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Ellipticines and 9-acridinylamines as inhibitors of D-alanine:D-alanine ligase

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ABSTRACT

D-Alanine:D-alanine ligase (Ddl), an intracellular bacterial enzyme essential for cell wall biosynthesis, is an attractive target for development of novel antimicrobial drugs. This study focused on an extensive evaluation of two families of Ddl inhibitors encountered in our previous research. New members of both families were obtained through similarity search and synthesis. Ellipticines and 9-acridinylamines were both found to possess inhibitory activity against Ddl from *Escherichia coli* and antimicrobial activity against *E. coli* and *Staphylococcus aureus*. Ellipticines with a quaternary methylpyridinium moiety were the most potent among all studied compounds, with MIC values as low as 2 mg/L in strains with intact efflux mechanisms. Antimicrobial activity of the studied compounds was connected to membrane damage, making their development as antibacterial drug candidates unlikely unless analogues devoid of this nonspecific effect can be discovered.

1. Introduction

Peptidoglycan is a cross-linked glycopeptide polymer that functions as a vital component of the bacterial cell wall. It provides bacteria with the necessary structural integrity to withstand the osmotic pressure gradient between the cytoplasm and the cell exterior. Disruption of peptidoglycan assembly is a known and validated mechanism for triggering bacterial cell lysis.[1]

D-Alanine:D-alanine ligase is a bacterial enzyme that supplies an essential building block in the intracellular steps of peptidoglycan assembly. Its product, D-alanyl–D-alanine, is the substrate for MurF, the final enzyme of the Mur ligase pathway which assembles the intracellular peptidoglycan precursor, UDP-MurNAc-pentapeptide. The terminal D-Ala–D-Ala dipeptide plays a crucial role in the extracellular steps of peptidoglycan assembly where its peptide bond is broken and a new bond is formed to cross-link adjacent peptidoglycan chains.[2–5]

Two Ddl isoforms, DdlA and DdlB, exist in *Escherichia coli* and *Salmonella typhimurium*. They share 35% amino-acid sequence identity and express similar kinetic characteristics, substrate specificity and susceptibility to known inhibitors. Our research was based on the more extensively studied DdlB isoform.[6,7]

D-Cycloserine, a structural analogue of D-Ala, is the only Ddl inhibitor that has been used as a therapeutic agent. Nevertheless, because of its psychotropic side effects it is only considered a second line drug for treatment of tuberculosis.[4,8] Several other Ddl inhibitors have been discovered, but none are routinely used in antibacterial therapy.[9–20] Most suffer from poor antibacterial activity, or toxicity. Therefore new inhibitors of Ddl would be desirable.

In our previous research, a structure-based virtual screen was performed to identify new inhibitors of DdlB using the AutoDock program with a ligand database of 1990 compounds from the NCI Diversity Set.[21] Several new and structurally diverse compounds were discovered that inhibit DdlB with IC_{50} values in the micromolar concentration range. Compound **1**, a 9-acridinylamine (Table 1), and compound **2**, a 9-methoxyellipticine derivative (Table 2), were also shown to possess antibacterial activity against both Gram-positive and Gram-negative bacteria. Both were selected as starting compounds for further drug design efforts.

In this study, compounds **1** and **2** were taken as templates for derivatisation and an exploratory structure–activity relationship study. Their analogues were obtained through similarity search and synthetic approaches and were tested for inhibitory activity against the DdlB enzyme as well as for antimicrobial activity against several strains of pathogenic bacteria.

2. Results and discussion

2.1. Similarity search

To extend the chemical space in our study beyond the NCI Diversity Set, a similarity search was performed for each of our

Abbreviations: Ddl, d-alanine:d-alanine ligase; MurNAc, *N*-acetyl-muramoyl; NCI, National Cancer Institute; NSC, National Service Center.

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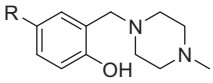
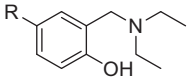
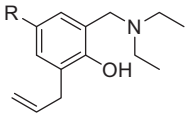
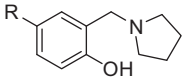
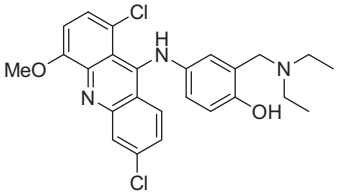
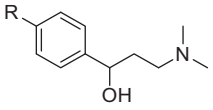
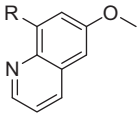
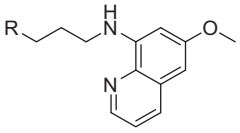
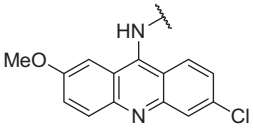
Table 1
9-Acridinylamine derivatives from similarity search and their antimicrobial activity

Compound	NSC no.	Similar to **1**,%	Structure[a]	IC_{50}, μM	MIC, mg/L			Membrane integrity in *S. aureus*, %
					E. coli 1411	*E. coli* SM1411	*S. aureus* SH1000	
1(reference)	130813	100		162	>256	64	32[b]	48.7 ± 9.1
3	10666	95		320	256	128	128	nd[c]
4	31711	95		161	>256	>256	32	47.7 ± 5.3
5	12516	94		102	256	256	64	nd[c]
6	130106	92		119	>256	>256	>256	nd[c]
7	130109	92		135	>256	128	256	nd[c]
8	28557	92		32	>256	>256	>256	nd[c]
9	192961	92		90	>256	>256	256	nd[c]

[a] R = 

[b] MIC determined for *S. aureus* 8325-4, the parent strain of SH1000.
[c] not determined because MIC for *S. aureus* ⩾64 mg/L.

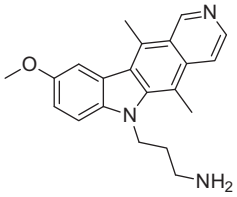
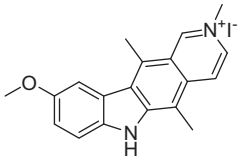
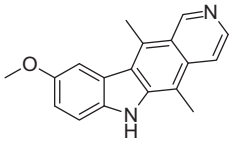
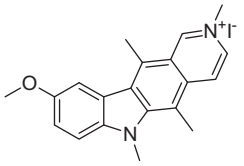
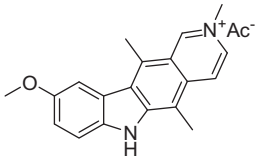
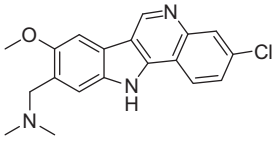
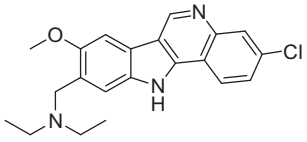
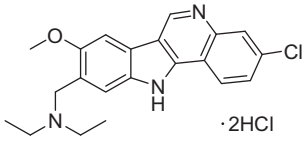
starting compounds to find sufficiently similar structures in the whole NCI database. Two series of compounds were the result: 13 analogues of 9-acridinylamine similar to compound **1** and 26 ellipticine analogues similar to compound **2**. Only 19 of these 39 compounds were available in sufficient quantities (>5 mg) from the National Cancer Institute and were obtained as solid samples. Five of the obtained compounds were excluded from further testing due to toxicity concerns, poor solubility or tendency to form aggregates. All data from this similarity search including structures of the unavailable and the excluded compounds are gathered in Supplementary Tables S1 and S2.

The remaining 14 compounds were evaluated for inhibition of DdlB from *E. coli* in 250 μM concentrations using the malachite green assay, followed by determination of IC_{50} values, and for antimicrobial activity through determination of minimal inhibitory concentrations on two strains of *E. coli* and one strain of *S. aureus*.

Six of the seven 9-acridinylamine analogues (Table 1) showed improved enzyme inhibition over their starting compound **1**. Compounds **8** and **9**, both with a 6-methoxy-8-quinolinyl moiety attached to the general 9-acridinylamine frame, showed promising IC_{50} values but lacked any significant microbiological activity. Compounds **4** and **5**, close analogues of **1** with a diethylamine group and a pyrollidine ring, respectively, replacing the original piperazine moiety, showed microbiological activity against *S. aureus* but failed to surpass the starting compound.

Out of seven ellipticine analogues (Table 2), compounds **10**–**13** exhibited similar or improved IC_{50} values against DdlB compared to their starting compound **2**. Compounds **10**, **12** and **13** showed promising antimicrobial activity against *S. aureus*. All three were also potent antimicrobial agents in the *E. coli* SM1411 strain deleted for the AcrAB efflux pump, with MIC values between 1 and 8 mg/L. Compound **12** was the only similarity search product to

Table 2
9-Methoxyellipticine derivatives from similarity search and their antimicrobial activity

Compound	NSC no.	Similar to **2**,%	Structure	IC_{50}, μM	MIC, mg/L			Membrane integrity in *S. aureus*, %
					E. coli 1411	*E. coli* SM1411	*S. aureus* SH1000	
2(reference)	176327	100		70	32	8	32[a]	Not determined
10	155693	96		65	256	8	32	16.0 ± 2.2
11	69187	95		60	>256	>256	>256	nd[b]
12	155694	95		43	64	1	4	17.8 ± 1.2
13	627505	93		89	256	4	64	nd[b]
14	352738	92		ra[c] 40%	128	32	256	nd[b]
15	317605	92		623	256	32	256	nd[b]
16	352737	92		330	>256	>256	>256	nd[b]

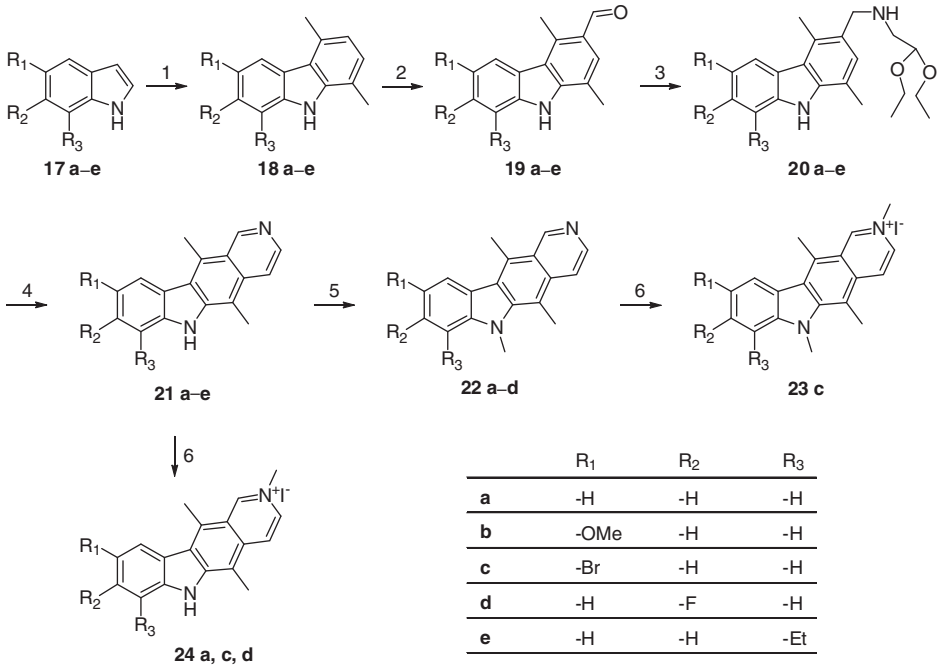
[a] MIC determined for *S. aureus* 8325-4, the parent strain of SH1000.
[b] not determined because MIC for *S. aureus* ⩾64 mg/L.
[c] residual activity at 500 μM; IC_{50} not measured.

possess significant antimicrobial activity against the native *E. coli* strain.

2.2. Synthesis of structural analogues

Based on promising MIC values demonstrated thus far, ellipticines were selected for further research via chemical synthesis. Several substitution patterns at positions 7–9 were explored (see Scheme 1): all-H (series **a**), 9-methoxy (**b**), 9-bromo (**c**), 8-fluoro (**d**) and 7-ethyl (**e**) derivatives were prepared. Additionally, methylation of one or both ellipticine nitrogen atoms was considered.

The synthetic route for production of ellipticine derivatives is depicted in Scheme 1 and follows the methods previously described.[22,23] The ellipticines were obtained from substituted indoles. First, indoles **17a–e** were condensed with acetonylacetone to obtain corresponding dimethylcarbazoles **18a–e**. Vilsmeier–Haack formylation by *N*-methylformanilide and $POCl_3$ gave **19a–e**. Schiff bases were formed with aminoacetaldehyde diethyl acetal, followed immediately by reduction to amine with $NaBH_4$.



	R_1	R_2	R_3
a	-H	-H	-H
b	-OMe	-H	-H
c	-Br	-H	-H
d	-H	-F	-H
e	-H	-H	-Et

Scheme 1. Synthetic route for production of ellipticine derivatives. Reagents and conditions used: (1) hexane-2,5-dione, *p*TSA, EtOH, reflux, 1.5 h; (2) $POCl_3$, *N*-methylformanilide, 1,2-dichlorobenzene, 105 °C, 4 h; (3) Aminoacetaldehyde diethyl acetal, 115 °C, 3 h; $NaBH_4$, EtOH, 1 h; (4) Na_2CO_3, TsCl, THF, 2 h; 6 M HCl, dioxane, reflux, 2 h; (5) NaH, MeI, DMF, 12 h; (6) MeI, acetone, 2 days.

Compounds **20a** and **20b** were then tosylated and cyclised in 6 M HCl in dioxane to yield ellipticine (**21a**) and 9-methoxyellipticine (**21b**), respectively. To obtain better yields, microwave irradiation was used for cyclisation of tosylated **20c–e**.[24] Methylation of indole nitrogens of **21a–d** was performed to afford *N*-methylated products **22a–d**. Finally, **21a**, **21c**, **21d** and **22c** were treated with iodomethane to give iodide salts **24a**, **24c**, **24d** and **23c**. Eleven ellipticine derivatives obtained through synthesis were evaluated for activity against DdlB (Table 3).

N^6-Methylated compounds **22b–d** were poorly soluble, causing difficulties in antimicrobial activity determination. Other synthesized ellipticines did exhibit inhibitory activity against DdlB and antimicrobial activity against several bacterial strains. Compounds **10**–**13** were considered in the following analysis as members of the 9-methoxy substituted series **b**.

N^2-Methylated quaternary compounds **24a**, **10**, **12**, **23c**, **24c** and **24d** showed improved MIC values over their analogues, as well as higher solubility. Notably, to achieve low MIC values against the native strain of *E. coli* and IC_{50} values under 100 μM against DdlB, a quaternary methylpyridinium moiety was necessary.

Non-methylated ellipticines **21a**, **21c** and **21d** surprisingly demonstrated improved MIC values compared with their 9-methoxy analogue **11**. Moreover, **21a** managed to match its quaternary counterpart **24a** and was the only ellipticine without a methylpyridinium moiety in this study to significantly inhibit growth of the native *E. coli* strain.

The all-H, 9-methoxy and 9-bromo substitution patterns generally resulted in active compounds, while 8-fluoro and 7-ethoxy ellipticines lacked either inhibitor activity or antimicrobial activity, or both. The exception to this rule was the non-methylated 8-fluoroellipticine (**21d**) with results largely comparable to its bioisostere **21a**.

Best overall activity was achieved by compounds **23c** and **24c**, both 9-bromo substituted ellipticines with quaternary methylpyridinium structure. Both demonstrated improved IC_{50} values against DdlB compared to top ellipticine derivatives from the similarity search. They also maintained similar antibacterial activity.

However, we noted a poor overall correlation between enzyme inhibitor activity and antimicrobial activity (Tables 1–3), suggesting the possibility that off-target activity[25] of the inhibitors might also contribute to antimicrobial activity. Since non-specific membrane disruption can contribute to the antimicrobial activity of experimental inhibitors[26] we determined whether the compounds described here could cause membrane damage in *S. aureus* SH1000 at 4 × MIC, measured by the *Bac*Light™ assay. We were unable to test inhibitors displaying high MICs because of solubility problems with the compounds in this assay. With the exception of **21a**, all other compounds that were tested decreased membrane integrity by >50% (Tables 1–3).

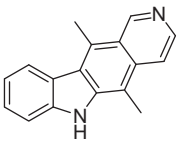
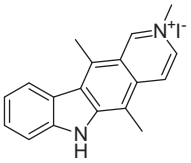
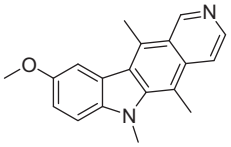
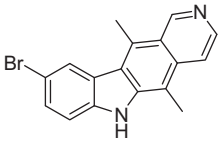
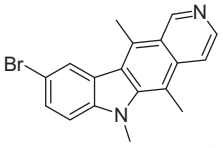
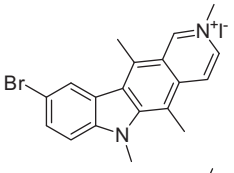
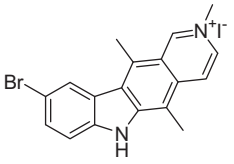
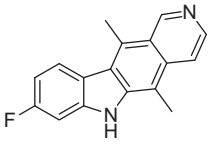
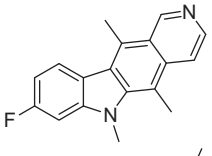
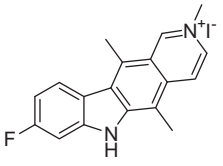
Some hints about the structure–activity relationship for ellipticines can already be concluded from this study: (i) the presence of a quaternary methylpyridinium ion at position 2 improves IC_{50} values, but exacerbates membrane damage; (ii) -OMe or -Br substitution at position 9 can be beneficial for inhibitor activity; (iii) -F substitution at position 8 or -Et substitution at position 7 can cause diminished antimicrobial activity.

3. Conclusions

A range of ellipticine and 9-acridinylamine derivatives have been obtained through similarity search and chemical synthesis. Both classes of compounds exhibit inhibitory activity against DdlB and antimicrobial activity against *E. coli* and *S. aureus*. Some of the compounds appear to be substrates for the AcrAB efflux pump in *E. coli* since their anti-bacterial activities were enhanced in the AcrAB-deficient mutant *E. coli* SM1411.

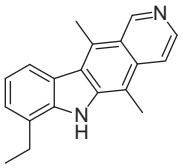
Ellipticines were generally more potent than 9-acridinylamines in all assays that were performed, but probably achieve part of their antimicrobial action by damaging the bacterial cytoplasmic membrane. This makes them unlikely to be developed as antibacterial drug candidates until this nonspecific effect can be diminished through structural changes, without compromising their inhibitory effect on DdlB.

Table 3
Antibacterial activity of synthesized ellipticines

Compound	Structure	IC_{50}, μM	MIC, mg/L			Membrane integrity in *S. aureus*, %
			E. coli 1411	*E. coli* SM1411	*S. aureus* SH1000	
21a		192	16	8	8	71.9 ± 7.2
24a		46	64	4	16	37.3 ± 6.2
22b		184	nd[a]	nd[a]	nd[a]	nd[a]
21c		86	>256	16	16	49.6 ± 6.9
22c		297	nd[a]	nd[a]	nd[a]	nd[a]
23c		36	32	1	2	42.4 ± 4.6
24c		23	16	4	8	29.8 ± 7.4
21d		103	>256	8	4	42.8 ± 5.4
22d		90	>256	>256	>256	nd[b]
24d		64	>256	4	>256	nd[b]

(*continued on next page*)

Table 3 (*continued*)

Compound	Structure	IC_{50}, μM	MIC, mg/L			Membrane integrity in *S. aureus*, %
			E. coli 1411	*E. coli* SM1411	*S. aureus* SH1000	
21e		125	>256	>256	8	34.2 ± 7.8

[a] Not determined because of poor solubility.
[b] Not determined because MIC for *S. aureus* ⩾64 mg/L.

4. Experimental

4.1. Similarity search

The National Cancer Institute (NCI) structural database is a library of over 450,000 compounds. The database has been optimized with use and at the moment over 250,000 compounds are available for searching at the Enhanced NCI Database Browser. To date, the main use of the NCI database has been in anticancer research. The compounds are available as solid samples to laboratories engaged in biomedical investigations.[27]

The search for similar structures within the NCI database was performed at the Classic Version of the Enhanced NCI Database Browser server (http://cactus.nci.nih.gov/ncidb2.1). The SMILES code for each input compound was extracted from the NCI database and copied into the 'Similarity Search' field of the server. The 2D-similarity search was performed using the Tanimoto Index[28] with a cut-off value of 92% as the measure of similarity.

4.2. Enzyme inhibition

Residual activity of DdlB ligase in the presence of potential inhibitors was monitored with the colorimetric malachite green method in which the orthophosphate generated during the enzyme reaction is measured. For the initial screening of compounds obtained through similarity search, each compound was tested in duplicate at a concentration of 250 μM. Assays were performed at 37 °C in a mixture (final volume: 50 μL) containing 50 mM Hepes (pH 8.0), 3.25 mM $MgCl_2$, 6.5 mM $(NH_4)_2SO_4$, 700 μM D-Ala, 500 μM ATP, purified *His*-tagged DdlB (diluted in 50 mM Hepes (pH 7.2) and 1 mM dithiothreitol), and the test compound. All compounds were soluble in the assay mixture containing 5% DMSO. After 20 min of incubation, 100 μL of Biomol reagent was added, and absorbance was read at 650 nm after 5 min. To exclude possible nonspecific (promiscuous) inhibitors, all compounds were also tested in the presence of Triton X-114 (0.005%).[29] IC_{50} values were determined by measuring the residual activity at seven different test compound concentrations, and they represent the test compound concentration where the residual activity was 50%.

4.3. Microbiological evaluation

MIC determinations were performed by broth microdilution against *S. aureus* SH1000 and *E. coli* 1411 (standard laboratory strains) and *E. coli* SM1411 (an *acrAB* deficient derivative of 1411) according to British Society for Antimicrobial Chemotherapy guidelines.[30] The *Bac*Light™ assay (Invitrogen) was used to measure staphylococcal membrane damage in *S. aureus* SH1000 induced by agents at 4 × MIC, compared to a drug free control, as described previously.[26,31]

4.4. Synthesis

4.4.1. General procedure for ellipticine synthesis

Starting compounds indole (**17**, 37 mmol), hexane-2,5-dione (4.2 g, 37 mmol) and *p*-toluenesulfonic acid (3.5 g, 18.5 mmol) were heated in ethanol (50 mL) at reflux for 1.5 h. The reaction mixture was cooled to room temperature and solvent volume was reduced to approximately 25 mL. Water (75 mL) was added and the aqueous mixture was extracted with ether (3 × 50 mL). The organic layer was washed with water (75 mL) and brine (75 mL), dried (Na_2SO_4) and concentrated in vacuo to give black residue. The residue was extracted with hot hexane. The combined hexane extracts were concentrated in vacuo. Crude residue was purified by flash chromatography (ethyl acetate:hexane 1:4) which yielded the product **18** as a white solid.

N-methylformanilide (2.4 mL, 19.2 mmol) and phosphorus oxychloride (1.45 mL, 16 mmol) in 1,2-dichlorobenzene (20 mL) were stirred for 0.5 h at room temperature. Then 1,4-dimethyl-carbazole (**18**, 11.3 mmol) in 1,2-dichlorobenzene (20 mL) was added and the reaction mixture was heated at 105 °C for 4 h. The reaction mixture was cooled to room temperature and sodium acetate (5 g) in water (50 mL) was added. The volume of solvent was reduced to ca. 20 mL and a black solid was collected by filtration. The black solid was dried over night at 80 °C and extracted with toluene (200 mL) in a Soxhlet extractor for 24 h. The hot extracts were decolorized (charcoal), filtered through Celite and concentrated to 30 mL in vacuo. The solid was collected by filtration to give the corresponding aldehyde **19**.

A mixture of the aldehyde (**19**, 4.5 mmol) and aminoacetaldehyde diethyl acetal (2.3 mL, 15.7 mmol) was stirred at 115 °C under argon atmosphere for 3 h. The reaction mixture was cooled to room temperature. Anhydrous ethanol (50 mL) with activated molecular sieves was added. Sodium borohydride (0.68 g, 18 mmol) was added and the reaction mixture was stirred for 1 h and concentrated to dryness in vacuo. The residue was dissolved in ethyl acetate, washed with water, dried and concentrated to give a brown viscous gum that was purified by flash chromatography (ethyl acetate:hexane 4:1) to give a yellowish product **20**.

To a mixture of amine (**20**, 3.7 mmol) and Na_2CO_3 (0.46 g, 4.4 mmol) in tetrahydrofuran (20 mL) and water (10 mL), *p*-toluenesulfonyl chloride (0.9 g, 4.8 mmol) was added at room temperature. The reaction mixture was stirred for 2 h, diluted with water (50 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with 0.1 M HCl (50 mL), water (50 mL), saturated solution of $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL) and dried (Na_2SO_4). The organic solvent was concentrated in vacuo. The crude residue was dissolved in dioxane (10 mL) and 6 M HCl (10 mL) was slowly added and the reaction mixture was stirred for 12 h. The separated solid was collected by filtration and suspended in water (20 mL). The stirred suspension was cooled to 0 °C and basified to pH 10 with 1 M NaOH.

The yellow suspension was collected by filtration and purified with flash chromatography to give **21**.

Sodium hydride (0.06 g, 2.5 mmol) was added to an ice cooled solution of ellipticine derivative (**21**, 1.9 mmol) in dry *N*,*N*-dimethylformamide (25 mL). After stirring for 30 min at 0 °C, methyl iodide (0.13 mL, 2.1 mmol) was added. The reaction mixture was stirred over night at room temperature. Water was added (50 mL) and extracted with dichloromethane (3 × 40 mL). The combined organic layers were extracted with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography (dichloromethane:methanol 9:1) to obtain **22** as a yellow solid.

The ellipticine derivative (**21** or **22**, 0.31 mmol) was dissolved in acetone (200 mL) and methyl iodide (1 mL) was added. The reaction mixture was stirred in the dark for 2 days. The yellow solid was collected by filtration and washed with ether to obtain **23** or **24**.

4.4.1.1. 1,4-Dimethyl-9*H*-carbazole (18a). White crystals. Yield = 54%, mp 93–94 °C (lit.,[22] 97–98 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.47 (s, 3H, CH_3), 2.78 (s, 3H, CH_3), 6.87 (d, J = 7.3 Hz, 1H, Ar–H), 7.06 (d, J = 7.3 Hz, 1H, Ar–H), 7.17 (m, 1H, Ar–H), 7.34 (m, 1H, Ar–H), 7.41 (d, J = 8.0 Hz, 1H, Ar–H), 7.91 (br s, 1H, NH), 8.10 (d, J = 7.9 Hz, 1H, Ar–H).

4.4.1.2. 1,4-Dimethyl-9*H*-carbazole-3-carbaldehyde (19a). Yellow crystals. Yield = 38%, mp 204–206 °C (lit.,[32] 216–218 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.56 (s, 3H, CH_3), 3.18 (s, 3H, CH_3), 7.31 (t, J = 7.3 Hz, 1H, Ar–H), 7.43–7.53 (m, 2H, Ar–H), 7.75 (s, 1H, Ar–H), 8.26 (d, J = 8.4 Hz, 1H, Ar–H), 8.31 (br s, 1H, NH), 10.44 (s, 1H, CHO).

4.4.1.3. *N*-((1,4-Dimethyl-9*H*-carbazol-3-yl)methyl)-2,2-diethoxyethanamine (20a). Yellow solid. Yield = 77 %, mp 87–88 °C (lit.,[24] 105–106 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.23 (t, J = 7.1 Hz, 6H, CH_3–CH_2), 1.64 (br s, 1H, CH_2–NH–CH_2), 2.51 (s, 3H, CH_3–Ar), 2.87–2.89 (m, 5H, CH_3–Ar + NH–CH_2–CH), 3.51–3.61 (m, 2H, CH_2–CH_3), 3.66–3.76 (m, 2H, CH_2–CH_3), 4.00 (s, 2H, NH–CH_2–Ar), 4.69 (t, J = 5.7 Hz, 1H, CH), 7.19 (s, 1H, Ar–H), 7.23–7.26 (m, 1H, Ar–H), 7.42–7,45 (m, 1H, Ar–H), 7.48 (d, J = 7.5 Hz, 1H, Ar–H), 8.14 (br s, 1H, NH), 8.26 (d, J = 7.9 Hz, 1H, Ar–H).

4.4.1.4. 5,11-Dimethyl-6*H*-pyrido[4,3-b]carbazole (21a). Yellow powder. Yield = 42%, mp 311–313 °C (lit.,[24] 309–310 °C); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.80 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 7.27 (t, J = 7.4 Hz, 1H, Ar–H), 7.53–7.59 (m, 2H, Ar–H), 7.92 (d, J = 6.0 Hz, 1H, Ar–H), 8.37–8.43 (m, 2H, Ar–H), 9.70 (s, 1H, Ar–H), 11.37 (s, 1H, NH).

4.4.1.5. 5,6,11-Trimethyl-6*H*-pyrido[4,3-b]carbazole (22a). Yellow powder. Yield = 49%, mp 208–210 °C (lit.,[33] 207–208 °C); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.06 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 4.14 (s, 3H, CH_3), 7.31–7.36 (m, 1H, Ar–H), 7.42 (d, J = 8.2 Hz, 1H, Ar–H), 7.57–7.63 (m, 1H, Ar–H), 7.91 (d, J = 6.1 Hz, 1H, Ar–H), 8.37 (d, J = 7.9 Hz, 1H, Ar–H), 8.53 (d, J = 5.3 Hz, 1H, Ar–H), 9.72 (s, 1H, Ar–H).

4.4.1.6. 2,5,11-Trimethyl-6*H*-pyrido[4,3-b]carbazol-2-ium iodide (24a). Yellow powder. Yield = 73%, mp 316–319 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.84 (s, 3H, CH_3), 3.29 (s, 3H, CH_3), 4.45 (s, 3H, CH_3-N), 7.37–7.42 (m, 1H, Ar–H), 7.67 (d, J = 3.32 Hz, 2H, Ar–H), 8.39–8.46 (m, 3H, Ar–H), 10.00 (s, 1H, Ar–H). 12.11 (br s, 1H, NH).

4.4.1.7. 6-Methoxy-1,4-dimethyl-9*H*-carbazole (18b). White crystals. Yield = 44%, mp 136–137 °C (lit.,[34] 136.5 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.54 (s, 3H, CH_3–Ar), 2.87 (s, 3H, CH_3–Ar), 3.97 (s, 3H, CH_3–O), 6.93 (d, J = 7.3 Hz, 1H, Ar–H), 7.10 (dd, J = 8.8, 2.5 Hz, 1H, Ar–H), 7.14 (d, J = 7.3 Hz, 1H, Ar–H), 7.40 (d, J = 8.7 Hz, 1H, Ar–H), 7.72 (d, J = 2.5 Hz, 1H, Ar–H), 7.86 (br s, 1H, NH).

4.4.1.8. 6-Methoxy-1,4-dimethyl-9*H*-carbazole-3-carbaldehyde (19b). White crystals. Yield = 42%, mp 205–207 °C (lit.,[34] 206 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.58 (s, 3H, CH_3–Ar), 3.21 (s, 3H, CH_3–Ar), 3.97 (s, 3H, CH_3–O), 7.14 (dd, J = 8.77, 2.44 Hz, 1H, Ar–H), 7.45 (d, J = 8.77 Hz, 1H, Ar–H), 7.76–7.81 (m, 2H, Ar–H), 8.17 (br s, 1H, NH), 10.47 (s, 1H, CHO).

4.4.1.9. 2,2-Diethoxy-N-((6-methoxy-1,4-dimethyl-9*H*-carbazol-3-yl)methyl)ethanamine (20b). Brown oil. Yield = 76%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.23 (t, J = 7.0 Hz, 6H, CH_3–CH_2), 1.68 (br s, 1H, NH), 2.49 (s, 3H, CH_3–Ar), 2.86 (s, 4H, CH_3–Ar + NH–CH_2–CH), 2.88 (s, 1H, NH–CH_2–CH), 3.51–3.61 (m, 2H, CH_2–CH_3), 3.6–3.77 (m, 2H, CH_2–CH_3), 3.95 (s, 3H, CH_3–O), 3.98 (s, 2H, NH–CH_2–Ar), 4.68 (t, J = 5.6 Hz, 1H, CH), 7.08 (dd, J = 8.7, 2.5 Hz, 1H, Ar–H), 7.17 (s, 1H, Ar–H), 7.37 (d, J = 8.7 Hz, 1H, Ar–H), 7.77 (d, J = 2.4 Hz, 1H, Ar–H), 7.95 (br s, 1H, NH).[35]

4.4.1.10. 9-Methoxy-5,11-dimethyl-6*H*-pyrido[4,3-b]carbazole (21b). Yellow solid. Yield = 43%, mp: 288–289 °C (lit.,[36] 276.3–278.5 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.77 (s, 3H, CH_3), 3.26 (s, 3H, CH_3), 3.91 (s, 3H, CH_3–O), 7.20 (dd, J = 8.71, 2.38 Hz, 1H, Ar–H), 7.49 (d, J = 8.71 Hz, 1H, Ar–H), 7.87–7.91 (m, 2H, Ar–H), 8.40 (d, J = 6.03 Hz, 1H, Ar–H), 9.68 (s, 1H, Ar–H), 11.19 (s, 1H, NH).

4.4.1.11. 9-Methoxy-5,6,11-trimethyl-6*H*-pyrido[4,3-b]carbazole (22b). Yellow powder. Yield = 55%, mp 278–281 °C (lit.,[34] 158 °C); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.91 (s, 3H, CH_3–Ar), 3.11 (s, 3H, CH_3–Ar), 3.89 (s, 3H, CH_3), 3.97 (s, 3H, CH_3), 7.12 (dd, J = 8.8, 2.3 Hz, 1H, Ar–H), 7.19 (d, J = 1.7 Hz, 1H, Ar–H), 7.76–7.78 (m, 2H, Ar–H), 8.40 (d, J = 6.1 Hz, 1H, Ar–H), 9.58 (s, 1H, Ar–H).

4.4.1.12. 6-Bromo-1,4-dimethyl-9*H*-carbazole (18c). White crystals. Yield = 55%, mp 137–138 °C (lit.,[37] 137–138 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.55 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 6.97 (d, J = 7.3 Hz, 1H, Ar–H), 7.18 (d, J = 7.3 Hz, 1H, Ar–H), 7.37 (d, J = 8.6 Hz, 1H, Ar–H), 7.52 (dd, J = 8.6, 1.9 Hz, 1H, Ar–H), 8.01 (br s, 1H, NH), 8.29 (d, J = 1.7 Hz, 1H, Ar–H).

4.4.1.13. 6-Bromo-1,4-dimethyl-9*H*-carbazole-3-carbaldehyde (19c). Yellow solid. Yield = 39%, mp 265–267 °C (lit.,[34] 267–268 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.60 (s, 3H, CH_3), 3.19 (s, 3H, CH_3), 7.43 (d, J = 8.5 Hz, 1H, Ar–H), 7.59 (dd, J = 8.5, 1.5 Hz, 1H, Ar–H), 7.81 (s, 1H, Ar–H), 8.31 (br s, 1H, NH), 8.41 (s, 1H, Ar–H), 10.49 (s, 1H, CHO).

4.4.1.14. *N*-((6-Bromo-1,4-dimethyl-9*H*-carbazol-3-yl)methyl)-2,2-diethoxyethanamine (20c). Pale yellow solid. Yield = 62%, mp 99–100 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.23 (t, J = 6.3 Hz, 6H, CH_3–CH_2), 1.51 (br s, 1H, NH), 2.49 (s, 3H, CH_3–Ar), 2.82 (s, 3H, CH_3–Ar), 2.86 (d, J = 5.6 Hz, 2H, CH–CH_2–NH), 3.53–3.59 (m, 2H, CH_2–CH_3), 3.69–3.76 (m, 2H, CH_2–CH_3), 3.97 (s, 2H, CH_2–Ar), 4.68 (t, J = 5.6 Hz, 1H, CH), 7.20 (s, 1H, Ar–H), 7.34 (d, J = 8.6 Hz, 1H, Ar–H), 7.49 (dd, J = 8.6, 1.9 Hz, 1H, Ar–H), 8.20 (br s, 1H, NH), 8.32 (d, J = 1.7 Hz, 1H, Ar–H); ^{13}C NMR (DMSO-d_6, 300 Hz) δ 15.37, 15.72, 16.37, 51.49, 51.79, 62.37, 102.08, 117.77, 111.88, 116.85, 121.09, 126.16, 126.16, 127.42, 129.06, 129.20, 138.34, 138.57; IR (KBr) ν_{max} 3528, 3318, 3153, 2973, 2931, 2853, 2366, 2345, 1610, 1458, 1438, 1406, 1374, 1362, 1335, 1278, 1242, 1226, 1147, 1124, 1112, 1082, 1057, 1010, 961, 930 cm^{-1}; ESI-HRMS *m/z* calcd. for $[M+(H)]^+$ 419.1334, found 419.1346. Anal.

Calcd. for $C_{21}H_{27}N_2O_2Br \times 0.6\ H_2O$: C, 58.63; H, 6.61; N, 6.51. Found C, 58.30; H, 6.62; N, 6.53.

4.4.1.15. 9-Bromo-5,11-dimethyl-6*H*-pyrido[4,3-b]carbazole (21c). Yellow powder. Yield = 72%, mp 329–331 °C (lit.,[24] 330–332 °C); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.79 (s, 3H, CH_3), 3.25 (s, 3H, CH_3), 7.53 (d, J = 8.6 Hz, 1H, Ar–H), 7.67 (dd, J = 8.6, 1.9 Hz, 1H, Ar–H), 7.94 (d, J = 6.1 Hz, 1H, Ar–H), 8.43–8.47 (m, 2H, Ar–H), 9.72 (s, 1H, Ar–H), 11.54 (s, 1H, NH).

4.4.1.16. 9-Bromo-5,6,11-trimethyl-6*H*-pyrido[4,3-b]carbazole (22c). Yellow powder. Yield = 35%, mp 208–210 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.97 (s, 3H, CH_3–Ar), 3.10 (s, 3H, CH_3–Ar), 4.02 (s, 3H, CH_3–N), 7.21 (d, J = 8.66 Hz, 1H, Ar–H), 7.64 (dd, J = 8.64, 1.89 Hz, 1H, Ar–H), 7.85 (d, J = 6.17 Hz, 1H, Ar–H), 8.34 (d, J = 1.83 Hz, 1H, Ar–H), 8.51 (d, J = 6.18 Hz, 1H, Ar–H), 9.64 (s, 1H, Ar–H); ^{13}C NMR (DMSO-d_6, 300 Hz) δ 13.42, 14.31, 33.70, 109.45, 110.98, 111.42, 116.12, 121.12, 122.72, 124.13, 125.58, 129.36, 129.53, 133.88, 140.17, 141.12, 143.27, 149.25; IR (KBr) ν_{max} 3448, 3067, 2926, 2365, 2345, 1588, 1508, 1498, 1474, 1458, 1439, 1388, 1379, 1355, 1340, 1299, 1287, 1241, 1200, 1160, 1144, 1107, 1024 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 339.0497, found 339.0492. Anal. Calcd. for $C_{18}H_{15}N_2Br \times 0.5\ H_2O$: C, 62.08; H, 4.63; N, 8.04. Found C, 62.06; H, 4.60; N, 7.88.

4.4.1.17. 9-Bromo-2,5,6,11-tetramethyl-6*H*-pyrido[4,3-b]carbazol-2-ium iodide (23c). Yellow powder. Yield = 57%, mp 299–301 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.11 (s, 3H, CH_3–Ar), 3.25 (s, 3H, CH_3–Ar), 4.24 (s, 3H, CH_3–N), 4.46 (s, 3H, CH_3–N), 7.77 (d, J = 8.81 Hz, 1H, Ar–H), 7.85 (dd, J = 8.78, 1.69 Hz, 1H, Ar–H), 8.47 (d, J = 7.20 Hz, 1H, Ar–H), 8.52 (d, J = 1.54 Hz, 1H, Ar–H), 8.58 (d, J = 7.29 Hz, 1H, Ar–H), 10.08 (s, 1H, Ar–H); ^{13}C NMR (DMSO-d_6, 300 Hz) δ 13.56, 15.11, 33.82, 46.61, 111.50, 111.95, 113.08, 120.12, 120.44, 123.04, 125.08, 126.23, 130.97, 132.36, 133.62, 133.80, 143.17, 144.07, 147.38; IR (KBr) ν_{max} 3448, 3039, 2368, 2346, 1636, 1609, 1586, 1566, 1458, 1392, 1364, 1334, 1300, 1288, 1243, 1189, 1142, 1104, 1078, 1053 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 353.0653, found 353.0642.

4.4.1.18. 9-Bromo-2,5,11-trimethyl-6*H*-pyrido[4,3-b]carbazol-2-ium iodide (24c). Yellow powder. Yield = 88%, mp 328–330 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.84 (s, 3H, CH_3–Ar), 3.29 (s, 3H, CH_3–Ar), 4.47 (s, 3H, CH_3–N), 7.61 (d, J = 8.4 Hz, 1H, Ar–H), 7.79 (d, J = 8.4 Hz, 1H, Ar–H), 8.43–8.56 (m, 3H, Ar–H), 10.09 (s, 1H, Ar–H), 12.26 (s, 1H, NH); IR (KBr) ν_{max} 3429, 3144, 2360, 1599, 1449, 1410, 1284, 1243, 1184, 1127, 1066 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 339.0497, found 339.0506.

4.4.1.19. 7-Fluoro-1,4-dimethyl-9*H*-carbazole (18d). White crystals. Yield = 46%, mp 114–115 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.55 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 6.96–7.04 (m, 2H, Ar–H), 7.13–7.19 (m, 2H, Ar–H), 8.01 (br s, 1H, NH), 8.09 (dd, J = 8.7, 5.4 Hz, 1H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 Hz) δ 16.49, 20.36, 97.20 (d, $^2J_{F,C}$ = 26.3 Hz), 107.47 (d, $^2J_{F,C}$ = 23.8 Hz), 117.02, 120.99, 121.31, 123.34 (d, $^3J_{F,C}$ = 10.3 Hz), 125.91, 130.30, 139.10, 139.86, 140.02, 161.42 (d, $^1J_{F,C}$ = 241.1 Hz); IR (KBr) ν_{max} 3423, 3080, 2975, 2944, 2920, 2865, 2371, 1870, 1617, 1593, 1522, 1492, 1458, 1447, 1387, 1375, 1335, 1315, 1296, 1274, 1218, 1141, 1121, 1099, 1040, 1007, 958 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 214.1032, found 214.1022. Anal. Calcd. for $C_{14}H_{12}NF$: C, 78.85; H, 5,67; N, 6.57. Found C, 79.12; H, 5.57; N, 6.48.

4.4.1.20. 7-Fluoro-1,4-dimethyl-9*H*-carbazole-3-carbaldehyde (19d). Grey powder. Yield = 42%, mp 197–200 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.60 (s, 3H, CH_3), 3.19 (s, 3H, CH_3), 7.08 (td, J = 9.1, 2.4 Hz, 1H, Ar–H), 7.22 (dd, J = 9.2, 2.3 Hz, 1H, Ar–H), 7.77 (s, 1H, Ar–H), 8.21 (dd, J = 8.8, 5.3 Hz, 1H, Ar–H), 8.33 (br s, 1H, NH), 10.47 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$, 300 Hz) δ 14.79, 16.14, 97.73 (d, $^2J_{F,C}$ = 26.26 Hz), 108.54 (d, $^2J_{F,C}$ = 23.64 Hz), 117.34, 122.42, 123.98 (d, $^3J_{F,C}$ = 10.22 Hz), 126.92, 127.11, 128.80, 135.59, 140.21, 142.38, 161.50 (d, $^1J_{F,C}$ = 243.43 Hz), 191.43; IR (KBr) ν_{max} 3482, 3311, 3098, 2920, 2862, 2767, 2373, 2346, 1734, 1718, 1664, 1648, 1636, 1624, 1594, 1577, 1458, 1438, 1371, 1339, 1330, 1282, 1224, 1202, 1160, 1140, 1112, 1044, 960 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 242.0981, found 242.0981. Anal. Calcd. for $C_{15}H_{12}NOF$: C, 74.67; H, 5.01; N, 5.81. Found C, 74.44; H, 4.76; N, 5.74.

4.4.1.21. 2,2-Diethoxy-*N*-((7-fluoro-1,4-dimethyl-9*H*-carbazol-3-yl)methyl)ethanamine (20d). Yellow solid. Yield = 51%, mp 65–67 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, J = 7.05 Hz, 6H, CH_3–CH_2), 1.78 (br s, 1H, NH), 2.41 (s, 3H, CH_3–Ar), 2.81 (s, 3H, CH_3–Ar), 2.92 (d, J = 5.59 Hz, 2H, CH–CH_2–NH), 3.55–3.60 (m, 2H, CH_2–CH_3), 3.71–3.77 (m, 2H, CH_2–CH_3), 3.99 (s, 2H, CH_2-Ar), 4.71 (t, J = 5.56 Hz, 1H, CH), 6.92–6.99 (m, 1H, Ar–H), 7.08 (dd, J = 10.06, 2.78 Hz, 2H, Ar–H), 8.09 (dd, J = 8.73, 5.43 Hz, 1H, Ar–H), 8.55 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 300 Hz) δ 15.37, 15.73, 16.39, 37.03, 51.52, 51.64, 62.43, 97.17 (d, $^2J_{F,C}$ = 26.09 Hz), 101.96, 107.26 (d, $^2J_{F,C}$ = 23.63 Hz), 116.71, 121.01, 121.66, 123.57 (d, $^3J_{F,C}$ = 10.25 Hz), 128.20, 128.68, 128.87, 138.64, 140.42 (d, $^3J_{F,C}$ = 12.32 Hz), 161.30 (d, $^1J_{F,C}$ = 241.17 Hz); IR (KBr) ν_{max} 3316, 3123, 3023, 2974, 2910, 2885, 2827, 1734, 1605, 1590, 1534, 1522, 1502, 1490, 1472, 1438, 1406, 1394, 1372, 1339, 1324, 1294, 1273, 1225, 1202, 1139, 1126, 1095, 1080, 1060, 1024, 973, 940, 928 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 359.2135, found 359.2140. Anal. Calcd. for $C_{21}H_{27}N_2O_2F \times 0.9\ H_2O$: C, 67.32; H, 7.75; N, 7.48. Found C, 67.05; H, 7.59; N, 7.85.

4.4.1.22. 8-Fluoro-5,11-dimethyl-6*H*-pyrido[4,3-b]carbazole (21d). Yield = 52%, mp 283–286 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.82 (s, 3H, CH_3), 3.24 (s, 3H, CH_3), 7.15 (td, J = 9.33, 2.38 Hz, 1H, Ar–H), 7.35 (dd, J = 9.50, 2.32 Hz, 1H, Ar–H), 8.28 (d, J = 6.70 Hz, 1H, Ar–H), 8.37–8.45 (m, 2H, Ar–H), 9.87 (s, 1H, Ar–H), 12.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 Hz) δ 11.82, 14.14, 97.15 (d, $^2J_{F,C}$ = 26.00 Hz), 106.59 (d, $^2J_{F,C}$ = 23.46 Hz), 108.25, 115.86, 119.78, 122.07, 122.72, 125.09 (d, $^3J_{F,C}$ = 10.58 Hz), 127.47, 132.29, 140.52, 140.89, 143.68 (d, $^3J_{F,C}$ = 12.79 Hz), 149.54, 161.73 (d, $^1J_{F,C}$ = 241.58 Hz); IR (KBr) ν_{max} 3904, 3854, 3839, 3690, 3368, 3163, 3004, 2924, 2375, 2345, 1605, 1591, 1490, 1458, 1449, 1420, 1398, 1388, 1333, 1299, 1283, 1170, 1150, 1111, 1034, 1010, 963 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 265.1141, found 265.1140.

4.4.1.23. 8-Fluoro-5,6,11-trimethyl-6*H*-pyrido[4,3-b]carbazole (22d). Yellow powder. Yield = 42%, mp 232–234 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.05 (s, 3H, CH_3–Ar), 3.21 (s, 3H, CH_3–Ar), 4.15 (s, 3H, CH_3–N), 7.11 (td, J = 9.33, 2.38 Hz, 1H, Ar–H), 7.55 (dd, J = 10.61, 2.35 Hz, 1H, Ar–H), 8.02 (d, J = 6.00 Hz, 1H, Ar–H), 8.38 (dd, J = 8.67, 5.60 Hz, 1H, Ar–H), 8.46 (d, J = 6.12 Hz, 1H, Ar–H), 9.71 (s, 1H, Ar–H); ^{13}C NMR (DMSO-d_6, 300 Hz) δ 13.25, 14.27, 33.90, 96.38 (d, $^2J_{F,C}$ = 27.20 Hz), 106.82 (d, $^2J_{F,C}$ = 23.39 Hz), 109.31, 116.10, 118.97, 122.14, 123.41, 125.01 (d, $^3J_{F,C}$ = 10.57 Hz), 127.91, 133.47, 140.34, 141.60, 145.96 (d, $^3J_{F,C}$ = 12.56 Hz), 149.19, 162.12 (d, $^1J_{F,C}$ = 243.73 Hz); IR (KBr) ν_{max} 3480, 3020, 2927, 2374, 2346, 1617, 1590, 1560, 1458, 1438, 1420, 1412, 1382, 1362, 1340, 1310, 1276, 1242, 1212, 1198, 1159, 1097, 1086, 1025, 998, 952, 896 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 279.1298, found 279.1291. Anal. Calcd. for $C_{18}H_{15}N_2F \times 0.3\ H_2O$: C, 76.20; H, 5.54; N, 9.87. Found C, 76.67; H, 5.34; N, 9.76.

4.4.1.24. 8-Fluoro-2,5,11-trimethyl-6*H*-pyrido[4,3-b]carbazol-2-ium iodide (24d). Yellow powder. Yield = 54%, mp 323–

325 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.81 (s, 3H, CH_3–Ar), 3.23 (s, 3H, CH_3–Ar), 4.47 (s, 3H, CH_3–N), 7.20 (td, J = 9.42, 9.40, 2.38 Hz, 1H, Ar–H), 7.36 (dd, J = 9.42, 2.33 Hz, 1H, Ar–H), 8.38–8.44 (m, 3H, Ar–H), 10.01 (s, 1H, Ar–H), 12.19 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 Hz) δ 11.94, 14.81, 46.69, 98.18 (d, $^2J_{F,C}$ = 26.22 Hz), 108.38 (d, $^2J_{F,C}$ = 23.90 Hz), 109.63, 110.52, 118.77, 120.25, 125.23, 126.01 (d, $^3J_{F,C}$ = 10.53 Hz), 131.93, 132.23, 143.65, 143.82, 144.60, 147.19, 162.38 (d, $^1J_{F,C}$ = 244.28 Hz); IR (KBr) ν_{max} 3436, 3169, 2346, 1637, 1603, 1508, 1492, 1458, 1451, 1420, 1399, 1364, 1322, 1293, 1242, 1185, 1144, 1109, 1070, 1032, 963, 922 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 279.1298, found 279.1287.

4.4.1.25. 8-Ethyl-1,4-dimethyl-9*H*-carbazole (18e). White crystals. Yield = 76%, mp 114–116 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.49 (t, J = 7.6 Hz, 3H, CH_3–CH_2), 2.61 (s, 3H, CH_3–Ar), 2.89 (s, 3H, CH_3–Ar), 3.02 (q, J = 7.6 Hz, 2H, CH_2), 6.98 (d, J = 7.3 Hz, 1H, Ar–H), 7.17 (d, J = 7.3 Hz, 1H, Ar–H), 7.24–7.33 (m, 2H, Ar–H), 7.88 (br s, 1H, NH), 8.09 (d, J = 7.6 Hz, 1H, Ar–H); ^{13}C NMR (DMSO-d_6, 300 Hz) δ 13.74, 16.68, 20.48, 24.18, 116.96, 119.63, 120.23, 120.86, 121.85, 123.58, 124.15, 125.62, 125.97, 130.82, 137.92, 138.56; IR (KBr) ν_{max} 3469, 3078, 3052, 3019, 2972, 2934, 2890, 2862, 2735, 1889, 1846, 1780, 1706, 1605, 1584, 1534, 1520, 1496, 1456, 1438, 1423, 1388, 1376, 1349, 1333, 1308, 1294, 1258, 1232, 1162, 1119, 1076, 1038, 1022, 988, 959, 948 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 224.1439, found 224.1430. Anal. Calcd. for $C_{16}H_{17}N$: C, 86.05; H, 7.67; N, 6.27. Found C, 85.85; H, 7.70; N, 6.20.

4.4.1.26. 8-Ethyl-1,4-dimethyl-9*H*-carbazole-3-carbaldehyde (19e). White solid. Yield = 35%, mp 191–194 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.47 (t, J = 7.6 Hz, 3H, CH_3–CH_2), 2.62 (s, 3H, CH_3–Ar), 3.02 (q, J = 7.7 Hz, 2H, CH_2), 3.21 (s, 3H, CH_3–Ar), 7.31–7.37 (m, 2H, Ar–H), 7.78 (s, 1H, NH), 8.16 (d, J = 7.4 Hz, 2H, Ar–H), 10.48 (s, 1H, CHO); IR (KBr) ν_{max} 3752, 3274, 2962, 1654, 1648, 1608, 1578, 1339, 1266, 1231, 1206, 1179, 1114 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 252.1388, found 252.1380. Anal. Calcd. for $C_{17}H_{17}NO \times 0.25\ H_2O$: C, 79.81; H, 6.89; N, 5.48. Found C, 80.09; H, 6.90; N, 5.54.

4.4.1.27. 2,2-Diethoxy-*N*-((8-ethyl-1,4-dimethyl-9H-carbazol-3-yl)methyl)ethanamine (20e). Yellow solid. Yield = 72%, mp 74–76 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.23 (t, J = 7.03 Hz, 6H, CH_3–CH_2–O), 1.46 (t, J = 7.56 Hz, 3H, CH_3–CH_2–Ar), 1.62 (br s, 1H, NH), 2.57 (s, 3H, CH_3–Ar), 2.86 (s, 2H, NH–CH_2–CH), 2.88 (s, 3H, CH_3–Ar), 3.00 (q, J = 7.56 Hz, 2H, CH_3–CH_2–Ar), 3.53–3.61 (m, 2H, CH_3–CH_2–O), 3.66–3.77 (m, 2H, CH_3–CH_2–O), 3.99 (s, 2H, Ar–CH_2–NH), 4.68 (t, J = 5.71 Hz, 1H, CH), 7.20–7.30 (m, 3H, Ar–H), 7.84 (br s, 1H, NH), 8.13 (d, J = 7.60 Hz, 1H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 Hz) δ 13.58, 15.32, 15.70, 16.49, 20.48, 23.99, 51.60, 51.69, 54.53, 62.14, 116.50, 119.43, 120.37, 120.71, 122.45, 123.45, 124.17, 125.52, 128.27, 128.88, 129.06, 137.91, 138.25; IR (KBr) ν_{max} 3502, 3338, 3227, 3178, 3109, 3058, 2972, 2930, 2857, 1676, 1608, 1584, 1508, 1459, 1436, 1397, 1366, 1353, 1337, 1321, 1285, 1262, 1233, 1206, 1152, 1130, 1104, 1087, 1058, 1016, 982, 954, 920 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 369.2542, found 369.2537. Anal. Calcd. for $C_{23}H_{32}N_2O_2 \times 1\ H_2O$: C, 71.47; H, 8.87; N, 7.25. Found C, 71.62; H, 8.62; N, 7.34.

4.4.1.28. 7-Ethyl-5,11-dimethyl-6*H*-pyrido[4,3-b]carbazole (21e). Yellow powder. Yield = 33%, mp 259–262 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.35 (t, J = 7.51 Hz, 3H, CH_3–CH_2), 2.88 (s, 3H, CH_3–Ar), 3.09 (q, J = 7.50 Hz, 2H, CH_2), 3.25 (s, 3H, CH_3–Ar), 7.22 (t, J = 7.62 Hz, 1H, Ar–H), 7.37 (d, J = 7.15 Hz, 1H, Ar–H), 7.93 (d, J = 6.03 Hz, 1H, Ar–H), 8.23 (d, J = 7.77 Hz, 1H, Ar–H), 8.43 (d, J = 6.00 Hz, 1H, Ar–H), 9.70 (s, 1H, Ar–H), 10.74 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 Hz) δ 12.09, 14.22, 14.45, 23.52, 108.51, 115.92, 119.56, 121.21, 121.94, 123.02, 123.84, 126.05, 126.36, 127.70, 132.44, 140.10, 140.60, 140.86, 149.44; IR (KBr) ν_{max} 3448, 3255, 3197, 3107, 3048, 2963, 2928, 2869, 2346, 1618, 1599, 1592, 1560, 1534, 1522, 1499, 1491, 1458, 1443, 1407, 1383, 1350, 1322, 1312, 1303, 1264, 1235, 1178, 1130, 1080, 1058, 1027, 1000, 916 cm^{-1}; ESI-HRMS m/z calcd. for [M+(H)]$^+$ 275.1548, found 275.1543. Anal. Calcd. for $C_{19}H_{18}N_2 \times 0.3\ H_2O$: C, 81.57; H, 6.70; N, 10.01. Found C, 81.49; H, 6.46; N, 9.76.

NMR spectra for compounds that were not previously characterized in literature are available in Supplementary data.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.07.020.

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