immediately turned dark purple and slowly turned a light brown color. After the reaction was stirred for 1 h the solvent was removed by passing N_2 over it. To the remaining residue was added CH_2Cl_2 (50 mL) and NH_3 (10 mL, 2.0 M in MeOH). This solution was stirred for 30 min and then poured into a separatory funnel containing 10% NaOH (aq.). The product was extracted with CH_2Cl_2 (3×) and combined organic layers were dried over anhydrous Na_2SO_4. Filtration and concentration in vacuo furnished 4.64 g (96%) the crude product (yellow solid) which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 2H), 7.23 (s, 1H), 7.05 (s, 1H), 4.32 (s, 2H), 4.11 (s, 3H), 4.10 (s, 3H), 4.02 (s, 3H), 3.99 (s, 3H), 3.31 (t, J = 5.9 Hz, 2H), 3.02 (t, J = 5.8 Hz, 2H), 2.17 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.81, 148.79, 148.60, 148.53, 126.96, 126.16, 125.98, 124.26, 123.54, 123.31, 103.71, 103.49, 103.41, 102.91, 56.15, 56.13, 55.95, 46.56, 43.67, 26.89; IR (neat) 3390, 2956, 2835, 1618, 1515, 1462, 1425, 1248, 1214, 1156, 1044, 1013, 843, 799, 764 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{21}H_{24}NO_4^+$: 354.1700, found 354.1698.

4.3. Synthesis of the *N*-alkylated phenanthropiperidine library

4.3.1. General procedure for reductive amination reactions

Phenanthropiperidine **6** (1.00 equiv, 0.05–0.08 mmol) and aldehyde (3.00 equiv) were dissolved in anhydrous DCE (0.02 M) and allowed to stir for 5 min at rt. $Na(OAc)_3BH$ (5.00 equiv) was added in one portion (as a solid) and the reaction was allowed to stir at room temperature until completion (6–24 h, monitored via TLC). The reaction was quenched with 10% NaOH and extracted with CH_2Cl_2 (3×). The combined organic layers were dried over anhydrous $MgSO_4$, concentrated, and purified via prep TLC (2.5–5% MeOH (2.0 M NH_3 in MeOH)/CH_2Cl_2).

4.3.2. 2-Benzyl-6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7a)

The title compound was isolated as an off-white solid (74%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 2H), 7.53 (d, J = 3.9 Hz, 2H), 7.38 (d, J = 7.1 Hz, 3H), 7.25 (s, 1H), 6.99 (s, 1H), 4.34 (s, 2H), 4.16 (s, 2H), 4.11 (d, J = 3.3 Hz, 6H), 4.03 (s, 3H), 3.97 (s, 3H), 3.30 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.08, 148.92, 133.04, 130.38, 130.23, 130.20, 128.92, 128.62, 128.40, 125.10, 124.66, 124.62, 123.99, 123.84, 103.95, 103.65, 103.48, 102.87, 59.98, 56.21, 56.19, 56.09, 56.03, 51.77, 48.13, 29.83; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{28}H_{30}NO_4^+$: 444.2169, found 444.2165.

4.3.3. 6,7,10,11-Tetramethoxy-2-(4-nitrobenzyl)-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline (7b)

The title compound was isolated as an off-white solid (71%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.7 Hz, 2H), 7.82 (s, 1H), 7.81 (s, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.27 (s, 1H), 7.05 (s, 1H), 4.11 (s, 3H), 4.11 (s, 3H), 4.07 (s, 2H), 4.03 (s, 3H), 3.99 (s, 3H), 3.96 (s, 2H), 3.19 (t, J = 5.6 Hz, 2H), 2.91 (t, J = 5.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.74, 148.71, 148.58, 148.47, 147.21, 146.64, 129.31, 125.75, 125.48, 125.21, 124.04, 123.61, 123.50, 123.42, 103.81, 103.46, 103.26, 102.82, 61.82, 55.99, 55.98, 55.90, 55.80, 54.42, 49.79, 27.14; IR (neat) 2917, 1516, 1344, 1248, 1146, 1046, 842 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{28}H_{29}N_2O_6^+$: 489.2026, found 489.2030.

4.3.4. 6,7,10,11-tetramethoxy-2-(4-methoxybenzyl)-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7c)

The title compound was isolated as an off-white solid (81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 2.6 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H), 7.27 (s, 1H), 7.10 (s, 1H), 6.91 (d, J = 8.6 Hz, 2H), 4.12 (dd, J = 6.3, 1.2 Hz, 6H), 4.03 (dd, J = 12.0, 5.1 Hz, 8H), 3.84–3.80 (m, 5H), 3.17 (t, J = 5.5 Hz, 2H), 2.90 (t, J = 5.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.96, 148.79, 148.76, 148.58, 148.48, 130.60, 130.45, 128.76, 126.17, 125.83, 124.44, 123.59, 123.51, 114.05, 113.85, 104.01, 103.53, 103.37, 103.11, 65.18, 62.28, 56.15, 56.03, 55.96, 55.44, 54.50, 49.54, 27.33; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{29}H_{32}NO_5^+$: 474.2280, found 474.2285.

4.3.5. 6,7,10,11-Tetramethoxy-2-(3-methoxybenzyl)-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7d)

The title compound was isolated as an off-white solid (85%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 1.6 Hz, 2H), 7.25 (d, J = 6.1 Hz, 2H), 7.08 (s, 1H), 7.04 (d, J = 6.7 Hz, 2H), 6.88–6.79 (m, 1H), 4.09 (d, J = 1.0 Hz, 6H), 4.04 (s, 2H), 4.00 (d, J = 5.7 Hz, 6H), 3.84 (s, 2H), 3.80 (s, 3H), 3.15 (d, J = 5.0 Hz, 2H), 2.89 (t, J = 5.6 Hz, 2H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.94, 148.84, 148.81, 148.63, 148.53, 140.32, 129.43, 126.15, 125.85, 125.82, 124.43, 123.63, 123.54,

121.55, 114.49, 112.95, 104.06, 103.60, 103.45, 103.14, 62.81, 56.17, 56.16, 56.04, 55.97, 55.38, 54.59, 49.68, 27.32; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841 cm^{-1}; *m/e* calcd for $[M+H]^+$ $C_{32}H_{32}NO_4^+$: 474.2280, found 474.2269.

4.3.6. 6,7,10,11-Tetramethoxy-2-(2-methoxybenzyl)-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7e)

The title compound was isolated as an off-white solid (56%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.81 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.32–7.27 (m, 1H), 7.28 (s, 1H), 7.10 (s, 1H), 7.01–6.87 (m, 2H), 4.16 (br s, 2H), 4.11 (s, 3H), 4.11 (s, 3H), 4.03 (s, 3H), 4.01 (s, 3H), 4.01 (br s, 2H), 3.87 (s, 3H), 3.22 (t, J = 5.1 Hz, 2H), 3.06 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.86, 148.65, 148.61, 148.42, 148.31, 130.45, 128.18, 126.33, 125.96, 125.73, 124.35, 123.43, 123.34, 120.47, 110.38, 103.92, 103.43, 103.30, 102.99, 56.01, 56.01, 55.88, 55.83, 55.81, 55.42, 54.14, 49.83, 27.16; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841 cm^{-1}; HRMS (ESI^+) *m/e* calcd for $[M+H]^+$ $C_{29}H_{32}NO_5^+$: 474.2280, found 474.2269.

4.3.7. *N*,*N*-Dimethyl-4-((6,7,10,11-tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]isoquinolin-2(1*H*)-yl)methyl)aniline (7f)

The title compound was isolated as an off-white solid (73%): ^1H NMR (400 MHz, CD_2Cl_2) δ 7.81 (d, J = 1.3 Hz, 2H), 7.29 (d, J = 8.6 Hz, 2H), 7.26 (s, 1H), 7.08 (s, 1H), 6.73 (d, J = 8.7 Hz, 2H), 4.04 (d, J = 1.2 Hz, 6H), 4.01 (s, 2H), 3.95 (d, J = 5.3 Hz, 6H), 3.77 (s, 2H), 3.13 (t, J = 5.6 Hz, 2H), 2.92 (s, 6H), 2.90–2.85 (m, 2H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 150.77, 149.49, 149.45, 149.30, 149.20, 130.73, 128.97, 126.47, 126.09, 124.72, 123.93, 123.85, 112.95, 112.64, 104.68, 104.18, 104.04, 103.78, 65.56, 62.44, 56.47, 56.45, 56.29, 56.23, 49.76, 41.03, 40.94, 30.25, 27.49; IR (neat) 2919, 1615, 1515, 1470, 1248, 1146, 1045, 840 cm^{-1}; HRMS (ESI^+) *m/e* calcd for $[M+H]^+$ $C_{30}H_{35}N_2O_4^+$: 487.2597, found 487.2604.

4.3.8. 2-Methoxy-6-((6,7,10,11-tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]isoquinolin-2(1*H*)-yl)methyl)phenol (7g)

The title compound was isolated as an off-white solid (67%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.81 (s, 1H), 7.24 (s, 1H), 7.05 (s, 1H), 6.92–6.84 (m, 1H), 6.81 (t, J = 7.7 Hz, 1H), 6.75 (d, J = 7.1 Hz, 1H), 4.20 (s, 2H), 4.11 (d, J = 1.4 Hz, 8H), 4.02 (s, 3H), 4.01 (s, 3H), 3.87 (s, 3H), 3.25 (d, J = 4.7 Hz, 2H), 3.06 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.97, 148.91, 148.76, 148.18, 147.41, 125.57, 125.37, 124.12, 123.99, 123.77, 123.67, 121.22, 121.12, 119.03, 111.35, 103.96, 103.67, 103.45, 102.98, 60.48, 56.19, 56.14, 56.03, 55.96, 53.66, 48.85, 26.48; IR (neat) 2933, 1619, 1514, 1474, 1249, 1144, 1044, 842 cm^{-1}; HRMS (ESI^+) *m/e* calcd for $[M+H]^+$ $C_{29}H_{32}NO_6^+$: 490.2230, found 490.2230.

4.3.9. 2-(4-(*tert*-Butyl)benzyl)-6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7h)

The title compound was isolated as an off-white solid (81%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.81 (s, 1H), 7.40 (s, 4H), 7.26 (s, 1H), 7.08 (s, 1H), 4.11 (s, 3H), 4.11 (s, 3H), 4.08 (s, 2H), 4.02 (s, 3H), 4.00 (s, 3H), 3.88 (s, 2H), 3.18 (t, J = 5.4 Hz, 2H), 2.95 (t, J = 5.3 Hz, 2H), 1.34 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.45, 148.82, 148.80, 148.64, 148.53, 138.12, 129.14, 126.99, 125.72, 125.59, 125.43, 124.35, 123.65, 123.55, 104.01, 103.58, 103.43, 103.11, 65.27, 56.15, 56.01, 55.93, 54.29, 49.37, 34.65, 31.53, 26.96; IR (neat) 2959, 1515, 1479, 1248, 1147, 1046, 839 cm^{-1}; HRMS (ESI^+) *m/e* calcd for $[M+H]^+$ $C_{32}H_{38}NO_4^+$: 500.2801, found 500.2789.

4.3.10. 2-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]isoquinolin-2(1*H*)-yl)methyl)phenol (7i)

The title compound was isolated as an off-white solid (65%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.82 (s, 1H), 7.25 (s, 1H), 7.25–7.19 (m, 1H), 7.10 (d, J = 7.3 Hz, 1H), 7.05 (s, 1H), 6.89–6.82 (m, J = 12.2, 4.7 Hz, 2H), 4.16 (br s, 2H), 4.12 (s, 3H), 4.12 (s, 3H), 4.09 (br s, 2H), 4.02 (s, 3H), 4.02 (s, 3H), 3.23 (t, J = 5.2 Hz, 2H), 3.03 (br s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.15, 148.97, 148.90, 148.77, 129.10, 128.94, 125.61, 125.38, 124.37, 123.99, 123.77, 123.66, 121.28, 119.33, 116.48, 103.97, 103.66, 103.45, 102.96, 61.16, 56.20, 56.19, 56.13, 55.97, 53.85, 48.95, 26.73; IR (neat) 1619, 1514, 1249, 1143, 1045, 845 cm^{-1}; HRMS (ESI^+) *m/e* calcd for $[M+H]^+$ $C_{28}H_{30}NO_5^+$: 460.2124, found 460.2137.

4.3.11. 2-(3,5-Dichlorobenzyl)-6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7j)

The title compound was isolated as an off-white solid (81%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 2H), 7.36 (s, 2H), 7.28 (s, 1H), 7.17 (d, J = 18.9 Hz, 1H), 7.04 (s, 1H), 4.10 (s, 6H), 4.01 (d, J = 6.7 Hz, 8H), 3.80 (s, 2H), 3.17 (s, 2H), 2.89 (d, J = 5.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.88, 148.86, 148.71, 148.59, 142.52, 135.09, 127.52, 127.34, 125.95, 125.68, 125.40, 124.93, 124.24, 123.66, 123.56, 104.00, 103.62, 103.43, 102.99, 61.79, 56.16, 56.05, 55.97, 54.47, 49.89, 27.30; IR (neat) 2932, 1619, 1569, 1514, 1425, 1249, 1147, 846 cm^{-1}; HRMS (ESI^+) *m/e* calcd for $[M+H]^+$ $C_{28}H_{28}NO_4Cl_2^+$: 512.1395, found 512.1400.

4.3.12. 2-(2,6-Dimethoxybenzyl)-6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7k)

The title compound was isolated as an off-white solid (55%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.80 (s, 1H), 7.27 (s, 1H), 7.24 (d, J = 8.3 Hz, 1H), 7.15 (s, 1H), 6.61 (d, J = 8.4 Hz, 2H), 4.10 (t, J = 2.3 Hz, 8H), 4.04 (s, 5H), 4.02 (s, 3H), 3.86 (s, 6H), 3.16 (d, J = 5.4 Hz, 2H), 3.08 (t, J = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.65, 148.72, 148.60, 148.42, 148.29, 128.98, 126.50, 125.96, 125.92, 124.65, 123.47, 123.42, 114.20, 104.07, 103.89, 103.55, 103.44, 103.15, 56.14, 55.93, 55.91, 55.82, 53.25, 49.94, 48.91, 27.05; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841 cm^{-1}; HRMS (ESI^+) *m/e* calcd for $[M+H]^+$ $C_{30}H_{34}NO_6^+$: 504.2386, found 504.2380.

4.3.13. 6,7,10,11-Tetramethoxy-2-methyl-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7l)

The title compound was isolated as an off-white solid (71%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.83 (s, 1H), 7.30 (s, 1H), 7.14 (s, 1H), 4.12 (s, 6H), 4.05 (s, 3H), 4.05 (s, 3H), 3.95 (s, 2H), 3.22 (t, J = 5.8 Hz, 2H), 2.90 (t, J = 5.9 Hz, 2H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.88, 148.66, 148.55, 125.79, 125.75, 125.64, 124.32, 123.63, 123.54, 104.08, 103.63, 103.49, 103.10, 56.19, 56.13, 56.07, 56.01, 52.51, 46.58, 27.51; IR (neat) 3373, 2975, 1515, 1425, 1366, 1249, 1150, 844 cm^{-1}; HRMS (ESI^+) *m/e* calcd for $[M+H]^+$ $C_{22}H_{26}NO_4^+$: 368.1862, found 368.1869.

4.3.14. 6,7,10,11-Tetramethoxy-2-phenethyl-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7m)

The title compound was isolated as an off-white solid (76%): ^1H NMR (400 MHz, CD_2Cl_2) δ 7.83 (s, 1H), 7.82 (s, 1H), 7.32 (dd, J = 5.0, 1.5 Hz, 4H), 7.29 (s, 1H), 7.24–7.19 (m, 1H), 7.09 (s, 1H), 4.06 (d, J = 1.5 Hz, 8H), 3.99 (d, J = 8.9 Hz, 6H), 3.21 (t, J = 5.6 Hz, 2H), 3.09–2.98 (m, 6H); ^1H NMR (400 MHz, CD_2Cl_2) δ 7.83 (s, 1H), 7.82 (s, 1H), 7.32 (dd, J = 5.0, 1.5 Hz, 4H), 7.29 (s, 1H), 7.24–7.19 (m, 1H), 7.09 (s, 1H), 4.06 (d, J = 1.5 Hz, 8H), 3.99 (d, J = 8.9 Hz, 6H), 3.21 (t, J = 5.6 Hz, 2H), 3.09–2.98 (m, 6H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 149.52, 149.50, 149.34, 149.21, 141.23, 129.32, 128.93, 126.58, 126.40, 126.03, 124.67, 123.94, 123.85, 104.67, 104.20, 104.04, 103.66, 60.45, 56.47, 56.46, 56.36, 56.26, 50.59, 34.25, 27.54; IR (neat) 1615, 1513, 1422, 1248, 1149, 1042, 840 cm^{-1}; HRMS (ESI^+) *m/e* calcd for $[M+H]^+$ $C_{29}H_{32}NO_4^+$: 458.2331, found 458.2329.

4.3.15. *tert*-Butyl (2-(6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]isoquinolin-2(1*H*)-yl)ethyl)carbamate (7n)

The title compound was isolated as an off-white solid (95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.80 (s, 1H), 7.27 (s, 1H), 7.10 (s, 1H), 4.10 (s, 6H), 4.03 (s, 6H), 4.00 (s, 2H), 3.45 (d, J = 5.0 Hz, 2H), 3.18 (d, J = 5.1 Hz, 2H), 2.95 (t, J = 5.6 Hz, 2H), 2.84 (t, J = 5.7 Hz, 2H), 1.48–1.43 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.24, 148.88, 148.71, 148.59, 125.94, 125.59, 125.35, 124.23, 123.64, 123.52, 103.97, 103.59, 103.43, 102.93, 79.30, 57.15, 56.15, 56.07, 55.98, 54.08, 49.98, 37.64, 29.81, 28.55, 28.49, 28.45, 28.10, 27.23; IR (neat) 3373, 2975, 1701, 1515, 1425, 1366, 1249, 1150, 844 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{28}H_{27}N_2O_6^+$: 497.2652, found 497.2665.

4.3.16. 2-(6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]isoquinolin-2(1*H*)-yl)ethanamine (7o)

The title compound was isolated as an off-white solid (84%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.80 (s, 1H), 7.26 (s, 1H), 7.07 (s, 1H), 4.10 (t, J = 5.6 Hz, 10H), 4.05 (s, 6H), 3.69 (d, J = 5.1 Hz, 2H), 3.22 (s, 2H), 3.03 (d, J = 21.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.08, 149.07, 148.99, 148.83, 125.29, 123.92, 123.80, 123.67, 103.92, 103.67, 103.46, 102.77, 56.22, 56.19, 56.13, 56.03, 55.39, 53.84, 49.72, 36.61, 29.84.; IR (neat) 3373, 2975, 1701, 1515, 1425, 1366, 1249, 1150, 844 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{23}H_{29}N_2O_4^+$: 397.2122, found 397.2135.

4.3.17. 4-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]isoquinolin-2(1*H*)-yl)methyl)pyridine 1-Oxide (7p)

The title compound was isolated as an off-white solid (55%): ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.13 (d, J = 6.3 Hz, 2H), 7.83 (s, 2H), 7.40 (d, J = 6.4 Hz, 2H), 7.29 (s, 1H), 7.06 (s, 1H), 4.13–3.92 (m, 14H), 3.83 (s, 2H), 3.19 (d, J = 5.9 Hz, 2H), 2.91 (t, J = 5.9 Hz, 2H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 149.57, 149.53, 149.40, 149.29, 139.41, 138.80, 126.51, 126.40, 126.00, 125.85, 124.56, 123.97, 123.88, 104.61, 104.22, 104.05, 103.63, 60.95, 56.47, 56.45, 56.35, 56.25, 54.75, 50.48, 27.77; IR (neat) 2918, 1620, 1514, 1474, 1249, 1146, 1043, 843 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{27}H_{29}N_2O_5^+$: 461.2076, found 461.2077.

4.3.18. 4-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]isoquinolin-2(1*H*)-yl)methyl)oxazole (7q)

The title compound was isolated as an off-white solid (91%): ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.91 (s, 1H), 7.82 (d, J = 2.1 Hz, 2H), 7.73 (s, 1H), 7.28 (s, 1H), 7.12 (s, 1H), 4.06 (d, J = 1.5 Hz, 8H), 3.99 (s, 3H), 3.97 (s, 3H), 3.85 (s, 2H), 3.17 (t, J = 5.9 Hz, 2H), 2.99 (t, J = 5.8 Hz, 2H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 151.67, 149.49, 149.47, 149.30, 149.20, 138.17, 137.20, 126.42, 126.14, 126.09, 124.70, 123.91, 123.83, 104.67, 104.19, 104.05, 103.75, 56.47, 56.45, 56.33, 56.24, 50.48, 27.77; IR (neat) 2935, 1620, 1514, 1425, 1248, 1146, 1045, 843 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{25}H_{27}N_2O_5^+$: 435.1920, found 435.1911; 91%.

4.3.19. 5-Methyl-3-((6,7,10,11-tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]isoquinolin-2(1*H*)-yl)methyl)isoxazole (7r)

The title compound was isolated as an off-white solid (72%): ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.82 (s, 1H), 7.82 (s, 1H), 7.27 (s, 1H), 7.10 (s, 1H), 6.12 (d, J = 1.1 Hz, 1H), 4.08–4.03 (m, 8H), 3.98 (s, 3H), 3.97 (s, 3H), 3.89 (s, 2H), 3.25–3.10 (m, 2H), 2.94 (t, J = 5.8 Hz, 2H), 2.41 (d, J = 0.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 170.20, 162.48, 149.51, 149.34, 149.24, 126.36, 126.04, 125.99, 124.63, 123.94, 123.84, 104.64, 104.19, 104.04, 103.72, 102.22, 56.46, 56.45, 56.37, 56.24, 54.60, 50.44, 27.77, 12.62; IR (neat) 2918, 1607, 1514, 1424, 1248, 1145, 1046, 841 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{26}H_{29}N_2O_5^+$: 449.2076, found 449.2062.

4.3.20. (5-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[*f*,*h*]isoquinolin-2(1*H*)-yl)methyl)furan-2-yl)methanol (7s)

The title compound was isolated as an off-white solid (72%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.79 (s, 1H), 7.24 (s, 1H), 7.07 (s, 1H), 6.30 (d, J = 3.1 Hz, 1H), 6.26 (d, J = 3.1 Hz, 1H), 4.60 (s, 2H), 4.10 (s, 3H), 4.10 (s, 3H), 4.04 (br s, 2H), 4.02 (s, 3H), 4.01 (s, 3H), 3.88 (s, 2H), 3.18 (t, J = 5.4 Hz, 2H), 2.98 (t, J = 5.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.04, 151.75, 148.70, 148.67, 148.51, 148.40, 125.65, 125.51, 125.13, 124.14, 123.48, 123.40, 109.79, 108.38, 103.85, 103.46, 103.29, 102.90, 57.53, 56.02, 55.91, 55.83, 54.64, 53.64, 49.59, 26.82; IR (neat) 3393, 1619, 1514, 1425, 1248, 1146, 1012 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{27}H_{30}NO_6^+$: 464.2073, found 464.2055.

4.3.21. 6,7,10,11-Tetramethoxy-2-((1-methyl-1*H*-imidazol-2-yl)methyl)-1,2,3,4-tetrahydrodibenzo[*f*,*h*]isoquinoline (7t)

The title compound was isolated as an off-white solid (62%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.82 (s, 1H), 7.28 (s, 1H), 7.11 (s, 1H), 6.99 (d, J = 1.3 Hz, 1H), 6.89 (d, J = 1.3 Hz, 1H), 4.12 (s, 3H), 4.11 (s, 3H), 4.03 (s, 7H), 3.98 (s, 2H), 3.75 (s, 3H), 3.17 (t, J = 5.8 Hz, 2H), 2.94 (t, J = 5.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.88, 148.83, 148.66, 148.56, 145.05, 127.24, 126.04, 125.68, 125.65, 124.30, 123.61, 123.44, 121.97, 103.97, 103.51, 103.38, 103.04, 56.17, 56.09, 55.97, 55.14, 54.35, 49.83, 33.19, 27.44; IR (neat) 2917, 1620, 1514, 1425, 1248, 1147, 1044 cm^{-1}; HRMS (ESI$^+$) *m/e* calcd for $[M+H]^+$ $C_{26}H_{30}N_3O_4^+$: 448.2236, found 448.2223.

4.4. Biological testing of the phenanthropiperidine library

4.4.1. Anti-proliferative activity

To improve solubility, each phenanthroindolizidine alkaloid was converted to its HCl salt by dissolving the free amine in CH_2Cl_2, adding a large excess (>10 equiv) of HCl (2.0 M in diethylether) and removing the solvent in vacuo. Stock solutions (10 mM) of the each compound were prepared in DMSO.

MCF-7, DU-145 and A549/NF-κB-luc cancer cells were harvested (125 G centrifuge for 5 min) from an exponential-phase maintenance culture. The cells were re-suspended in new culture media (RPMI1640 (GIBCO11875) with 10% FBS, EMEM (ATCC P/N 30-2003) with 10% FBS, and DMEM (ATCC P/N 30-2002) with 10% FBS, penicillin, streptomycin and hygromycin) and the cell density was adjusted to 10^5 cells/mL and dispensed in 96-well culture plates at a density of 5000 cells per well (50 μL). The cells were incubated overnight to allow cells to adhere to the wells. Fresh culture medium (50 μL) containing varying concentrations of the test and control compounds was added to each well. The cultures were grown for an additional 72 h and alamarBlue® (10 μL) was added. After 1.5 h, the fluorescence excitation (530 nm) and emission (590 nm) of each well were measured to determine the optical density. Each compound was tested in triplicate with less than 5% variation. Compound **7h** was tested in duplicate.

4.4.2. NF-κB activity assay

A549/NF-κB-luc cells were cultured using complete growth medium (ATCC P/N 30-2002, 10% FBS, penicillin and streptomycin) containing hygromycin at 37 °C with 5% CO_2. After trypsinizing and centrifuging the cells (150 × G for 5 min), the cell density was adjusted to 10^5 cells/mL and dispensed in 96-well culture plates at a density 5000 cells per well (50 μL). The cells were incubated for 24 h, allowing cells to attach. The appropriate compound in the complete growth medium (50 μL) was added to each well and the cells were incubated for another 30 min. TNF-α (5 μL of TNF-α solution in 1 × PBS (1:667 dilution of TNF-α in 1X PBS)) was then added to each well. After incubating for another 7 h, luciferin reagent (100 μL; Bright-Glo™ from Promega) was added to each well. After 2 min, luciferase activity was measured

by luminescence detection according to the manufacturer's instructions. Each compound was tested in triplicate with less than 5% variation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.07.044.

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