Synthesis and Anti-HSV Activity of A-Ring-Deleted Mappicine Ketone Analog

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Mappicine ketone (MPK) **1** is an analog of mappicine **2** (Figure 1) , a naturally-derived alkaloid isolated from *Mapia foetida* Miers (family; Olacaceae).[1] MPK has been identified as an antiviral lead compound with selective activity against herpesviruses HSV-1, HSV-2, and human cytomegalovirus (HCMV) with PR_{50}'s of 2.9, 0.5 and 13.2 μM, respectively. MPK appears to be herpesvirus selective in that it does not inhibit other DNA or RNA viruses. Although the mechanism of action of MPK has not been determined, it is different from that of Acyclovir (ACV) as demonstrated by the observation that ACV-resistant HSV-1 and HSV-2 are inhibited by MPK and that MPK resistant mutants are sensitive to ACV at HSV-1 wild-type virus levels.[2]

The synthesis of MPK was initially achieved by thermolysis of the naturally occurring alkaloid camptothecin.[3] However, the limited supplies of camptothecin and the desire to expand the SAR within the MPK series made it necessary to develop new synthetic routes for this class of compounds. In order to determine the minimal structural requirements of MPK necessary for antiviral activity and to prepare more-soluble derivatives of MPK, the A-ring deleted analog **3** was prepared.[4]

Results and Discussion

Scheme 1 outlines a retrosynthetic approach for the synthesis of A-ring-deleted MPK (**3**). Construction of the D-ring could proceed by methods described by Sugasawa[5] for the total synthesis of camptothecin. The required pyridine-lactam intermediate, which contains the B- and C-ring of MPK, could be prepared from a 1,2,4-triazine via an inverse-demand intramolecular Diels–Alder reaction.[6]

The synthesis of compound **3** is shown in Scheme 2. Reaction of ethyl cyanoformate with hydrogen sulfide afforded ethyl thioamidooxalate which upon reaction with

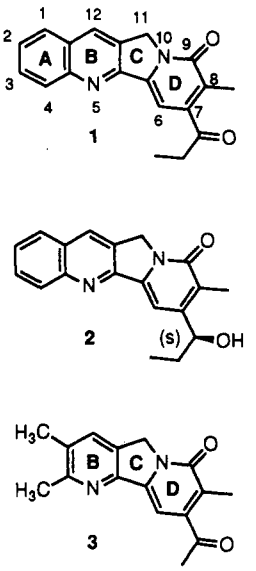
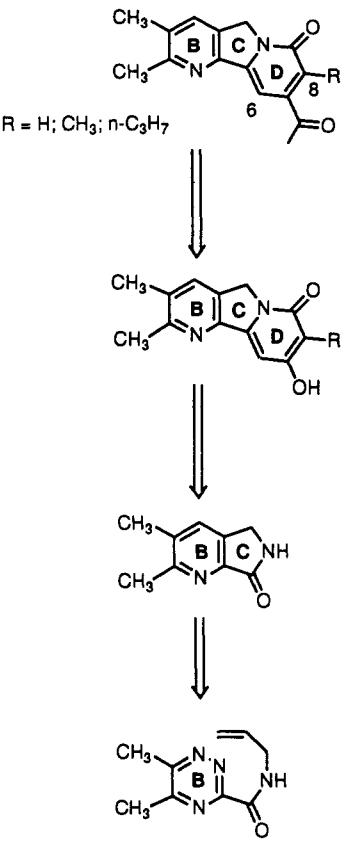


Figure 1.

Scheme 1



hydrazine hydrate and 2,3-butanedione afforded the triazine ester **4**. Initially, **4** was hydrolyzed with potassium hydroxide to provide a water-soluble triazine acid which was converted to the desired amide **5** via standard carbodiimide (EDC) coupling. However, yields for this step were low and unpredictable due to difficulties encountered in isolating the acid. Subsequently, we found that the enzymatic conversion of the ester **4** to amide **5**, using yeast lipase, was more efficient.[7] Intramolecular Diels–Alder reaction of **5** in refluxing xylene followed by

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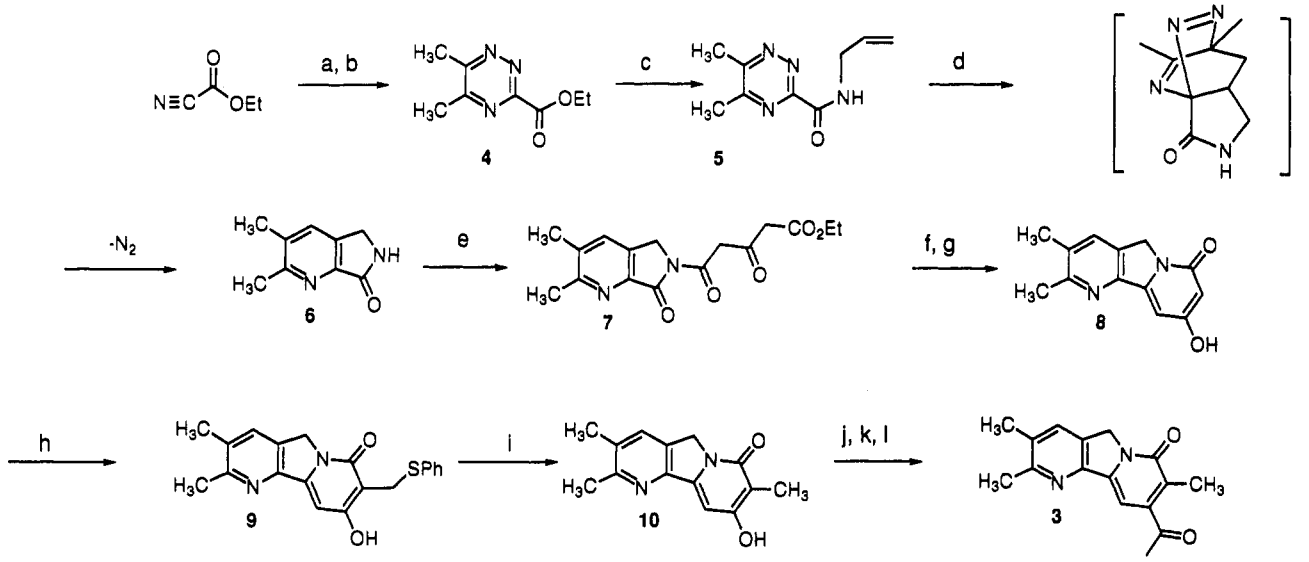
Scheme 2



[a] (a) H_2S, Et_2NH, toluene, 0 °C–rt (74%); (b) NH_2NH_2, 2,3-butanedione, EtOH, rt–reflux (83%); (c) yeast lipase, allylamine, hexane/CCl_4, rt (77%); (d) xylene, 130 °C (71%); (e) diethyl 1,3-acetonedicarboxylate, xylene, reflux (56%); (f) piperidine, DMF, 100 °C (80%); (g) concd HCl, sealed tube, 165 °C (50%); (h) 37% aqueous formaldehyde, thiophenol, AcOH, piperidine, EtOH, 55 °C (67%); (i) Raney nickel, EtOH, 75 °C (45%); (j) Tf_2NPh, DMF, rt (57%); (k) butyl vinyl ether, Et_3N, $Pd(OAc)_2$, dppp, CH_3CN, 70 °C (30%); (l) AcOH, HCl (55%).

loss of nitrogen gave lactam **6** which was converted to **7** by acylation with 1,3-diethyl acetonedicarboxylate. Cyclization of **7** with piperidine in DMF and subsequent hydrolysis and decarboxylation in concentrated hydrochloric acid gave the tricyclic compound **8**. Earlier in the study, it was established that a methyl group at position C-8 of mappicine ketone is critical for antiviral activity; the unsubstituted compound (R = H) and the propyl derivative (R = *n*-propyl) both lack antiviral activity (Scheme 1).[8] Direct attempts to introduce a methyl group at position C-8 (e.g., MeI/base; CH_2N_2) failed. However, insertion of the methyl group was accomplished in a two-step process via a thio-Mannich reaction.[9] Thus, **8** was converted to intermediate **9** which was reduced with Raney nickel catalyst to afford the desired C-8 methyl derivative **10**. It is noteworthy that of the two possible sites for alkylation (C-8 and C-6) only the C-8 substituted product was formed because of greater nucleophilicity of this site. Compound **10** was converted to the triflate using *N*-phenyltrifluoromethanesulfonimide and triethylamine in dimethylformamide and the resulting triflate was converted to ketone **3** in two steps via palladium-catalyzed Heck reaction[10] followed by hydrolysis of the intermediate enol vinyl ether.

Compound **3** was evaluated for antiviral activity using a plaque reduction assay (HSV-1 and HSV-2)[11] in which confluent African green monkey kidney cells (Vero) were infected with virus for 1 h, unattached virus removed, and the test compound then added. After 24 h, the plaques caused by HSV-induced cell lysis were quantified. A compound is considered to be active if it reduces the number of plaques; the PR_{50} is defined as the concentration of compound that reduces this number by 50%. Compound **3** was found to be inactive (PR_{50} = >100 μM) in this assay.

The synthesis of A-ring-deleted MPK (**3**) was achieved in 12 steps and the requirement of the aromatic A-ring for antiviral activity has been demonstrated. Research is currently in progress to expand the SAR for this class of compounds.

Experimental Section

General Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained on a Bruker AM 250 instrument in $CDCl_3$ solvent unless otherwise stated; all values are reported in parts per million (δ) from $(CH_3)_4Si$ unless otherwise stated. Elemental analyses were performed in the Analytical and Physical Chemistry Department of SmithKline Beecham Pharmaceuticals. Mass spectra were obtained by the Physical and Structural Chemistry Department at SmithKline Beecham Pharmaceuticals.

Analytical thin-layer chromatography (TLC) was caried out with Analtech silica gel GF plates. Column chromatography was performed with silica gel (Merck, 230–400 mesh grade). Compounds were named following IUPAC rules as applied by AUTONOM, a PC software for systematic names in organic chemistry, Beilstein-Institute and Springer-Verlag.

Ethyl 5,6-Dimethyl-1,2,4-Triazine-3-carboxylate (4). To a 100-mL flask equipped with a chlorox scrubber was added ethyl cyanoformate (10 g, 62 mmol) in toluene (15 mL). The solution was cooled to 0 °C and hydrogen sulfide was bubbled into the reaction for 10 min. Diethylamine (0.2 mL) was added and the mixture was stirred at room temperature for 14 h. A yellow solid precipitated in the course of the reaction. The product was collected by filtration, washed with toluene (2 × 5 mL), and dried in vacuo to give ethyl thioamidooxalate as a yellow solid (9.6 g, 74%): mp 62–66 °C; ^1H NMR δ 7.30–8.30 (br s, 2H, NH_2), 4.33 (q, 2H, J = 8 Hz, CH_2), 1.39 (t, 3H, J = 8 Hz, CH_3).

To a solution of ethyl thioamidooxalate (4 g, 30 mmol) in absolute ethanol (150 mL) was added hydrazine hydrate (100%, 1.5 g, 30 mmol). The reaction mixture was stirred at room temperature for 1 h. 2,3-Butanedione (2.9 g, 36 mmol) was added, and the mixture was heated at reflux for 1 h. The resulting

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solution was cooled, filtered, and concentrated. Flash chromatography (10–60% EtOAc–hexane) gave **4** as a yellow oil (4.53 g, 83%): ^{1}H NMR δ 4.5 (m, 2H, ethyl ester), 2.8 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 1.5 (t, 3H, ethyl ester).

***N*-2-Propenyl-5,6-dimethyl-1,2,4-triazine-3-carboxamide (5).** To a solution of the ester prepared above (4.5 g, 24 mmol) in hexane/CCl_4 (60:40, 100 mL) was added allylamine (1.87 mL, 24 mmol). The resulting mixture was stirred at room temperature for 5 min. Yeast *Candida cylindracea* lipase (9 g, type VII crude, Sigma) was added and the mixture was stirred at room temperature for 24 h. The resulting mixture was filtered through Celite and washed with CH_2Cl_2. Flash chromatography (0–2% MeOH–CH_2Cl_2) afforded **5** as an oil (3.7 g, 77%): ^{1}H NMR δ 8.1 (br s, 1H, NH), 6.0 (m, 1H, olefin), 5.3 (dd, J = 7 Hz, 2H, olefin), 4.2 (m, 2H, $NHCH_2$), 2.8 (s, 3H, CH_3), 2.6 (s, 3H, CH_3).

5,6-Dimethyl-3-oxo-1*H*-pyrrolo[3,4-*b*]pyridine (6). A solution of the amide prepared above (3.7 g, 19.2 mmol) in xylene (400 mL) was heated at 130 °C under a stream of argon for 48 h. The resulting solution was cooled and the solid precipitated. The precipitate was washed with hexane and dried in vacuo to give **6** as pale solid (2.25 g, 71%): ^{1}H NMR (CD_3CN) δ 7.65 (s, 1H, pyridyl), 7.0 (br s, 1H, NH), 4.3 (s, 2H, CH_2NHCO), 2.6 (s, 3H, CH_3), 2.4 (s, 3H, CH_3); MS (CI) 163 (M + H). Anal. Calcd for $C_9H_{10}N_2O \cdot {}^{13}/_{16}H_2O$: C, 61.13; H, 6.63; N, 15.84. Found: C, 61.46; H, 6.17; N, 15.39.

Ethyl 3,5-Dioxo-5-[5,6-dimethyl-3-oxo-1*H*-pyrrolo[3,4-*b*]pyridin-2-yl]pentanoate (7). To the solution of lactam prepared above (2.25 g, 14 mmol) in xylene (50 mL) was added diethyl 1,3-acetonedicarboxylate (7.6 mL,42 mmol). The resulting mixture was heated at reflux for 48 h. The mixture was cooled and diluted with EtOAc/hexane. The precipitated solid was filtered, washed with hexane, and dried in vacuo (2.5 g, 56%): ^{1}H NMR δ 7.65 (s, 1H, pyridyl), 4.8 (s, 2H, CH_2NHCO), 4.35 (s, 2H, $NCOCH_2$), 4.2 (m, 2H, ethyl ester), 3.75 (s, 2H, CH_2-ester), 2.7 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 1.3 (t, 3H, ethyl ester); MS (ES) 319 (M + H).

9-Hydroxy-2,3-dimethylpyrido[2,3-*a*]indolizin-7-(5*H*)-one (8). To a solution of the pentanoate **7** prepared above (2.5 g, 7.8 mmol) in DMF (60 mL) was added piperidine (0.93 mL, 9.4 mmol). The resulting mixture was heated at 100 °C for 3 h. Solvent was removed in vacuo and the resulting residue was suspended in CH_2Cl_2/EtOAc (50:50, 100 mL). The precipitated 10-carbethoxy-9-hydroxy-2,3-dimethylpyrido[2,3-*a*]indolizin-7(5*H*)-one was filtered (1.88 g, 80%). ^{1}H NMR (CD_3OD) δ 7.7 (s, 1H, pyridyl), 5.9 (s, 1H, pyridone ring), 5.0 (s, 2H, CH_2N), 4.5 (q, 2H, ethyl ester), 2.6 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 1.4 (t, J = 5 Hz, 3H, ethyl ester); MS (FAB) 304 (M + H deuterated).

A solution of the intermediate prepared above (1.88 g, 6.3 mmol) in concd HCl (50 mL) was heated in the sealed tube at 165 °C for 3 h. The mixture was cooled and the pH was carefully adjusted to 6.5 with 2 N NaOH. The precipitated solid was filtered and dried in vacuo to give **8** (0.72 g, 50%): ^{1}H NMR (CD_3OD) δ 7.8 (s, 1H, pyridyl), 6.8 (d, 1H, J = 3 Hz, pyridone ring), 5.85 (d, 1H, J = 3 Hz, pyridone ring), 5.0 (s, 2H, CH_2N), 2.65 (s, 3H, CH_3), 2.4 (s, 3H, CH_3); MS (CI) 229 (M + H).

9-Hydroxy-8-[(phenylthio)methyl]-2,3-dimethylpyrido-[2,3-*a*]indolizin-7(5*H*)-one (9). To a solution of 37% aqueous formaldehyde (0.24 mL, 3 mmol) and thiophenol (0.92 mL, 9 mmol) in EtOH (15 mL) was added piperidine (0.7 mL) and glacial acetic acid (0.7 mL). The resulting mixture was stirred at 55 °C for 5 min. To the resulting mixture was added a suspension of indolizine **8** (0.72 g, 3 mmol) in EtOH (100 mL). The mixture was heated with stirring at 55 °C for 1 h. The solution was cooled and the solid precipitated, washed with hexane, and dried in vacuo to give **9** as a solid (0.75 g, 67%): ^{1}H NMR (CD_3OD) δ 7.8 (s, 1H, pyridyl) 7.4 (d, 2H, aromatic), 7.3 (m, 3H, aromatic), 6.85 (s, 1H, pyridone ring), 5.0 (s, 2H, CH_2N), 4.25 (s, 2H, benzylic), 2.65 (s, 3H, CH_3), 2.4 (s, 3H, CH_3); MS (CI) 351 (M + H).

9-Hydroxy-2,3,8-trimethylpyrido[2,3-*a*]indolizin-7(5*H*)-one (10). To a solution of **9** (0.75 g, 2 mmol) in EtOH (50 mL) was added Raney nickel (50 mg, deactivated by boiling in acetone) in EtOH (20 mL). The resulting mixture was heated at 75 °C for 1 h. The resulting suspension was extracted repeatedly with MeOH/CH_2Cl_2. The mixture was filtered and the solvent removed in vacuo to give **10** as a solid (0.23 g, 45%): ^{1}H NMR (CD_3OD) δ 7.75 (s, 1H, pyridyl), 6.9 (s, 1H, pyridone ring), 5.0 (s, 2H, CH_2N), 2.6 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 2.1 (s, 3H, CH_3); MS (ESI) 243 (M + H). Anal. Calcd for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.14; H, 5.90; N, 11.39.

9-Acetyl-2,3,8-trimethylpyrido[2,3-*a*]indolizin-7-(5*H*)-one (3). To a solution of **10** (0.22 g, 0.9 mmol) in DMF (10 mL) was added triethylamine (0.4 mL, 2.8 mmol) and *N*-phenyltrifluoromethanesulfonimide (0.5 g, 1.3 mmol). The resulting mixture was stirred at room temperature for 30 min. Solvent was removed in vacuo and the mixture chromatographed (CH_2Cl_2) to give 9-[[(trifluoromethane)sulfonyl]oxy]-2,3,8-trimethylpyrido[2,3-*a*]indolizin-7(5*H*)-one as a solid (0.17 g, 57%): ^{1}H NMR δ 7.6 (s, 1H, pyridyl), 7.1 (s, 1H, pyridone ring), 5.1 (s, 2H, CH_2N), 2.6 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 2.25 (s, 3H, CH_3).

To a solution of the triflate prepared above (0.15 g, 0.4 mmol) in CH_3CN (10 mL) was added triethylamine (0.12 mL, 0.8 mmol) and butyl vinyl ether (0.26 mL, 2 mmol). To the resulting mixture was added $Pd(OAc)_2$ (3 mg, 3% mol) and 1,3-bis(diphenylphosphino)propane (6 mg, 4% mol). The resulting mixture was heated at 70 °C for 28 h. The solvent was removed in vacuo and the mixture chromatographed (0–3% MeOH–CH_2Cl_2) to give 9-[(butyloxy)vinyl]-2,3,8-trimethypyrido[2,3-a]indolizin-7(5H)-one as oil (33 mg, 30%): ^{1}H NMR δ 7.65 (s, 1H, pyridyl), 7.1 (s, 1H, pyridone ring), 5.0 (s, 2H, CH_2N), 4.4 (d, 1H, J = 3 Hz, olefin), 4.3 (d, 1 H, J = 3 Hz, olefin), 3.8 (t, J = 5 Hz, 2H, $-OCH_2-$), 2.6 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 1.75 (m, 2H, alkyl), 1.5 (m, 2H, alkyl), 1.0 (t, 3H, alkyl).

To a solution of the vinyl ether prepared above (32 mg, 0.1 mmol) in glacial acetic acid (1 mL) was added 3 N HCl (4 drops) and the resulting mixture was stirred at room temperature for 1 h. Mixture was diluted with H_2O, extracted with EtOAc, washed with $NaHCO_3$ and NaCl, and dried (Na_2SO_4). Flash chromatography (0–3% MeOH–CH_2Cl_2) gave **3** as a solid (11 mg, 55%): mp 195–197 °C, ^{1}H NMR δ 7.65 (s, 1H, pyridyl), 7.1 (s, 1H, pyridone ring), 5.1 (s, 2H, CH_2N), 2.6 (s, 3H, CH_3), 2.55 (s, 3H, CH_3CO), 2.4 (s, 3H, CH_3), 2.3 (s, 3H, CH_3); MS (ESI): 269 (M + H). Anal. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.24; H, 5.90; N, 10.12.

Plaque Reduction Assays. For HSV-1 and HSV-2 assays, confluent Vero cell monolayers in 24-well plates were infected with 100 pfu/well in Hanks Balanced Salt Solution (HBSS) at 37 °C. Following 1 h of adsorption, EMEM containing 20% FBS, antibiotics, Human IgG (50 mg/mL, filter sterilized through a 0.45-μm filter and then mixed 1:1 with 4× complete EMEM), and the appropriate amount of compound in HBSS were added to each well. At 24 h post-infection, plaques were visualized and quantified after staining plates with crystal violet. The HCMV assay was carried out on MRC-5 cell monolayers for 7–10 days under the same liquid overlay described above. For all antiviral assays, plaques were counted and compound effectiveness evaluated in terms of percent plaque reduction compared to untreated, infected controls. Calculations for 50% plaque reduction values for antiviral compounds (PR_{50}) were mathematically derived from dose–response data using the Kärber method.

Supplementary Material Available: Copies of ^{1}H NMR spectra of **4, 5, 7–9** (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.