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Stereoselective synthesis of a natural product inspired tetrahydroindolo[2,3-*a*]-quinolizine compound library

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ABSTRACT

A natural product-inspired synthesis of a compound collection embodying the tetrahydroindolo [2,3-*a*]quinolizine scaffold was established with a five step synthesis route. An imino-Diels–Alder reaction between Danishefsky's diene and the iminoesters derived from tryptamines was used as a key reaction. Reductive amination of the ketone function and amide synthesis with the carboxylic acid derived from the ethyl ester, were used to decorate the core scaffold. Thus a compound library of 530 tetrahydroindolo[2,3-*a*]quinolizines was generated and submitted to European lead factory consortium for various biological screenings.

1. Introduction

One of the important obstacles in the discovery of hit and lead structures from high throughput screening (HTS) campaigns of compound collections is the redundancy of the core-structures or scaffolds of the libraries.[1] In many cases, library synthesis efforts are driven by the availability of inexpensive substrates or synthesis routes which often result in heavily compromised structural features of molecules and yield for instance relatively flat heterocycles.[2] Such compound collections may fail to provide interesting starting points for drug discovery research.[3] Biological relevance of the molecular frameworks or scaffolds used in the synthesis of a compound collection bequeaths their ability to modulate different biological functions.[4] Scaffolds of natural products for instance, encode evolutionary selected properties for interacting with proteins and, therefore, represent biologically prevalidated frameworks.[5] Natural product based synthesis thus employs the core structures of natural products as scaffolds for compound collections.[6] Natural product derived molecules behold the frameworks identical with the core structure of a natural product in which different substituents are introduced at exactly the same positions as predetermined by nature. However, in natural-product-inspired synthesis, closely related structural frameworks of natural products may be used for library synthesis.[6a] In this approach, not only the relative positions and nature of substituents can be varied but also different relative stereochemistry patterns can be generated covering a broader chemical space of a particular structural class.

The European lead factory[7] (ELF) is a consortium and a platform where academic research groups have joined industrial partners to build up compound collections from novel chemical space and with structural features that pharmaceutical compound collections have been lacking so far. In order to enrich the ELF compound collection with natural product based structural features, we set out to develop a synthetic access to the indole alkaloid-inspired tetrahydroindolo[2,3-*a*]quinolizine scaffold and a compound collection based on this framework (Fig. 1).[8] Many natural products embodying the indolo[2,3-*a*]quinolizine framework display a wide range of biological activities. For instance, the antibacterial lercheine (**I**), the antiviral natural product hirsutine (**II**), the cytotoxic compound 10-hydroxyngustine (**III**) as well as the antiplasmodial agent glabratine (**IV**, Fig. 1).[9] We have previously reported the synthesis and biology of anticancer centrocountins, that is, indoloquinolizines (**V**) and related phosphatase inhibitors (**VI**, Fig. 1).[10] These findings provide further validation to employing the indolo[2,3-*a*]quinolizine scaffold as a promising structural framework to build compound libraries for drug discovery endeavours.

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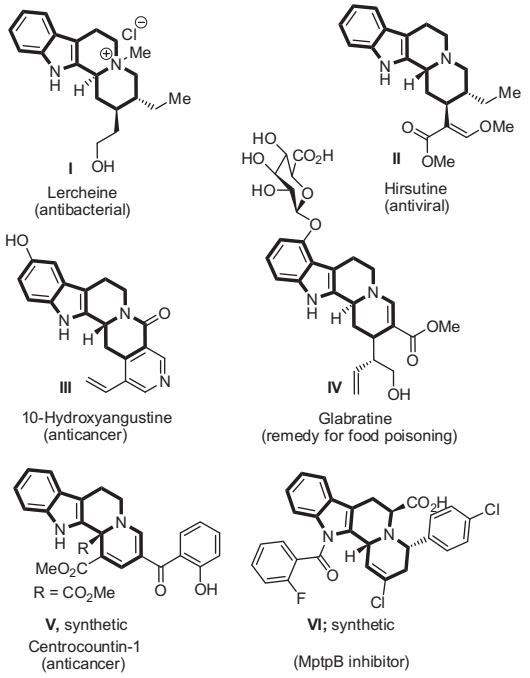
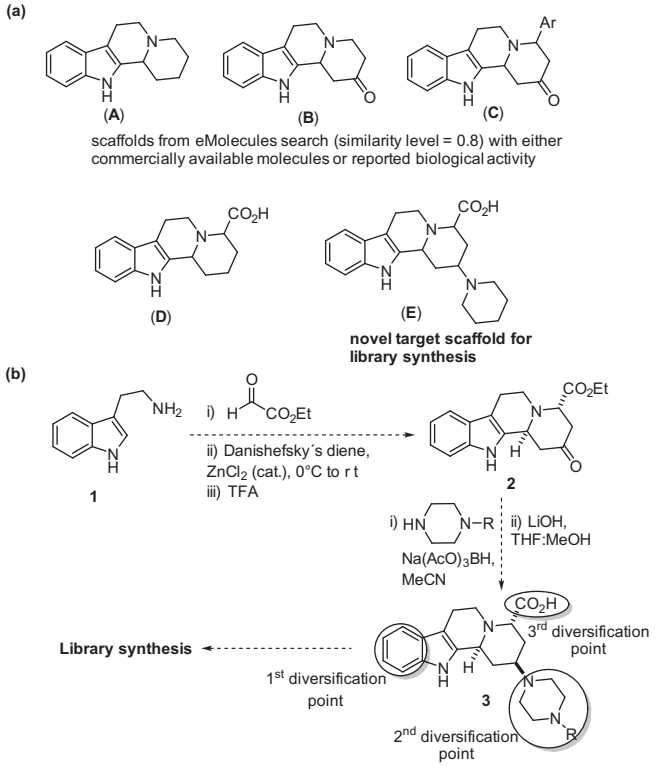


Figure 1. Biologically active natural and synthetic molecules embodying the tetrahydroindolo[2,3-*a*]quinolizine scaffold.



Scheme 1. Design and synthetic planning of tetrahydroindolo[2,3-*a*]quinolizines library.

2. Results and discussion

2.1. Synthesis design and planning of the indoloquinolizine library

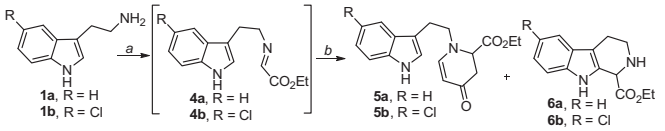
Among different criteria set up in ELF for accepting a synthetic proposal for a library, the most important criterion is the novelty of the scaffold. An inspection of the molecules emanating from an eMolecules search revealed that tetrahydro-indoloquinolizine scaffold **A** or ketone **B** derivatives were either reported or commercially available and thus were not suitable for library synthesis. Scaffold **C** (Scheme 1a), though was not commercial but was part of molecules with reported biological activities.[8] Interestingly, scaffold **D** embodying a carboxylic acid ester or derivatives of it as well as scaffold **E** were found to be novel frameworks with no hits found either in an eMolecules search or in other common search engines (Scifinder etc.) and were therefore chosen as the target scaffolds for the library synthesis.

A synthetic plan for the library with three diversification points is presented in Scheme 1b. A hetero-Diels–Alder reaction between Danishefsky's diene and imines, formed from tryptamine derivatives (**1**) and ethyl glyoxylate, would provide the tetracyclic indoloquinolizines (**2**) on acidic treatment. The ketone function in compound **2** can be elaborated further by reductive amination with various amines. Ester saponification would lead to acid derivatives **3** which can provide a handle for further combinatorial synthetic steps to build up a compound library (Scheme 1b).

2.2. Synthesis optimization of the tetrahydroindolo[2,3-*a*]quinolizine scaffold

As depicted in the synthesis plan (Scheme 1b), tryptamine **1a** was treated with freshly distilled ethyl glyoxylate to obtain imine **4a**[11] which was used directly without purification for imino-Diels–Alder (IDA) reactions. In the absence of Lewis acid no product formation was observed (Table 1, entry 1). Mediation by Lewis acids such as $ZnCl_2$, and $Cu(OTf)_2$ at 0 °C afforded Pictet–Spengler products **6** as the major product (Table 1, entries 2 & 3). The IDA reaction was also tested with a Lewis base (LiOMe) at 0 °C based on a recent report by Mukaiyama and co-workers,[12] but did not yield any desired product (Table 1, entry 4). The reaction with $ZnCl_2$ at −78 °C encouragingly yielded a slight excess of the desired product **5a** (Table 1, entry 5). However, the isolation of **5a** from the crude reaction mixture was highly tedious and contaminations from different impurities remained with cycloadduct **5a** after column chromatographic purification. Interestingly, IDA reaction catalyzed by $Yb(OTf)_3$ at −30 °C was not only high yielding for **5a** but also the purification of **5a** was simple in the absence of side reactions (Table 1, entry 6). Further reaction condition optimization led to

Table 1
Optimization of imino-Diels Alder reaction. The bold numbers are highlight the best conditions observed in the optimization reactions



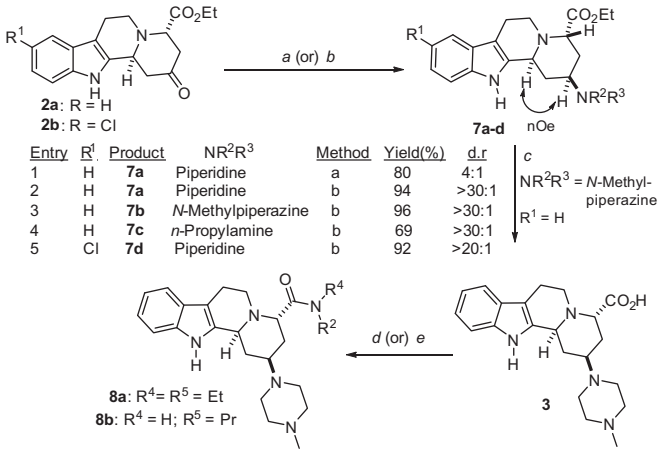
Entry	R	Lewis acid/base	Mole (%)	Temp (°C)	Yield (%)	
					5	**6**
1	H	–	–	0	–	16
2	H	$ZnCl_2$	5	0	32	44
3	H	$Cu(OTf)_2$	5	0	31	36
4	H	LiOMe	5	0	–	–
5	H	$ZnCl_2$	5	−78	38	32
6	H	$Yb(OTf)_3$	5	−30	54	34
7	**H**	**$Yb(OTf)_3$**	**5**	**−78**	**77**[c]	**8**
8	**Cl**	**$Yb(OTf)_3$**	**5**	**−78**	**74**[c]	**6**

Conditions: [a] ethyl glyoxylate, dry CH_2Cl_2, molecular sieves 4 Å, 0 °C, 1 h; [b] Danishefsky's diene, Lewis acid, CH_3CN, 4–8 h; [c] imine formation was done at −40 °C.

the following protocol for the synthesis of **5a**: formation of imine **4a** from tryptamine **1a** and ethyl glyoxylate at −40 °C was followed by treatment with Danishefsky's diene in the presence of catalytic $Yb(OTf)_3$ (5 mol %) at −78 °C in a one pot fashion affording the vinylogous amide **5a** in 77% yield on a 1.8 mmol scale (Table 1, entry 7). Reaction sequence was further scaled up to 18.1 mmol scale using similar reaction conditions which afforded the product **5a** in 67% yield. Under the optimized reaction conditions and employing 5-chlorotryptamine **1b** derived imine **4b** (4.2 mmol), the expected vinylogous amide **5b** was obtained in 74% yield (Table 1, entry 8).

Our attempts to get the tetracyclic indoloquinolizines (**2**) from tryptamine **1a** in one pot as shown in the Scheme 1, however, remained unsuccessful. Hence the cyclization of the vinylogous amide **5a** to provide **2a** was tested with different acids as a separate step. Treatment of compound **5a** with trifluoroacetic acid, triflic acid and sulfuric acid did not yield the desired product **2a** (Table 2, entries 1–4). Treatment of compound **5a** with hydrochloric acid in ethanol did not provide the expected compound (Table 1, entry 5), but resulted in the formation of a 1:1 mixture of the diethyl ketal and enol ethyl ether derivative of compound **2a**. Interestingly, when the amide **5a** (0.5 mmol scale) was treated with a 4 M solution of hydrochloric acid in dioxane (10 equiv) the expected tetracyclic compound **2a** was obtained in 69% yield with 4:1 diastereomeric ratio (Table 2, entry 6). No change in the diastereoselectivity was observed when the same reaction was performed at higher scale (4.1 mmol).

However, the cyclization reaction of the amide **5b** with 4 M solution of HCl in dioxane (10 equiv) led to little conversion (<10%) at room temperature (Table 2, entry 7). Under reflux condition, the cyclization did proceed but afforded **2b** in a poor yield and with 1:1 diastereoselectivity (Table 2, entry 8). Interestingly, treatment of the amide **5b** (1.3 mmol scale) with continuous and constant flow of HCl (g) in dioxane resulted in the formation of the tetracycle **2b** in good yield and with a 5:1 diastereomeric ratio (Table 2, entry 9). The relative stereochemistry of the tetracyclic compound **2b** was unambiguously confirmed by single crystal X-ray analysis.[13]

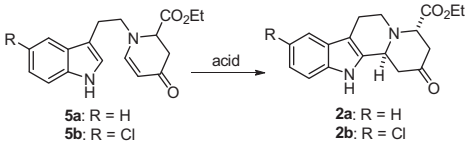


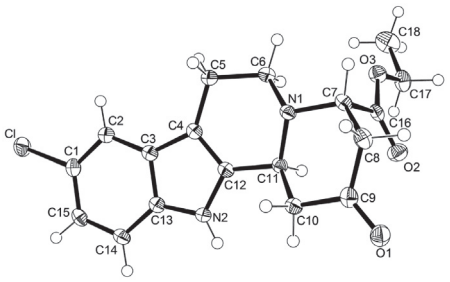
Entry	R^1	Product	NR^2R^3	Method	Yield(%)	d.r
1	H	**7a**	Piperidine	a	80	4:1
2	H	**7a**	Piperidine	b	94	>30:1
3	H	**7b**	*N*-Methylpiperazine	b	96	>30:1
4	H	**7c**	*n*-Propylamine	b	69	>30:1
5	Cl	**7d**	Piperidine	b	92	>20:1

Scheme 2. Reductive amination and amide synthesis. Reagents and conditions: (a) piperidine, $NaBH_3(CN)$, AcOH, dry THF, molecular sieves 4 Å, rt, 6 h; (b) piperidine, $NaBH(OEh)_3$, EhOH, THF/CH_2Cl_2, molecular sieves 4 Å, rt, 6 h. Eh = 2-ethylhexanoyl; (c) concd HCl, reflux, 10 h; (d) diethylamine, EDC, HOBt, DIPEA, DMF, 0 °C to rt, 63%; (e) *n*-propylamine, EDC, HOBt, DIPEA, DMF, 0 °C to rt, 67%.

Reductive amination of the tetracyclic compound **2a** by a standard protocol using sodium cyanoborohydride in acetic acid afforded the amine derivative **7a** in very good yield with 4:1 diastereomeric ratio (Scheme 2, entry 1). While the separation of the two isomers was very difficult, we resorted to a highly stereoselective reductive amination. To this end, we observed that the use of bulkier trialkoxyborohydride can provide the desired adduct **7** in a very good yield and in a highly stereoselective manner.[13] As expected, the reductive amination of **2a** with the piperidine, using sodium tri(2-ethylhexanoyloxy) borohydride as reducing agent afforded the amine compound **7a** in an excellent yield and diastereoselectivity (>30:1) (Scheme 2, entry 2). The relative stereochemistry of the product **7a** was established by NOESY analysis (see the Supporting information). Similarly, the stereoselective reductive amination of **2a** with *N*-methylpiperazine employing

Table 2
Cyclization of vinylogous amide **5**. The bold numbers are highlight the best conditions observed in the optimization reactions





Entry	R	Acid[a]	Solvent	Product	Yield (%)	dr
1	H	CF_3CO_2H[b]	CH_2Cl_2	**2a**	0	–
2	H	CF_3CO_2H	CH_2Cl_2	**2a**	0	–
3	H	CF_3SO_3H	CH_2Cl_2	**2a**	0	–
4	H	H_2SO_4	CH_3CN	**2a**	0	–
5	H	HCl (g)	EtOH	**2a**	0[c]	–
6	**H**	**HCl**	**Dioxane**	**2a**	**69**	**4:1**
7	Cl	HCl	Dioxane	**2b**	<10	–
8	Cl	HCl	Dioxane	**2b**	28[d]	1:1
9	**Cl**	**HCl (g)**	**Dioxane**	**2b**	**62**	**5:1**

[a] Reactions were performed at 0 °C to rt unless otherwise specified.
[b] Reaction was performed at −78 °C.
[c] 1:1 mixture of diethyl ketal and enol ethyl ether derivative of compound **2a** was observed.
[d] The reaction was performed at reflux temperature for 4 h.

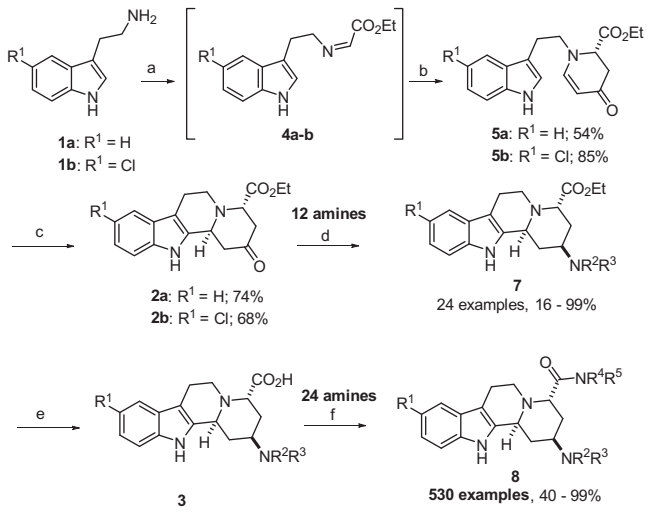
above reaction conditions afforded the amine compound **7b** in an excellent yield and diastereoselectivity (>30:1) (Scheme 2, entry 3). The reductive amination of **2a** with primary amines for instance, *n*-propylamine was also high yielding as well as highly stereoselective (**7c**, Scheme 2, entry 4). Likewise, the chloro derivative **2b** upon treatment with piperidine under similar reaction conditions provided the desired product **7d** in 92% yield with a very good diastereoselectivity (>20:1) (Scheme 2, entry 5).

In order to have a carboxylic acid function, which can be explored in a combinatorial step to build a compound collection, we explored the hydrolysis of the ethyl ester avoiding any possible epimerization. Thus, compound **7b** was hydrolyzed to the corresponding carboxylic acid **3** under acidic conditions (Scheme 2). The crude carboxylic acid (**3**) was used in the amide coupling with diethyl- and *n*-propylamine using EDC, HOBt and DIPEA affording the amide derivative **8a** and **8b** respectively in good yields (63–67%) over two steps.

2.3. Generation of a tetrahydroindolo[2,3-*a*]quinolizine library

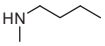
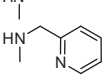
Synthesis optimization of the core-scaffold followed the design of a library containing 3 points of diversity, as shown in Scheme 3. A matrix of $2 \times 12 \times 24$ using tryptamine derivatives, secondary amines and amines, respectively, was selected to ensure that at least 80% of the final compounds have 'lead-like' properties. The physicochemical constraints, commercial availability, cost and reactivity considerations prompted us to select monoatomic substituents (H, Cl) at position 6 of the starting tryptamine and small or polar secondary amines for the reductive amination step. This allowed for a wider structural diversity to be installed via amines at the final amide coupling step (Table 3).

The key one-pot imination/imino Diels–Alder reaction proceeded well at higher scales too. The use of HPLC quality DCM proved a better choice over the distilled solvent. Acidic cyclization of the vinylogous amides **5** was afforded by using 4 M HCl in dioxane instead of HCl (g). Although this required a longer reaction time (144 vs 36 h), this procedure maintained the adequate conversion to the products (68–74%). Importantly, the diastereomeric ratio in the formation of the IDA products was not affected (dr = 4:1, Scheme 3).



Scheme 3. Production of the tetrahydroindolo[2,3-*a*]quinolizine library. Reagents and conditions: (a) ethyl glyoxylate, dry CH_2Cl_2, molecular sieves 4 Å, −40 °C, N_2 atmosphere, 1 h; (b) Danishefsky's diene, Yb(OTf)$_3$, CH_3CN, −78 °C, N_2 atmosphere, overnight; (c) HCl 4 M in dioxane, 0 °C to rt, 7 days or HCl(g), dioxane, 0 °C to rt, 36 h; (d) amine, NaBH(OEh)$_3$, EhOH, THF/CH_2Cl_2, molecular sieves 4 Å, N_2 atmosphere, rt, 6–24 h; (e) concd HCl, reflux, 10–36 h (f) amine, HOBt, EDCHCl, DIPEA, DMF, 50 °C, N_2 atmosphere, 18 h.

Table 3
Representative examples for the reductive amination step

7	R^1	R^2	Yield (%)	7	R^1	R^2	Yield (%)
7a	H		81	**7i**	Cl		78
7b	H		62	**7j**	Cl		59
7c	H		99	**7k**	Cl		68
7d	H		57	**7l**	Cl		48
7e	H		77	**7m**	Cl		72
7f	H		16	**7n**	Cl		42
7g	H		66	**7o**	Cl		70

The chemistry optimized in the synthetic validation of the scaffold tetrahydroindolo[2,3-*a*]quinolizine (Tables 1 and 2 and Scheme 2) was generally reproducible in the library production phase across various substrates used and yields were overall comparable to the ones reported at smaller scale, as summarized in Table 3 for a subset of amines used in the reductive amination step.

The validated procedure for the last diversification step, using HATU as amide coupling agent was found not to be general enough for the parallel synthesis using 15 mL reaction tubes in a 24 position Mettler Toledo Miniblock®. A different protocol using EDC and HOBt in DIPEA proved to be efficient. Initially, an EDC: HOBt ratio of 1.5:2.0 equiv was employed, but the detection of significant amounts of by-products in the uHPLC analysis with the mass of corresponding carboxylic acid/HOBt intermediate led us to a final stoichiometry of 1.8:1.5 equiv With this scaffold-optimized amidation protocol, 530 compounds of sufficient quantity (>5 μmol) and purity (LC–MS >85%), were synthesized and isolated (92% success rate) after HPLC/MS-based analysis and purification.

3. Conclusion

A synthetic route to structurally complex tetrahydroindolo[2,3-*a*]quinolizines was established using an imino-Diels–Alder reaction as the key step and leading to a compound library of more than 500 members. Synthetic validation of the scaffold was performed in multigram scale reactions and successfully transferred in the production phase of the library. In the latter case, reductive amination of cyclic ketones and amide synthesis were used as synthetic steps to build up a compound collection for the European lead factory consortium. The library is currently being exposed to various HTS campaigns and the results will be made public in due course of time.

4. Experimental

4.1. General methods

Unless otherwise noted, all reagents and solvents were purchased commercial suppliers and were used without further purification without further purification. Thin layer chromatography (TLC) was performed on Merck silica gel 60F254 aluminum sheet. For flash chromatography Baker silica gel (40–70 μm) was used. ^{1}H and ^{13}C NMR spectroscopic data were recorded on a Varian Mercury VX 400 or Varian 500-inova500 spectrometer at RT. ^{1}H and ^{13}C NMR spectra were calibrated to the solvent signals of $CDCl_3$ (=7.26 and 77.00 ppm) and the coupling constants are displayed in Hz. Fast atom bombardment (FAB)-MS measurements were taken on

Finnigan MAT MS70 spectrometer and electrospray ionization (ESI)-MS were measured by using an Agilent 1100 series binary pump together with a reversed-phase HPLC column (Macherey-Nagel).

4.2. Ethyl 1-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-1,2,3,4-tetrahydropyridine-2-carboxylate (5a)

Activated molecular sieves 4 Å (500 mg/1.00 mmol substrate) were taken in a dry 250 mL round bottom (RB) flask, and to this dry CH_2Cl_2 (10 mL) was added followed by tryptamine (1.872 mmol) under argon atmosphere. The solution was cooled to −40 °C and stirred for 10 min. Then, freshly distilled ethyl glyoxylate (2.0 mmol, 1.05 equiv) was added dropwise. The reaction mixture was continued to stir at the same temperature for 2 h. TLC analysis showed complete conversion of tryptamine. The reaction mixture was again cooled down to −78 °C and after stirring for another 10 minutes at −78 °C, Danishefsky's diene (2.0 mmol, 1.05 equiv) was added dropwise over 10 minutes followed by a dropwise addition of the solution of ytterbium triflate (0.094 mmol, 0.05 equiv) in acetonitrile (1 mL). The reaction mixture was continued to stir at −78 °C for 3–6 h. After completion of the reaction, it was warmed to room temperature and filtered through sintered funnel and washed with CH_2Cl_2 (50 mL). The combined filtrate was washed with saturated sodium bicarbonate solution (10 mL), water (10 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a crude compound. The crude compound was purified by flash column chromatography over silica gel (0.04–0.06 mm particle size, 60 times of the weight of the crude compound) using 2–5% methanol in dichloromethane (DCM) as a gradient eluent to afford the pure IDA adduct **5a** in 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.11 (t, J = 7.6 Hz, 1H), 7.00 (d, J = 2.2 Hz, 1H), 6.92 (d, J = 7.6 Hz, 1H), 4.89 (d, J = 7.5 Hz, 1H), 4.15 (q, J = 7.1 Hz, 2H), 3.93 (dd, J = 7.4, 2.8 Hz, 1H), 3.71–3.61 (m, 1H), 3.58–3.46 (m, 1H), 3.04 (t, J = 6.6 Hz, 2H), 2.71 (dd, J = 16.7, 2.8 Hz, 1H), 2.64 (dd, J = 16.7, 7.5 Hz, 1H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.3, 170.3, 154.3, 136.8, 127.0, 123.1, 122.3, 119.6, 118.3, 112.0, 111.4, 98.4, 62.3, 59.6, 56.1, 37.9, 26.1, 14.3. HRMS [ESI]: calculated for $C_{18}H_{21}N_2O_3$ [M+H$^+$]: 313.15467. Found: 313.15463.

4.3. Ethyl 1-(2-(5-chloro-1*H*-indol-3-yl)ethyl)-4-oxo-1,2,3,4-tetrahydropyridine-2-carboxylate (5b)

Following the similar procedure above (Section 4.2), 5-chlorotryptamine (4.37 mmol) was converted to an imine at −40 °C followed by the IDA reaction of the imine with Danishefsky's diene (4.58 mmol) at −78 °C that gave compound **5b** in 74% yield after flash column chromatography purification using 2–5% methanol in dichloromethane as an eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.49 (d, J = 2.0 Hz, 1H), 7.29 (d, J = 8.6 Hz, 1H), 7.12 (dd, J = 8.6, 2.0 Hz, 1H), 7.04 (d, J = 2.3 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 4.87 (d, J = 7.6 Hz, 1H), 4.16 (q, J = 7.1 Hz, 2H), 3.94 (dd, J = 7.1, 2.6 Hz, 1H), 3.65 (dt, J = 13.7, 8.6 Hz, 1H), 3.51 (dt, J = 20.2, 6.4 Hz, 1H), 2.99 (t, J = 6.6 Hz, 2H), 2.75–2.69 (m, 1H), 2.65 (dd, J = 16.7, 7.5 Hz, 1H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.2, 170.2, 154.2, 135.1, 128.0, 125.4, 124.5, 122.7, 117.8, 113.0, 111.2, 98.6, 62.4, 59.6, 55.9, 37.9, 25.9, 14.3. HRMS [ESI]: calculated for $C_{18}H_{20}ClN_2O_3$ [M+H$^+$]: 347.11570. Found: 347.11572.

4.4. Ethyl 2-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-4-carboxylate (2a)

The IDA adduct **5a** (4.16 mmol) was taken in a dry 50 mL RB flask and to this dry dioxane (2 mL) was added. It was stirred for 10 minutes at room temperature to make a clear solution. Then it was cooled to 0 °C and stirred for 15 min. To this reaction mixture 21 mL of HCl in dioxane (4 N, 83.24 mmol) was added dropwise at 0 °C and the reaction mixture was stirred at the same temperature for 1 h. Then it was allowed to stir at rt for 24 h. The solvent was removed under reduced pressure and was diluted with DCM (50 mL). This solution was washed with saturated sodium bicarbonate solution (20 mL) followed by water (20 mL) and dried over anhydrous sodium sulfate. It was concentrated under reduced pressure to give crude product as mixture of isomers (~4:1 diastereomeric ratio). The crude compound was purified by flash column chromatography over silica gel (0.04–0.06 mm particle size, 100 times of the weight of crude compound) using 1–4% ethyl acetate in dichloromethane as an eluent to afford pure tetracyclic compound **2a** in 69% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.50 (d, J = 7.7 Hz, 1H), 7.34 (d, J = 7.9 Hz, 1H), 7.17 (t, J = 7.4 Hz, 1H), 7.11 (t, J = 7.4 Hz, 1H), 4.50 (d, J = 10.1 Hz, 1H), 4.26–4.14 (m, 2H), 4.11 (dd, J = 6.7, 1.9 Hz, 1H), 3.31 (dd, J = 12.2, 4.5 Hz, 1H), 3.14–2.96 (m, 2H), 2.95–2.77 (m, 3H), 2.66 (d, J = 15.0 Hz, 1H), 2.56 (dd, J = 14.4, 11.1 Hz, 1H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.48, 170.80, 136.53, 133.70, 127.11, 122.09, 119.85, 118.41, 111.27, 108.30, 63.87, 61.39, 52.46, 50.49, 45.57, 43.16, 22.21, 14.65. HRMS [ESI]: calculated for $C_{18}H_{21}N_2O_3$ [M+H$^+$]: 313.15467. Found: 313.15469.

4.5. Ethyl 9-chloro-2-oxo-1,2,3,4,6,7,12,12b-octahydroindolo-[2,3-*a*]quinolizine-4-carboxylate (2b)

To a solution of IDA adduct **5b** (1.3 mmol) in dry dioxane (10 mL), dry HCl (g) (generated by the reaction between 5 g of NaCl and 10 mL of Conc. H_2SO_4) was purged in to the solution for 60 min at 0 °C and was stirred at the same temperature for 1 h. Then it was allowed to stir at rt for 36 h until completion. The solvent was removed under reduced pressure and was diluted with DCM (30 mL). This solution was washed with saturated sodium bicarbonate solution (10 mL) followed by water (10 mL) and dried over sodium sulfate and concentrated under reduced pressure to give crude product (~5:1 diastereomeric ratio). The crude compound was purified by column chromatography over flash silica gel (0.04–0.06 mm particle size, 100 times the weight of the crude compound) using 0.0–0.6% methanol in dichloromethane to give the title compound **2b** in 62% yield. ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.28 (s, 1H), 7.47 (s, 1H), 7.29 (d, J = 8.6 Hz, 1H), 7.10 (dd, J = 8.7, 1.5 Hz, 1H), 4.45 (d, J = 11.2 Hz, 1H), 4.27–4.15 (m, 2H), 4.11 (d, J = 6.6 Hz, 1H), 3.30 (dd, J = 11.3, 4.0 Hz, 1H), 3.05 (td, J = 11.1, 3.8 Hz, 1H), 3.00–2.79 (m, 3H), 2.76 (dd, J = 14.8, 1.6 Hz, 1H), 2.65–2.50 (m, 2H), 1.29 (tt, J = 7.1, 1.3 Hz, 3H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 205.4, 170.9, 135.9, 134.9, 128.3, 125.1, 121.8, 117.8, 112.2, 108.1, 63.9, 61.3, 52.2, 50.3, 45.5, 43.1, 22.2, 14.4. HRMS [ESI]: calculated for $C_{18}H_{20}ClN_2O_3$ [M+H$^+$]: 347.11570. Found: 347.11568.

4.6. Ethyl 2-(piperidin-1-yl)-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-4-carboxylate (7a)

The tetracyclic keto compound **2a** (0.42 mmol) was dissolved in dry THF (3 mL), and an equivalent weight of activated, cooled 4 Å molecular sieves was added followed by addition of 2-ethylhexanoic acid (0.42 mmol) and piperidine (0.62 mmol). The flask was flushed with argon and the solution was stirred at room temperature for 1 h. $NaBH(OEh)_3$ (0.83 mmol) dissolved in DCM (3 mL) was then added under argon atmosphere and the mixture was stirred for further 4 h at room temperature. Saturated aqueous $NaHCO_3$ solution (10 mL) was added to the reaction mixture followed by DCM (20 mL). The extracted organic layer was collected and washed with water (10 mL), dried over anhydrous sodium sulfate

and concentrated in vacuum to give the crude product that was purified by flash column chromatography using 4–8% methanol in dichloromethane as an eluent to afford pure compound **7a** (150 mg, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.46 (d, J = 7.5 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.17–7.12 (m, 1H), 7.11–7.06 (m, 1H), 4.72 (s, 1H), 4.32–4.17 (m, 2H), 3.53 (dd, J = 11.3, 2.9 Hz, 1H), 3.36 (dd, J = 14.1, 5.6 Hz, 1H), 3.25–3.16 (m, 1H), 3.04–2.91 (m, 1H), 2.68–2.61 (m, 2H), 2.55 (dd, J = 16.0, 4.5 Hz, 1H), 2.50–2.38 (m, 3H), 2.35–2.19 (m, 2H), 1.97–1.91 (m, 1H), 1.72 (dd, J = 23.2, 11.7 Hz, 1H), 1.67–1.53 (m, 4H), 1.48–1.41 (m, 2H), 1.30 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.0, 173.5, 136.2, 132.8, 127.7, 121.7, 119.6, 118.1, 111.5, 108.3, 61.2, 57.9, 57.6, 55.2, 49.8, 48.6, 30.6, 29.2, 25.8, 24.5, 17.1, 14.5. HRMS [ESI]: calculated for $C_{23}H_{32}N_3O_2$ [M+H$^+$]: 382.24890. Found: 382.24888.

4.7. Ethyl 2-(4-methylpiperazin-1-yl)-1,2,3,4,6,7,12,12b-octa-hydroindolo[2,3-*a*]quinolizine-4-carboxylate (7b)

Following the similar procedure (Section 4.6), the tetracyclic keto compound **2a** (0.32 mmol) was treated with *N*-methyl piperazine (0.48 mmol) in the presence of ethylhexanoic acid (0.32 mmol) and $NaBH(OEh)_3$ (0.64 mmol) to get the crude product that was purified by flash column chromatography using 4–8% methanol in dichloromethane as an eluent to afford pure compound **7b** (122 mg, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.16 (t, J = 7.5 Hz, 1H), 7.10 (t, J = 7.4 Hz, 1H), 4.72 (s, 1H), 4.31–4.18 (m, 2H), 3.54 (dd, J = 10.7, 2.7 Hz, 1H), 3.34 (dd, J = 13.9, 5.6 Hz, 1H), 3.27–3.17 (m, 1H), 3.04–2.92 (m, 1H), 2.89–2.74 (m, 1H), 2.73–2.64 (m, 2H), 2.59–2.38 (m, 6H), 2.28 (s, 3H), 2.25–2.19 (m, 2H), 1.95 (d, J = 12.5 Hz, 1H), 1.75 (q, J = 11.7 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.2, 173.5, 136.0, 133.0, 127.7, 121.8, 119.8, 118.3, 111.3, 108.7, 61.1, 58.1, 56.7, 55.2, 54.6, 48.7, 48.6, 46.1, 46.0, 31.0, 29.9, 17.4, 14.5. HRMS [ESI]: calculated for $C_{23}H_{33}N_4O_2$ [M+H$^+$]: 397.25980. Found: 397.25982.

4.8. Ethyl 2-(propylamino)-1,2,3,4,6,7,12,12b-octahydroindolo-[2,3-*a*]quinolizine-4-carboxylate (7c)

Following the similar procedure (Section 4.6), the tetracyclic keto compound **2a** (0.16 mmol) was treated with *n*-propylamine (0.24 mmol) in the presence of ethylhexanoic acid (0.16 mmol) and $NaBH(OEh)_3$ (0.32 mmol) to get the crude product that was purified by flash column chromatography using 5–10% methanol in dichloromethane as an eluent to afford pure compound **7c** (40 mg, 69% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.17–7.12 (m, 1H), 7.12–7.07 (m, 1H), 4.70 (s, 1H), 4.30–4.15 (m, 2H), 3.58 (dd, J = 8.7, 4.0 Hz, 1H), 3.35–3.21 (m, 2H), 3.02–2.89 (m, 1H), 2.72–2.64 (m, 1H), 2.64–2.54 (m, 2H), 2.53–2.46 (m, 1H), 2.23–2.06 (m, 2H), 2.02–1.96 (m, 2H), 1.74 (dt, J = 12.3, 8.6 Hz, 1H), 1.54–1.42 (m, 2H), 1.30 (t, J = 7.1 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 136.1, 134.0, 127.8, 121.7, 119.7, 118.2, 111.1, 108.5, 60.9, 58.4, 52.5, 50.7, 49.2, 48.8, 35.4, 34.9, 23.6, 18.7, 14.5, 12.0. HRMS [ESI]: calculated for $C_{21}H_{30}N_3O_2$ [M+H$^+$]: 356.23325. Found: 356.23321.

4.9. Ethyl 9-chloro-2-(piperidin-1-yl)-1,2,3,4,6,7,12,12b-octa-hydroindolo[2,3-*a*]quinolizine-4-carboxylate (7d)

Following the similar procedure Section 4.6, the tetracyclic keto compound **2b** (0.09 mmol) was treated with piperidine (0.13 mmol) in the presence of ethylhexanoic acid (0.09 mmol) and $NaBH(OEh)_3$ (0.18 mmol) to get the crude. The crude compound was purified by flash column chromatography using 4–8% methanol in dichloromethane as eluent to afford pure compound **7d** (122 mg, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H), 7.40 (d, J = 1.5 Hz, 1H), 7.29 (d, J = 8.6 Hz, 1H), 7.08 (dd, J = 8.6, 2.0 Hz, 1H), 4.69 (s, 1H), 4.32–4.17 (m, 2H), 3.49 (dd, J = 11.2, 2.8 Hz, 1H), 3.34 (dd, J = 14.3, 5.9 Hz, 1H), 3.25–3.14 (m, 1H), 2.98–2.87 (m, 1H), 2.82–2.71 (m, 2H), 2.68–2.58 (m, 2H), 2.57–2.48 (m, 3H), 2.23 (td, J = 13.1, 4.8 Hz, 1H), 1.93 (dd, J = 11.8, 2.6 Hz, 1H), 1.82–1.62 (m, 5H), 1.53–1.43 (m, 2H), 1.30 (td, J = 7.1, 0.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.2, 173.0, 134.7, 134.0, 128.7, 125.3, 122.0, 117.7, 112.7, 108.1, 61.4, 58.2, 57.8, 55.1, 49.7, 49.5, 48.4, 30.3, 28.7, 25.2, 24.1, 16.9, 14.5. HRMS [ESI]: calculated for $C_{23}H_{31}ClN_3O_2$ [M+H$^+$]: 416.20993. Found: 416.20991.

4.10. *N*,*N*-Diethyl-2-(4-methylpiperazin-1-yl)-1,2,3,4,6,7,12,-12b-octahydroindolo[2,3-*a*]quinolizine-4-carboxamide (8a)

A mixture of reductive amination product **7b** (0.09 mmol) and conc. HCl (2 mL) was refluxed overnight. TLC and LCMS analysis were used to monitor the reaction. After completion, the solvent was removed under vacuum to give the crude product **3** (quantitative) and was used directly for the next step without purification. The crude acid **3** was dissolved in dry DMF (2 mL) and treated with DIPEA (0.45 mmol) followed by HOBt (0.226 mmol), diethylamine (0.1 mmol) and EDC (0.2 mmol) at 0 °C and the reaction mixture was allowed to stir at room temperature for 10 h. TLC was used to monitor the completion of the reaction. After completion, the solvent was removed in vacuo to give crude. The crude mixture was purified by flash column chromatography using 5–10% methanol in DCM as an eluent to afford pure compound **8a** (0.06 mmol) in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.12 (t, J = 7.3 Hz, 1H), 7.07 (t, J = 7.4 Hz, 1H), 3.55–3.31 (m, 6H), 3.21–3.05 (m, 1H), 2.98–2.85 (m, 1H), 2.82–2.52 (m, 11H), 2.41–2.49 (m, 1H), 2.36 (s, 3H), 2.32–3.24 (m, 2H), 1.99–1.91 (m, 1H), 1.22–1.19 (m, 3H), 1.16 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 136.2, 134.5, 127.4, 121.7, 119.6, 118.3, 111.2, 108.6, ^{13}C NMR (101 MHz, cdcl$_3$) δ ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 136.2, 134.5, 127.4, 121.7, 119.6, 118.3, 111.2, 108.6, 61.4, 55.0, 48.8, 48.7, 48.4, 48.3, 45.5, 44.2, 44.0, 42.1, 40.8, 30.9, 30.5, 22.1, 13.2, 13.1. HRMS [ESI]: calculated for $C_{25}H_{38}N_5O$ [M+H$^+$]: 424.30709. Found: 424.30711.

4.11. 2-(4-Methylpiperazin-1-yl)-*N*-propyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-4-carboxamide (8b)

A mixture of reductive amination product **7b** (0.09 mmol) and conc. HCl (2 mL) was refluxed overnight. TLC and LCMS analysis were used to monitor the reaction. After completion, the solvent was removed under vacuum to give the crude product **3** (quantitative) and was used directly for the next step without purification. The crude acid **3** was dissolved in dry DMF (2 mL) was treated with DIPEA (0.45 mmol) followed by HOBt (0.226 mmol), *n*-propylamine (0.135 mmol) and EDC (0.2 mmol) at 0 °C and was allowed to stir at room temperature for 10 h. TLC was used to monitor the completion of reaction. After completion, the solvent was removed in vacuo to give crude product that was purified by flash column chromatography using 5–10% methanol in dichloromethane as an eluent to afford pure compound **8b** (0.06 mmol) in 67% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.39 (d, J = 7.3 Hz, 1H), 7.28 (d, J = 7.8 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 7.02 (t, J = 7.0 Hz, 1H), 6.59 (t, J = 5.6 Hz, 1H), 3.36 (d, J = 11.2 Hz, 1H), 3.33–3.21 (m, 2H), 3.15–2.98 (m, 3H), 2.90 (d, J = 11.8 Hz, 1H), 2.83–2.74 (m, 1H), 2.73–2.62 (s, 4H), 2.60–2.49 (m, 3H), 2.48–2.32 (m, 3H), 2.30 (s, 3H), 2.22–2.15 (m, 1H), 1.55–1.39 (m, 4H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.6, 136.2, 134.4, 127.3, 121.9, 119.8, 118.3, 111.3, 108.3, 67.8, 60.6, 58.5, 55.0, 51.2, 48.7, 45.6,

40.9, 33.5, 32.2, 23.1, 22.4, 11.7. HRMS [ESI]: calculated for $C_{24}H_{36}N_5O$ [M+H$^+$]: 410.29144. Found: 410.29140.

4.12. General procedure for EDC/HOBt-based amide coupling reactions

Reactions were performed in parallel in 15 mL reaction tubes in a 24 position Mettler-Toledo Miniblock® equipped with a heat transfer block and inert gas manifold. Each reaction tube was loaded with a solution of the appropriate carboxylic acid **3a**-**z** (40.0 mg, 1 equiv, 0.5 mL as a 0.18 M stock solution in dry DMF), and DIPEA (9 equiv, 1.62 M) and stirred for 5 min at room temperature under nitrogen. HOBt (1.5 equiv, 0.1 mL as a 1.3 M stock solution in dry DMF) was added then and after 2 min the amine (3 equiv of primary amine or 5 equiv if secondary amine, as a DMF solution) and neat EDC·HCl (2 equiv). The Mettler Block was closed and the reactions were stirred at 50 °C for 18 h. Volatiles were evaporated in a centrifugal evaporator and the residue was redissolved in MeOH (1.0 mL). Crude methanolic solutions were transferred to 96 position filtration plates and centrifuged into 96 deep-well plates for uHPLC/MS analysis and HPLC purification.

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Supplementary data

Supplementary data (NMR data of all new compounds and single crystal X-ray diffractional analysis of compound **2b**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.01.019.

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13. Crystallographic data for **5b** (CCDC 1029458) can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.