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Synthesis of 5-methyl phenanthridium derivatives: A new class of human DOPA decarboxylase inhibitors

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ABSTRACT

DOPA decarboxylase (DDC) is responsible for the decarboxylation of L-DOPA and related aromatic amino acids and correlates closely with a number of clinical disorders. Sanguinarine, a natural quaternary benzophenanthridine alkaloid (QBA), was reported to be inhibitor of rat DDC and possessed a different inhibitory mechanism. In this study, several natural QBAs were assayed as human DDC inhibitors for the first time. A series of 5-methyl phenanthridium derivatives that contain the basic core structure of QBAs were also synthesized and evaluated as human DDC inhibitors. The title compounds still possessed DDC inhibitory potential. Among the synthesized compounds, 2-hydroxyl-8-methoxy-5-methylphenanthridinium chloride (**11k**) showed good inhibitory activity with an IC_{50} value of 0.12 mM. Preliminary structure–activity relationship indicated that DDC inhibitory potential of 5-methyl phenanthridium derivatives correlated with the π-electro densities on C=N double bond of iminium cation. The hydroxyl group on compound **11k** possibly contributed to the formation of hydrogen bond between DDC and the inhibitor.

Dopa decarboxylase (DDC; EC 4.1.1.28) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme that possesses broad substrate specificity.[1] DDC recognized substrates, such as L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan (L-5-HTP), are decarboxylated to form the important neurotransmitters dopamine and serotonin, respectively. In mammalian tissue, DDC was found to decarboxylate other aromatic L-amino acids, so it is also known as aromatic L-amino acid decarboxylase (AADC).[2]

DDC correlates closely with a number of clinical disorders such as Parkinson's disease (PD) and hypertension.[3] PD is reported to be the result of degeneration of dopamine-producing cells in the substantia nigra of the brain.[4] Dopamine, the decarboxylated product of L-DOPA, cannot pass the blood–brain barrier. Thus, dopamine is not administered as a drug in the treatment of PD independently. On the other hand, L-DOPA is rapidly converted to dopamine in the blood stream when administered as a drug, and only a small percentage of a given dose reaches central nervous system. Peripheral decarboxylation of L-DOPA to dopamine causes prominent nausea and vomiting.[5] As a result, L-DOPA is administered with a DDC inhibitor to prevent side effects and reduce the catabolism of L-DOPA by the peripheral dopaminergic system.

CarbiDOPA[6] (**1**, Scheme 1) and benserazide[7] (**2**), the most commonly used DDC inhibitor, are analogues of L-DOPA. These two commercialized DDC inhibitors possess two characteristic structure motifs: catechol unit and hydrazine group. The crystal structures of DDC complex with carbiDOPA[8] has indicated that the catechol unit generated a hydrogen bonding interaction between the inhibitor and the active side of DDC. Meanwhile, the hydrazine group reacted with the coenzyme PLP and formed an imine adduct. Based on these findings, a series of synthetic catechin derivatives such as the analogues of compound **3** have been synthesized and evaluated for their DDC inhibitory activities.[9] As to the natural DDC inhibitors, epigallocatechin-3-gallate (EGCG) (**4**, Scheme 1), the most abundant catechin in green tea, was also proved to be the inhibitor of DDC.[10] Docking experiments[11] have shown that EGCG bound inside the DDC active site and formed important hydrogen bonds between the catechin motif and several residues in the binding pocket.

Quaternary benzophenanthridine alkaloids (QBAs) are a small family of natural *N*-containing compounds that are widely distributed in papaveraceous and rutaceous plants and display obvious

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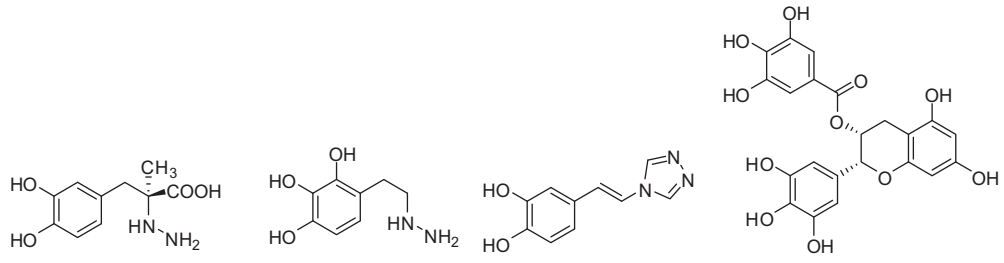


Scheme 1. DDC inhibitors with catechin motif.

antitumor[12] and antibacterial activities.[13] In previous study,[14] QBAs such as sanguinarine (**5**, Scheme 2) and chelerythrine (**6**) were also reported to be inhibitors of rat DDC. In this study, sanguinarine, chelerythrine and nitidine (**7**) were selected for human DDC inhibition experiments. For comparison, dihydrosanguinarine (**8**) and berberine (**9**) were also assayed for their inhibitory activities. QBAs showed obvious inhibitory activities under our experimental conditions. When the C=N double bond was reduced, dihydrosanguinarine lost inhibitory activity, which suggested that the positive charge on QBAs was crucial to the inhibitory potential. Nevertheless, berberine, an analogue of QBAs, was inactive although that berberine possessed a positive charge and conjugated planar structure similar to QBAs. The preliminary findings gave us an impetus to synthesize 5-methyl phenanthridium (**10**, Scheme 2) and related derivatives (**11**) which possessed the core structure of QBAs.

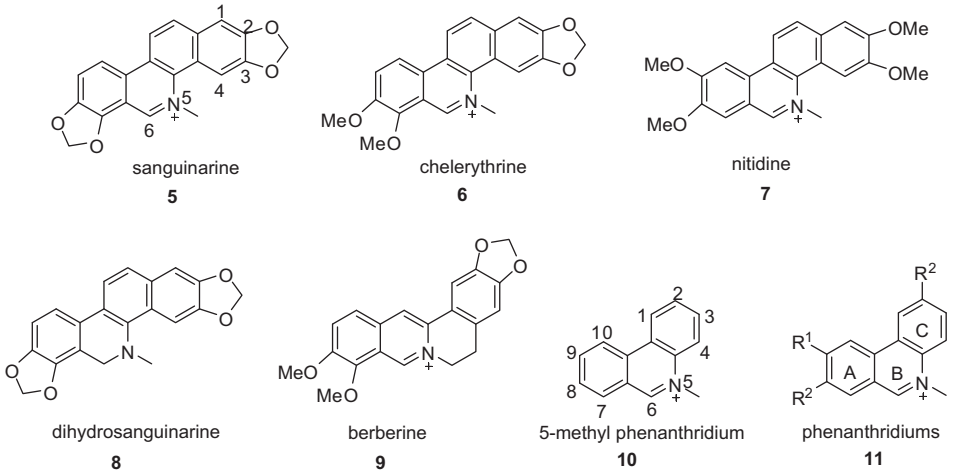
As shown in Scheme 3, commercially available phenanthridine (**12**) was used as the starting material to synthesize 5-methyl phenanthridium chloride (**10**). Compound **12** underwent *N*-methylation with dimethyl sulphate to form 5-methyl phenanthridium sulphate salt (**13**). Without further purification, compounds **13** was refluxed in basic alcoholic solution to provide 6-ethoxyl-5,6-dihydrophenanthridine (**14**), which lost a ethanol molecule in dilute HCl to give target compound **10**.

The general synthetic methodology for the synthesis of substituted phenanthridium chloride (**11**) was shown in Scheme 4. The bromobenzoic acids (**15**) were converted to corresponding acid chlorides (**16**) in situ by refluxing in $SOCl_2$. After evaporation of $SOCl_2$, the acid chlorides were directly reacted with the various anilines to provide the benzamides **17** in high yields. Before the intramolecular Heck-type cyclization of the benzamides to the corresponding phenanthridinones, it was necessary to convert the secondary amides to the tertiary amides. Direct intramolecular Heck coupling of benzamides **17** gave corresponding phenanthridinones in poor yields. Thus, the nitrogen atom in benzamides **17** was protected by methoxylmethyl group (MOM) firstly by reacting the benzamides with NaH and MOMBr in dry THF to give the corresponding *N*-MOM protected benzamides. The *N*-MOM benzamides (**18**) were cyclized to the phenanthridinones (**19**) successively via an intramolecular Heck reaction using $Pd(OAc)_2$/PPh_3/Na_2CO_3/DMF catalytic system.[15]

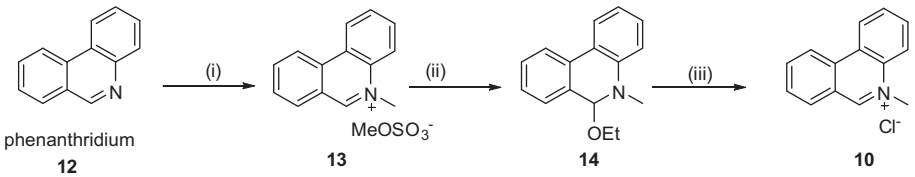
5-Methyl-5,6-dihydrophenanthridin-6-ols (**20**) were obtained through diisobutylaluminium hydride (DIBAL) mediated reduction of compound **19**. Without purification, treatment of compound **20** with dilute HCl afforded the desired phenanthridinium chloride salts (**11**) in moderate yields.

It was worth noting that when *ortho*-substituted anilines were used for synthesis of 4-substituted phenanthridium **22** (Scheme 5), intramolecular Heck cyclization could not be accomplished effectively (yield <5%). It was suggested that the interaction between group R^3 and bulky *N*-MOM group could affect the formation of transition state in intramolecular Heck coupling. A newly developed KO^{t}Bu promoted intramolecular homolytic aromatic substitution (HAS) methodology[16,17] was also applied for synthesis of compound **22**, but the target compound was isolated with very low yield (<5%) in present study.

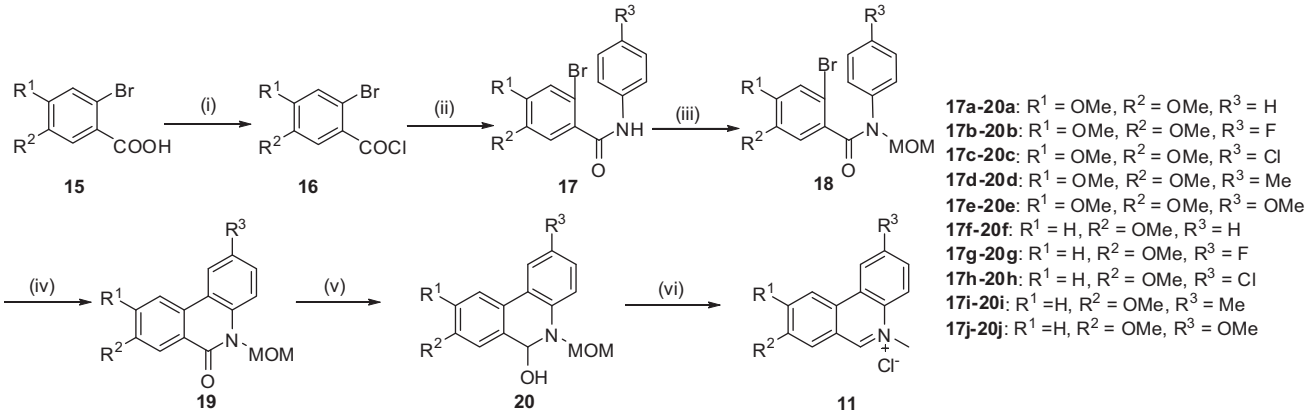
In previous literature, DDC inhibitory activity was determined by measuring the production of dopamine with a spectrophotometric



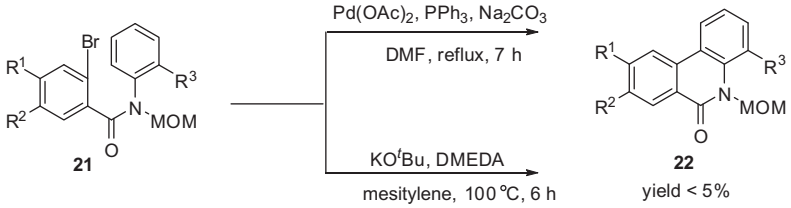
Scheme 2. Structures of QBAs (**5**–**7**), dihydrosanguinarine (**8**), berberine (**9**) and synthetic quaternary phenanthridine derivatives (**10**–**11**).



Scheme 3. Reagents and conditions: (i) Me_2SO_4, 160 °C, 1 h; (ii) NaOEt, EtOH, reflux, 2 h; (iii) 2 M HCl, 30 min.



Scheme 4. Reagents and conditions: (i) $SOCl_2$, reflux, 2 h; (ii) phenyl anilines, TEA, DCM, rt, 3 h, 80–95%; (iii) NaH, THF, 0 °C–rt, 78–90%; (iv) $Pd(OAc)_2$, PPh_3, Na_2CO_3, dry DMF, reflux 7 h, 65–85%; (v) DIBAL, 0°C–rt; (vi) 2 M HCl, 20 min, 42–61% in two steps.



Scheme 5.

assay outlined by Sherald et al.[18] and modified by Charteris and John.[19] The decarboxylased product dopamine reacted with chromogenic agent trinitrobenzenesulphonic acid (TNBS) to yield a condensation product which could be detected by UV spectroscopy at the wavelength of 340 nm. In present study, QBAs and the synthetic phenanthridium salts had a maximum UV absorption at 340 nm. To overcome the perturbation, we developed an HPLC method to analyze the conversion rate of L-DOPA in this study.[20]

The natural QBAs (**5**–**7**), dihrdrosanguinarine (**8**), berberine (**9**) and all synthesized analogues (**10**, **11a**–**11j**) were evaluated for their human DDC inhibitory activities. An overview of the results of all compounds was given in Table 1. The lead compound sanguinarine chloride (**5**) showed strong inhibitory activity (IC_{50} = 0.20 mM) to recombinant human DDC under the experimental conditions. Chelerythrine (**6**) and nitidine (**7**), the analogues of sanguinarine, exhibited approximately 6-times decreased activities with IC_{50} values of 1.28 and 1.34 mM respectively. Dihydrosanguinarine (**8**) lost the inhibitory activity to DDC. It was suggested that the C=N$^+$ double bond or the positive charge on the quaternary *N*-atom was crucial to inhibitory potential.

It was worth mentioning that berberine (**9**) was inactive to inhibit DDC. Thus, 5-methyl phenanthridium (**10**), the basic core structure of QBAs, was synthesized and included for a comparison purpose. Interestingly, compound **10** showed better inhibitory activity (IC_{50} = 0.40 mM) than chelerythrine and nitidine.

Table 1
Human DDC inhibitory activities of compounds **5**–**10** and **11a**–**11k**

Compounds	R^1	R^2	R^3	IC_{50}[a] mM	$\delta_{H\text{-}6}$ (ppm)
5 (Sanguinarine)	—	—	—	0.20	—
6 (Chelerythrine)	—	—	—	1.28	—
7 (Nitidine)	—	—	—	1.34	—
8 (Dihyrosanguinarine)	—	—	—	n.a.[b]	—
9 (Berberine)	—	—	—	n.a.	—
10	H	H	H	0.40	10.10
11a	OMe	OMe	H	3.50	9.71
11b	OMe	OMe	F	2.69	9.73
11c	OMe	OMe	Cl	1.39	9.71
11d	OMe	OMe	Me	1.49	9.55
11e	OMe	OMe	OMe	1.17	9.52
11f	H	OMe	H	0.95	10.00
11g	H	OMe	F	0.57	10.10
11h	H	OMe	Cl	0.93	10.10
11i	H	OMe	Me	0.60	9.87
11j	H	OMe	OMe	0.19	9.80
11k	H	OMe	OH	0.12	9.68

[a] IC_{50} was the mean value of two separated experiments.
[b] n.a.: not active.

The inhibitory activities of ten synthesized quaternary phenanthridiums (**11a**–**11e**) were assayed and outlined in Table 1. Compounds **11a**–**11e** were synthesized with 2-bromo-4,5-dimethoxyl benzoic acid as the starting material and carried two methoxyl

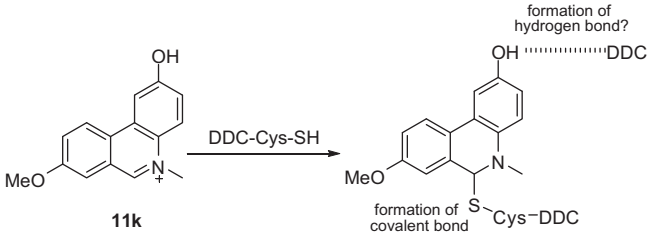
groups at position 8 and 9 on ring A of phenanthridine core. The inhibitory activities of compounds **11a**–**11e** were obviously lower than compound **10**. For example, the IC_{50} value of compound **11a** was 9-times higher than compound **10**, which indicated that two methoxyl groups on ring A of phenanthridine core generated negative effect on inhibitory activity. Different substituents on ring C of phenanthridine core also affected the inhibitory potential. The electro-donating groups such as methyl and methoxyl at position 2 of ring C could increase the inhibitory activity. For example, compounds **11d** (R^3 = Me) and **11e** (R^3 = OMe) showed increased activities compared with compound **11a**.

Compounds **11f**–**11j** possessed one methoxyl group at position 8 on ring A. An overall comparison of inhibitory activities between compounds **11f**–**11j** and compounds **11a**–**11e** strongly suggested that electro-donating group on ring A could decrease the inhibitory potential. IC_{50} values of compounds **11f**–**11j** were lower than compounds **11a**–**11e**. Substituents on ring C of compounds **11f**–**11j** exhibited similar effect on the activities: electro-donating group, for example methoxyl, on ring C could increase the inhibitory potential. For example, IC_{50} value of compound **11j** was 5-times lower than compound **11f**. Compound **11j** (IC_{50} = 0.19 mM) exhibited good inhibition potential as sanguinarine.

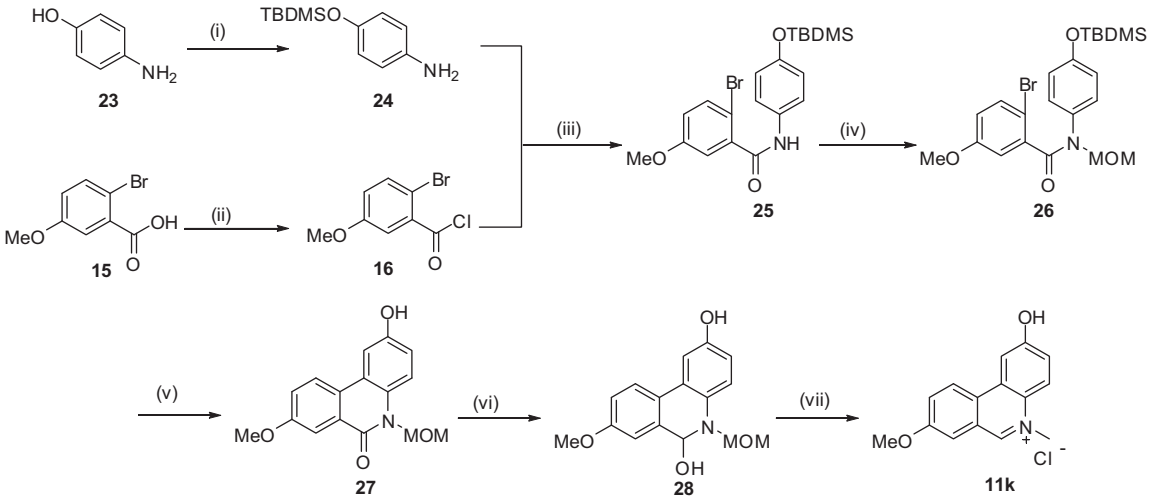
To further confirm the effect of substituent on ring C of compound **11j**, the 2-demethylated derivative **11k** was also synthesized as outlined in Scheme 6. An *O*-TBDMS temporary protected 4-animophenol (**24**) was synthesized[21] and reacted with corresponding acid chloride to afford *O*-TBDMS protected benzamide (**25**) which was further transferred to *N*-MOM protected benzamide **26**. Intramolecular Heck cyclization of compound **26** afforded the desired phenanthridinone **27** accompanied with the O-deprotection process. In the same condition, compound **27** was transferred to **11k** in two steps. DDC inhibitory activity of compound **11k** slightly increased compared with compound **11j** and possessed IC_{50} value of 0.12 mM.

The biological results in this study suggested a different DDC inhibitory mechanism of QBAs and quaternary phenanthridines. In previous literature,[22] the iminium cation C=N^+ of the QBAs was reported to interact with nucleophilic groups, especially SH groups in enzymes and other proteins. For example, it inhibited Na^+/K^+ and Ca^{2+}-ATPases by blocking the SH-groups essential for their activities.[23] According to quantum chemistry calculation, π-electron densities on the heterocyclic nitrogen atom at position 5, and on the carbon atom at position 6 in sanguinarine, are 1.660 and 0.646, respectively.[24] Thus, the double bond C=N^+ of sanguinarine is polar and sensitive to the attack by nucleophiles.[22] In present study, an overall comparison of the inhibitory activities for phenanthridiums **11a**–**11j** has indicated that the π-electro densities on ring A could influence the DDC inhibitory potential. The chemical shift values of H-6 in ^{1}H NMR spectroscopy of the phenanthridium derivatives reflected the π-electro densities. As outlined in Table 1, chemical shift values of compounds **11g**–**11j** were higher than the corresponding compounds **11b**–**11f**. It was suggested that low π-electron densities of C=N^+ of QBAs and phenanthridiums could facilitate the neuleophilic attack from SH groups of enzyme.

DDC contains 11–13 cysteine (Cys) residues according to the species of animals. The crystal structures of DDC complex[8] with carbiDOPA has indicated that Ile 101′ residue in active side of human DDC belongs to the substrate binding pocket and is in Van der Waals contact with the catechol ring of the inhibitor carbiDOPA. The SH-group of Cys 100′, the residue preceding Ile 101′, is only 4.1 Å apart from that of Cys 111′. Replacement of Cys 111′ by an Ala residue or formation of a disulfide bridge between these residues would perturb the active site geometry and reduce or even abolish enzyme activity.[25] In this study, it was assumed that QBAs and synthetic phenanthridiums could block the SH-groups of Cys 100′ or Cys 111′ and change the geometry of DDC active side. Compound **11k** was more active than compound **11j** which indicated that free hydroxyl group on the phenanthridium core could possibly contribute to the formation of hydrogen bond with residue of DDC active side. Based on above analysis, the possible binding mode of compound **11k** with DDC was provided as shown in Scheme 7. However, the acurate binding mode of QBAs and phenanthridiums as DDC inhibitors needs to be further investigated.



Scheme 7. Possible binding mode of active compound **11k** with DDC.



Scheme 6. Reagents and conditions: (i) TBDMSCl, imidazole, THF, rt, 89%; (ii) $SOCl_2$, reflux, 2 h; (iii) TEA, DCM, rt, 3 h, 85%; (iv) NaH, THF, 0 °C–rt, 77%; (v) $Pd(OAc)_2$, PPh_3, Na_2CO_3, dry DMF, reflux 7 h, 44%; (vi) DIBAL, 0 °C–rt; (vii) 2 M HCl, 20 min, 31% in two steps.

In summary, several natural QBAs and synthetic quaternary phenanthridines were assayed for the human DDC inhibitory activities for the first time. QBAs and phenanthridium salts were suggested to possess the same DDC inhibitory mechanism by blocking the SH-groups close to the active side of enzyme. DDC inhibitory potential of 5-methyl phenanthridium derivatives correlated with the π-electro densities on C=N double bond of iminium cation. Further modification of quaternary phenanthridium is under way in our lab.

Acknowledgments

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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.bmcl.2014.04.047. These data include MOL files and InChiKeys of the most important compounds described in this article.

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