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Chemistry of platensimycin

Sheo B. Singh,* Kithsiri B. Herath, Jun Wang, Nancy Tsou and Richard G. Ball

Merck Research Laboratories, Rahway, NJ 07065, USA

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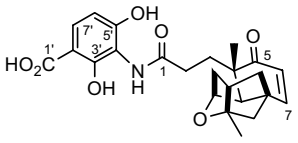
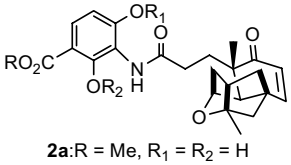
Abstract—Platensimycin is a novel natural product antibiotic that inhibits bacterial growth by inhibiting fatty acid synthesis specifically inhibiting the elongation condensing enzyme FabF. Reaction with diazomethane at controlled temperatures led to selective methylation of the phenolic groups. Methylation, halogenation, reduction, epoxidation, Bayer–Villiger oxidation and details of the conversion of dihydroplatensimycin to the cyclic enamino-amido forms have been described.

Platensimycin (**1**) is a novel antibiotic isolated from various strains of *Streptomyces platensis*.[1,2] It was discovered by a novel antisense differential sensitivity screening strategy in which FabH/FabF was sensitized.[3–6] Platensimycin selectively inhibits the elongation condensing enzyme FabF of the bacterial fatty acid synthesis pathway by interacting with the malonyl binding site of the catalytic triad of the FabF acyl-enzyme intermediate. It exhibits potent in vitro activity against both cell-free and whole-cell systems. The in vitro activity could not be directly translated to an in vivo mouse model when the drug was administered by conventional routes. However, when administered by continuous infusion the drug was highly efficacious. The poor in vivo activity under conventional administration is attributed to its pharmacokinetic properties which could potentially be improved by chemical modification. The present study describes some chemical modifications of platensimycin.

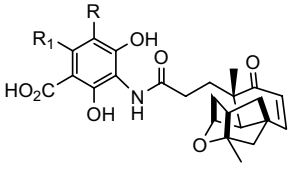
Treating platensimycin (**1**) with an excess of ethereal diazomethane at −78 °C selectively methylated the carboxyl group and produced the methyl ester (**2a**)[7] in quantitative yield. A similar reaction at 0 °C produced a mixture of mono- and dimethyl ethers **2b** and **2c** in a ratio of 4:1 in 30 min. These compounds were readily separated by reversed phase HPLC affording a combined yield of 95%. Compound **2b** was completely converted to **2c** when the reaction was allowed to continue for 48 h. The rate of methylation of the C-3′ phenolic group was slower due to steric factors or the formation of a six-center hydrogen bond with the carbonyl oxygen of the benzoic acid. The location of the methyl group of **2b** was deduced by HMBC correlations of the methyl hydrogens and both of the aromatic hydrogens to C-5′ and confirmed by NOEs between H-6′ and C-5′-OMe. The methyl ester was readily hydrolyzed by treatment with LiOH in THF–water overnight affording **2d** (from **2b**) and **2e** (from **2c**).



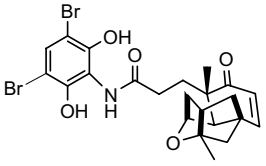
1 (Platensimycin)



2a:R = Me, R_1 = R_2 = H
2b:R = Me, R_1 = Me, R_2 = H
2c:R = Me, R_1 = R_2 = Me
2d:R = H, R_1 = Me, R_2 = H
2e:R = H, R_1 = R_2 = Me



3a:R = Cl, R_1 = H
3b:R = H, R_1 = Cl
3c:R = Br, R_1= H



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Reaction of **1** with a 10-fold excess of *N*-chlorosuccinimide in a 5:1 mixture of acetone and acetic acid produced a 7:9 mixture of the two mono-chloro derivatives, **3a** and **3b**, in a combined yield of 97%; they were readily separated by reversed phase HPLC. No reaction

* Corresponding author. Fax: +1 732 594 6880; e-mail: sheo_singh@merck.com

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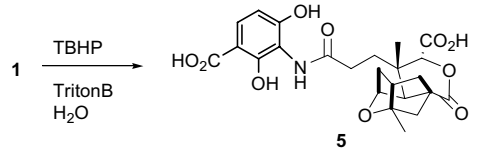
was observed in the absence of acetic acid. Adding 1.1 equiv of *N*-bromosuccinimide (NBS) to platensimycin in a 4:1 mixture of acetone and THF produced exclusively 6′-bromoplatensimycin (**3c**) in 2 h with an isolated yield of 85%. When the reaction was performed with 5 equiv of NBS it produced a 5:6 mixture of **3c** and the dibromo-*des*-carboxyl derivative **4** in less than 1 h. The structure of **4** was confirmed by 2D NMR experiments.

Epoxidation of **1** with *m*-CPBA in a 2:1 mixture of methylene chloride–THF failed to produce any product and left the unreacted starting material intact; a common feature of most enones. However, epoxidation under basic conditions using triton B and *tert*-butylhydroperoxide (TBHP) in a 1:1 mixture of MeOH–THF gave an oxidized product identified as lactone acid **5** (Scheme 1).[7] The structure and configuration of compound **5** was elucidated by HMBC (**6**) and NOEDS (**7**) experiments (Fig. 1). For example, H-6 (δ_H 4.59, s; δ_C 84.3) exhibited HMBC correlations to C-18 (δ_C 19.5), acid C-5 (δ_H 170.4) and lactone carbonyl C-7 (δ_C 174.6). H_3-18 (δ_H 1.31, s) exhibited HMBC correlation to C-6; irradiation of H_3-18 showed strong NOE to H-6 and H-13 while irradiation of H-6 showed reciprocal NOE to H_3-18 thus suggesting the equatorial orientation of H-6.[7]

We recently reported[2] that the catalytic hydrogenation of platensimycin produced dihydroplatensimycin (**8**), which converted to an inseparable 2:1 mixture of atropisomers of the enamino-amido cyclic product (**9**), particularly in the presence of acid (e.g., TFA), via intermediate **10**. This intermediate could not be detected by LCMS probably because of spontaneous dehydration. Recently, we crystallized **9** from a nitromethane–CH_2Cl_2–MeOH mixture and confirmed the structure of one of the atropisomers by single-crystal X-ray crystallography (Fig. 2).[8] Examination of the remaining crystals by ^{1}H NMR in C_5D_5N indicated that these crystals were still a mixture of the two atropisomers; the selection of a crystal with the shown atropisomeric form was fortuitous.



Scheme 1. Epoxidation of platensimycin (**1**).

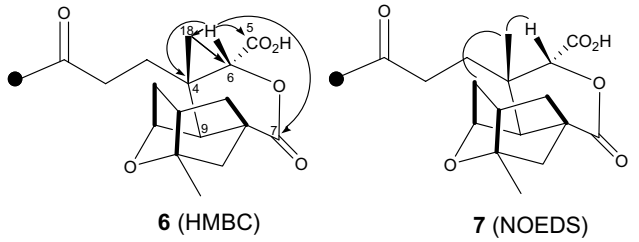
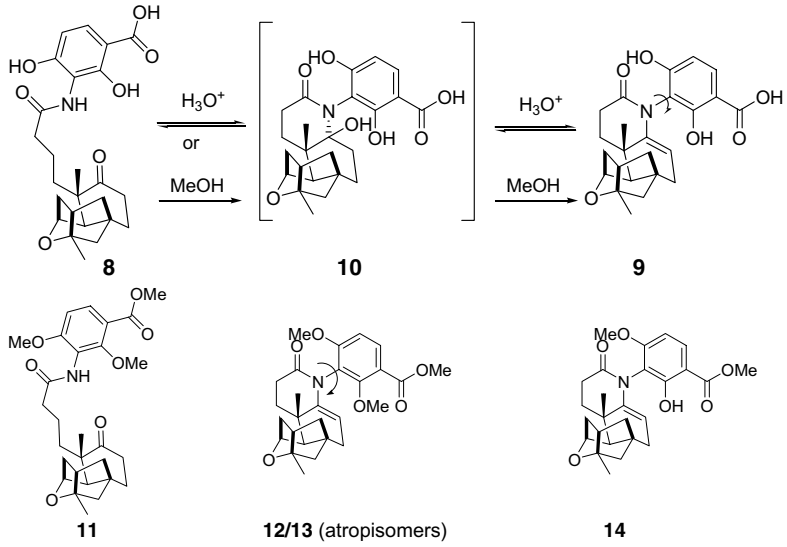


Figure 1. Key HMBC and NOEDS correlations of compound **5** in 3:1 $CDCl_3$–CD_3OD.

Methylation of **8** with diazomethane at 0 °C for 48 h produced a trimethyl derivative **11**. Similar methylation of the atropisomeric mixture of **9** produced a mixture of three products **12**/**13**, and **14** (listed in the order of RPHPLC elution) that were separated by HPLC.

Following our initial report[2] we decided to investigate the details of the interesting conversion reactions and stability of compounds **8** and **9**. Both compounds were found to be completely stable in pyridine for a period of two months. Compounds **8** and **9** do interconvert and reach a 60/40 (**8**:**9**) equilibrium mixture after standing for 4–5 days in an acetonitrile–water mixture containing 0.1% TFA. This inter-conversion can be accelerated so equilibrium is reached in 1 h if the solution is changed to aqueous MeOH with 1% PTSA or TFA.

Larger scale hydrogenation of **1** (1 g in 40 mL MeOH) was sluggish and required 48 h for completion. During this reaction 31% of **9** was produced in the absence of any added acid prompting further investigation of the conversion. The keto compound **8** was completely converted to enamino-amido product **9** in MeOH. However, the rate of the conversion was scale dependent. For example, on a small analytical scale (mg quantities) and at a concentration of 5 mg/mL the conversion was relatively fast and 98% of the cyclic product (**9**) was formed at room temperature (Fig. 3) in about 17 h. However, on a larger scale (grams) the conversion was slower and required heating at 50 °C for 24 h for complete conversion (Fig. 4). Once the enamino-amido cyclic product was produced it was completely stable in MeOH. Addition of hydrated PTSA to MeOH increased the rate of the formation of the cyclic product so 97% conversion occurred in less than 4 h. However, after about 15 h the cyclic product slowly hydrolyzed (Fig. 3) producing about 23% of the keto product. Complete conversion of **8** to **9** was achieved in less than 4 h in the presence of PPTS and no conversion back to the keto product was observed indicating that the hydration of PTSA provided just enough water content for the hydrolysis to take place. This result prompted us to screen for other solvents which affect the stability of these compounds. At 5 mg/mL, the rate of conversion of **8** was significantly slower in acetonitrile, THF and DMSO producing 30%, 10%, and 30%, respectively, of **9** in 4 h without any increase over the next 20 h. These observations, together with the observed stability of compounds **8** and **9** in pyridine, led us to the assumption that the acidity of the carboxylic acid was sufficient for protonation and formation of the enamino-amido product **9**. We tested this assumption by converting **8** quantitatively to a sodium salt by treatment with NaOH. This salt was completely stable in MeOH at room temperature for one week indicating that the acidity of the original carboxyl group was sufficient to catalyze the conversion whereas the acidities of the phenolic groups were insufficient for such catalysis. Likewise the trimethyl keto derivative (**11**), was completely stable in



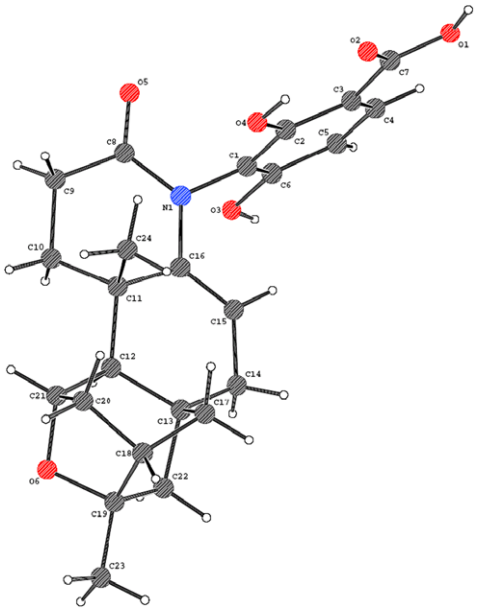


Figure 2. X-ray crystal structure of an atropisomer of **9**.

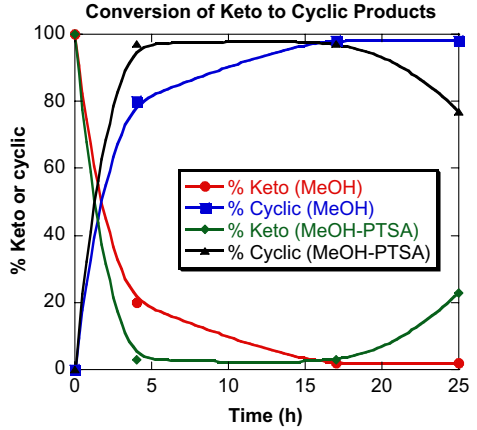


Figure 3. Conversion of keto (**8**) to cyclic (**9**) compounds on an analytical scale.

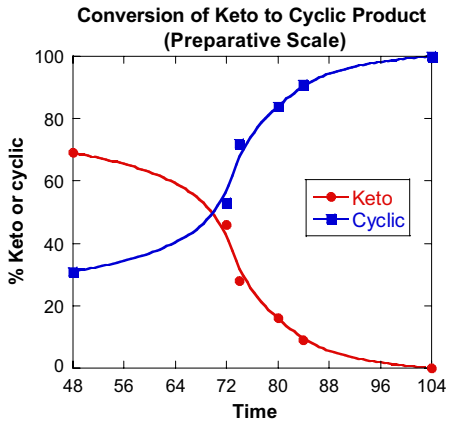
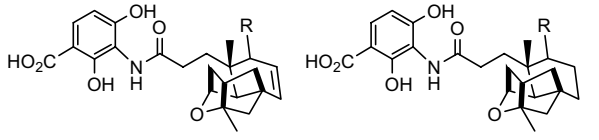
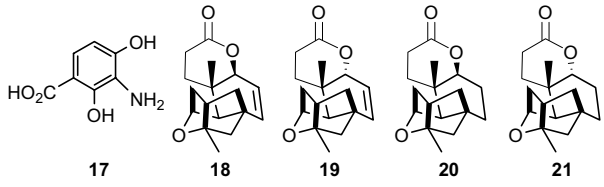


Figure 4. Conversion of keto (**8**) to cyclic (**9**) compounds at a preparative scale.

MeOH but the cyclization reaction could be catalyzed by addition of either aqueous 1% PTSA or 1% TFA leading to a 65/35 ratio of the keto and two HPLC-separable atropisomers of enamino-amido products **12** and **13** (ratio 20/15, respectively). Each of the purified atropisomers **12** and **13** were highly stable in MeOH but hydrolyzed cleanly to the keto form, **11**, in MeOH–water (2:1) with 1% PTSA reaching an identical equilibrium ratio as that produced from **8**. While heating of atropisomers **9**, **12/13**, in DMSO (NMR) at 120 °C did not show any inter-conversion, **12/13** could be converted via acidic hydrolysis to the keto form. The same ~20/15 ratio observed for **11** was seen when starting with **12/13**. The ratio of the two products was identical whether the hydroxy and carboxyl groups were protonated or methylated.





Reaction of **1** with 15 equiv of sodium borohydride in MeOH yielded a mixture of di- and tetra-hydro derivatives **15a**, **15b**, **16a**, and **16b** in a ratio of 50:5.8:21:22 (HPLC), respectively, in over 90% combined yield before HPLC purification from each other. Acid workup followed by concentration of the reaction mixture to dryness lead to rapid conversion to 3-amino-2,4-dihydroxybenzoic acid **17** and corresponding lactones **18**–**21**. The lactone formation was instantaneous when methanolic solution of amides was treated with 1 N HCl. In fact compounds **15b**, **16a**, and **16b** could not be isolated until the reaction mixture was quenched with sodium phosphate buffer (pH 7) and purified as a sodium salt by RP HPLC at pH 7 using the phosphate buffer. While the sodium salt of **15a** was stable the sodium salts of **15b**, **16a**, and **16b** were not as stable and the purified samples were always contaminated with the corresponding lactones after concentration with a ratio reaching as high as 1:1.

In summary, we have described herein selected functional-group chemistries of platensimycin. It is noteworthy that the reduction of the enone olefin or ketone leads to such a facile formation of the cyclic products **9** and **18**–**21**. Remarkably nature has protected this antibiotic by introduction of this enone olefin. None of these compounds showed better activity than platensimycin. Biological activity and SAR will be reported elsewhere.

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7. All compounds were characterized by ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR and ESIMS analyses. Key assignments of selected compounds are listed except for compound **5** where full assignments are listed. Compound **4b**: (C_5D_5N) δ_H 7.81 (1H, s, new H at original C-2′); δ_C 150.4 (C-3′,5′), 133.1 (C-2′), 127.6 (C-2′), 120.1 (C-6′), 103.5 (C-4′). Compound **5**: ($CDCl_3$–CD_3OD, 3:1) δ_H 7.54 (1H, d, $J = 8.5$ Hz, H-7′), 6.41 (1H, d, $J = 8.5$ Hz, H-6′), 4.59 (1H, s, H-6), 4.35 (1H, br s, H-10), 2.76 (1H, ddd, $J = 14$, 11.5, 4 Hz, H-2), 2.53 (1H, ddd, $J = 14$, 11.5, 5.5 Hz, H-2), 2.34 (1H, t, $J = 6.5$ Hz, H-12), 2.28 (1H, br s, H-9), 2.12 (1H, dd, $J = 12$, 3.5 Hz, H-13), 2.08 (1H, d, $J = 12$ Hz, H-14), 2.04 (1H, ddd, $J = 14$, 11.5, 5 Hz, H-3), 1.96 (1H, m, H-11), 1.94 (1H, m, H-13), 1.92 (1H, m, H-11), 1.86 (1H, d, $J = 12$ Hz, H-14), 1.68 (1H, ddd, $J = 14$, 11.5, 5 Hz, H-3), 1.37 (3H, s, H-17), 1.31 (3H, s, H-18) δ_C 173.5 (C-1), 30.1 (C-2), 33.2 (C-3), 36.6 (C-4), 170.4 (C-5), 84.3 (C-6), 174.6 (C-7), 48.3 (C-8), 46.7 (C-9), 75.7 (C-10), 39.6 (C-11), 43.9 (C-12), 45.5 (C-13), 51.2 (C-14), 86.8 (C-15), 22.2 (C-17), 19.5 (C-18), 172.0 (C-1′), 104.8 (C-2′), 155.0 (C-3′), 113.4 (C-4′), 155.0 (C-5′), 109.8 (C-6′), 128.4 (C-7′); ESIMS (*m*/*z*) 488 [M−H], 512 [M+Na], 490 [M+H]. Compound **11**: (C_5D_5N) δ_H 7.95 (1H, d, $J = 9$ Hz, H-7′), 6.83 (1H, d, $J = 9$ Hz, H-6′), 4.45 (1H, br s, H-10), 4.10, 3.81, 3.76 (3H each, s). Compound **12**: (C_5D_5N) δ_H 8.13 (1H, d, $J = 9$ Hz, H-7′), 6.95 (1H, d, $J = 9$ Hz, H-6′), 4.59 (1H, dd, $J = 4.5$, 3 Hz, H-6), 4.54 (1H, br t, $J = 3.5$ Hz, H-10), 3.96, 3.82, 3.74 (3H each, s). Compound **13**: (C_5D_5N) δ_H 8.09 (1H, d, $J = 9$ Hz, H-7′), 6.87 (1H, d, $J = 9$ Hz, H-6′), 4.69 (1H, t, $J = 4$ Hz, H-6), 4.56 (1H, br s, H-10), 4.03, 3.84, 3.70 (3H each, s). Compound **14**: ^{1}H (C_5D_5N) δ_H 7.92 (1H, d, $J = 9$ Hz, H-7′), 6.72 (1H, d, $J = 9$ Hz, H-6′), 4.81 (1H, t, $J = 4$ Hz, H-6), 4.54 (1H, br s, H-10), 3.77, 3.74 (3H each, s).

8. Compound **9** $C_{24}H_{27}NO_6$, $M_r = 425.470$, hexagonal, $P6_1$, $a = 16.4900(8)$, $c = 13.4962(13)$ Å, $V = 3178.2(4)$ Å^3, $Z = 6$, $D_x = 1.334$ g cm^{-3}, monochromatized radiation $\lambda(\text{Mo}) = 0.71073$ Å, $\mu = 0.10$ mm^{-1}, $F(000) = 1356$, $T = 100$ K. Data were collected on a Bruker CCD diffractometer to a θ limit of 30.48°, which yielded 51,312 reflections. There are 6438 unique reflections with 5308 observed at the 2σ level. The structure was solved by direct methods (SHELXS-97, Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467–473) and refined using full-matrix least-squares on F^2 (SHELXL-97, Sheldrick, G. M. SHELXL-97. *Program for the Refinement of Crystal Structures*. Univ. of Göttingen, Germany). The final model was refined using 285 parameters and all 6438 data. All non-hydrogen atoms were refined with anisotropic thermal displacements. The final agreement statistics are: $R = 0.043$ (based on 5308 reflections with $I > 2\sigma(I)$), $wR = 0.095$, $S = 1.02$ with $(\delta/\sigma)_{\max} < 0.01$. The maximum peak height in a final difference Fourier map is 0.305 e Å^{-3} and this peak is without chemical significance. CCDC 650857 contains the supplementary crystallographic data for this Letter. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.