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Synthesis and Activity of a C-8 Keto Pleuromutilin Derivative

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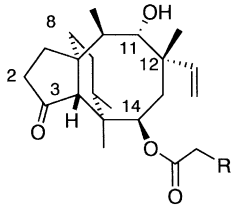
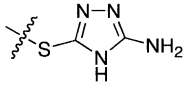
Abstract—A C-8 keto pleuromutilin derivative has been synthesized from the biotransformation product 8-hydroxy mutilin. A key step in the process was the selective oxidation at C-8 of 8-hydroxy mutilin using tetrapropylammonium perruthenate. The presence of the C-8 keto group precipitated interesting intramolecular chemistry to afford a compound (**10**) with a novel pleuromutilin-derived ring system.

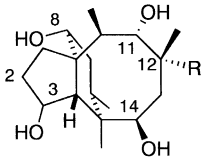


1 Pleuromutilin R = OH

2 Tiamulin R = $SCH_2CH_2N(CH_2CH_3)_2$

3 Azamulin R =





4a R = CH=CH
4b R = CH_2CH_3

Pleuromutilin (**1**) derivatives have attracted considerable pharmaceutical and academic attention due to their potent activity against drug-resistant Gram-positive bacteria, and the fascinating chemistry exhibited by the tricyclic nucleus.[1,2] Tiamulin (**2**) is a derivative discovered by Sandoz that is currently employed in veterinary medicine for promoting the growth of turkey poults, suckling pigs, and other newborn animals. Tiamulin is added to foodstuffs or injected ip in these animals to prevent deaths due to gastrointestinal tract infections such as dysentery, and respiratory tract infections.[3] Azamulin (**3**) was under development by Sandoz in the 1980's for potential use in humans. Unfortunately, while determined to be safe in healthy volunteers, azamulin displayed poor oral absorption and a short half-life due to rapid metabolism and subsequent excretion.[4] These poor PK characteristics are the bane of the pleuromutilin class, and efforts to overcome them have been largely fruitless to date.

A significant contributor to the metabolism of pleuromutilin derivatives is cytochrome P_{450}-mediated hydroxylation at C-2 and C-8 of the core structure. These metabolites are described in the pleuromutilin literature as being poorly active molecules.[2c,5] However, it is not clear from the reports if the inactivity is due to lack of intrinsic antibacterial activity in vitro (MIC), or a reflection of poor in vivo activity due to conjugation of the hydroxyls at C-2 and C-8 and subsequent elimination. The Sandoz group attacked the metabolism problem by synthesizing a number of C-2 and C-8 analogues designed to either sterically shield these sites, or electronically alter them to disfavor hydrogen atom abstraction by the iron–oxo complex of the cytochrome. Their chemical efforts at C-2 utilized pleuromutilin as starting material, readily available in bulk quantities from fungal

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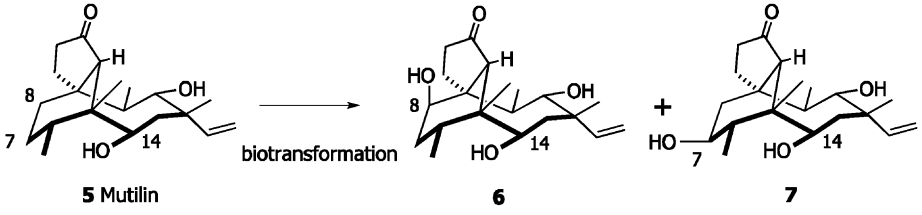
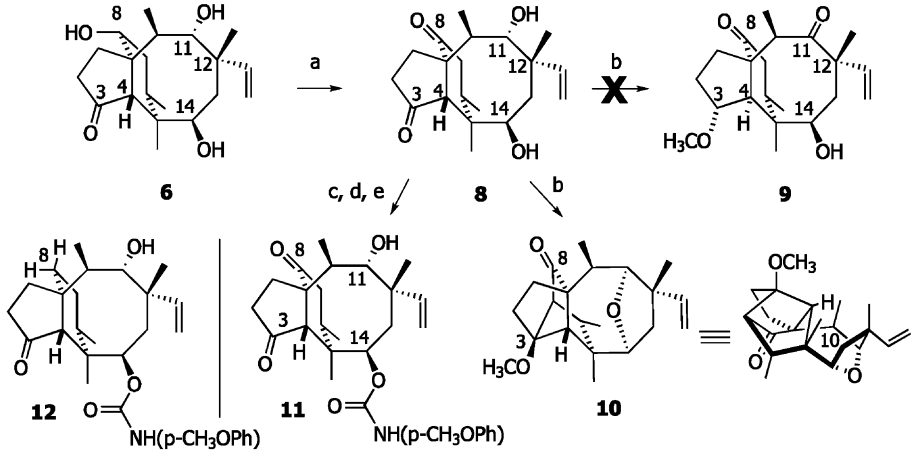
cultures. Their C-8 work proceeded from **4a** (described as being a by-product in the fermentation leading to pleuromutilin), and **4b**, available presumably by hydrogenation of **4a**.[6] While the Sandoz group utilized the C-8 hydroxyl functional group of **4a** and **4b** to synthesize a significant number of derivatives with alterations in the cyclohexyl ring of the system, no biological data has emerged from these derivatives.[6,7]

As part of a program at Bristol-Myers Squibb to develop a novel pleuromutilin derivative for human use, we wished to have chemical access to the six-membered ring to manipulate this under-exploited region for the purpose of extending pleuromutilin SAR. In one approach to this goal, we decided to produce C-8 oxidized pleuromutilin derivatives by initial biotransformation of mutilin (**5**) and subsequent conversion to the desired targets (Scheme 1). The successful biotransformation efforts generated very good yields of C-8 hydroxy mutilin **6**, and depending on the exact conditions, significant amounts of C-7 hydroxy mutilin **7** as well.[8] With ample quantities of **6** in hand we decided to synthesize the C-8 keto, C-14 carbamate analogue **11** (Scheme 2) as one of our initial targets. A group at GlaxoSmithKline has recently published a series of C-14 carbamates including carbamate **12**.[9] We had previously evaluated **12** in vitro and found it to be extremely potent. We believed a head-to-head comparison of **11** and **12** might prove useful in assessing the effect of the C-8 keto group on antibacterial potency.

We began our synthesis of **11** by exploring the selective oxidization of the C-8 hydroxyl of **6** to provide diketone **8**. After trying a number of oxidizing agents we found that tetrapropylammonium perruthenate gave the best yield of desired **8** (48%) relative to other oxidized products. We next attempted a familiar stratagem of pleuromutilin chemistry, the acid-catalyzed conversion of **8** to **9**. This process works well in most pleuromutilin derivatives, and serves to protect the C-3 ketone and C-11 hydroxyl groups during further transformations.[10] [The intramolecular hydride migration process is reversed using Lukas' reagent (concd HCl saturated with $ZnCl_2$) to reform the ketone at C-3 and the hydroxyl group at C-11.] Disconcertingly, we observed that the expected ^1H NMR resonances for a derivative such as **9** were not present in the major product of the reaction. After scrutiny of ^1H–^1H COSY, HMBC, and HMQC NMR spectra, we were able to assign the structure of **10** to the product of this reaction.[11] The formation of this novel ring structure is a direct consequence of the presence of a C-8 keto group in **8**. Once a methyl oxonium ion is formed at C-3, it is trapped by a C-7/C-8 enol to form the norbornane substructure of **10**. Hand-held models are useful in revealing that the cyclooctane ring of such a norbornane derivative has considerably more flex than a normal pleuromutilin. The normal pleuromutilin skeleton restricts the cyclooctane ring to predominately a boat-chair conformation. The boat-boat conformer is precluded by an intense 'prow-like' interaction between the C-4 proton protruding out over the ring, and the methyl group at C-12. Crown-like cyclooctane ring structures in this system are disfavored by a steric interaction between the C-10 methyl group and the C-4 methine proton. However, the norbornane structure of **10** causes the C-4 carbon atom (and its proton) to be splayed back away from the cyclooctane ring, allowing increased mobility of C-10 and C-12 towards C-4. A



Scheme 1.

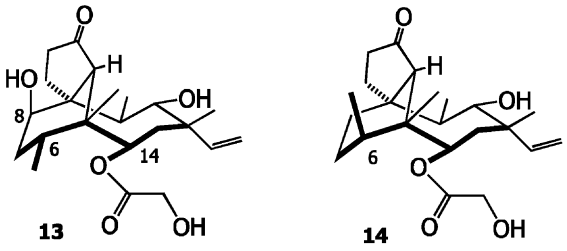


Scheme 2. Reaction conditions: (a) NMO, TRAP, CH_3CN, 4 Å sieves, 0 °C, 42%; (b) $HC(OMe)_3$, MeOH, concd H_2SO_4, 51%; (c) Ac_2O, DMAP, pyr, rt, 54%; (d) 4-MeO-C_6H_4NCO, *i*-Pr_2NEt, $CHCl_3$, reflux, 60%; (e) 1 N NaOH, rt, 30%.

Table 1.

	Organism	A no.	Mic of compounds (μg/mL)	
			11	**12**
1	*S. pneumoniae*/Pen. Resist.	A28272	32	0.003
2	*S. aureus*/Homo MR	A27223	32	0.003
3	*M. catarrhalis*/Pen. +	A22344	64	0.125
4	*H. influenzae*/Pen. +	A21515	64	4

conformation can now be accessed that allows the hydroxyl at C-11 to approach the backside of the C-14 carbon's hydroxyl bond to displace it via acid catalysis, forming the cyclic ether between C-11 and C-14.



We employed new tactics to arrive at the desired C-8 keto derivative **11**. We found that diol **8** could be selectively acylated at C-11. Introduction of the carbamate at C-14 was then followed by hydrolysis of the acetate at C-11 to deliver **11**.[12] Quite surprisingly, C-8 keto derivative **11** was found to be ~10,000 times less active than C-8 methylene derivative **12** in vitro against bacteria of interest to our program (see Table 1). This staggering erosion of activity after such a slight structural change, coupled with the observations that 8-hydroxy azamulin **13** and the C-6 epimeric pleuromutilin **14** are poorly active, clearly indicates that the environment of the cyclohexyl ring of the system is crucial to the molecule's interactions with the ribosomal complex. Altering this portion of the molecule (to improve pharmacokinetic parameters and avoid metabolic liabilities) while maintaining antibacterial potency will be a significant challenge to further investigations in the pleuromutilin class.

Acknowledgements

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References and Notes

1. (a) Kavanagh, F.; Hervey, A.; Robbins, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 570. (b) Egger, H.; Reinshagen, H. *J. Antibiot.* **1976**, *29*, 923.
2. (a) Arigoni, D. *Gazz. Chim. Ital.* **1962**, *92*, 884. (b) Birch, A. J.; Holzapfel, C. W.; Rickards, R. W. *Tetrahedron* **1966**, *Suppl. 8*, 359. (c) Berner, H.; Vyplel, H.; Schulz, G. *Tetrahedron* **1987**, *43*, 765 and references cited therein.
3. (a) Anderson, M. D. *Vet. Med. Small Anim. Clin.* **1983**, *78*, 98. (b) Burch, D. G. S.; Jones, G. T.; Heard, T. W.; Tuck, R. E. *Veterinary Record* **1986**, *119*, 108.
4. (a) Ganzinger, U.; Stephen, A.; Obenaus, H.; Baumgartner, R.; Walzl, H.; Bruggemann, S.; Schmid, B.; Racine, R.; Schatz, F.; Haberl, H. In *Proc. Int. Congr. Chemother. 13th*; Spitzey, K. H., Karrer, K., eds.; H. Egermann: Vienna, Austria, 1983, Vol. 5, p 108/53. (b) Nefzger, M.; Battig, F.; Czok, R. In *Proc. Int. Congr. Chemother. 13th*; Spitzey, K. H., Karrer, K., eds.; H. Egermann: Vienna, Austria, 1983; Vol. 5, p 108/47. (c) Schatz, F.; Haberl, Czok, R. In *Proc. Int. Congr. Chemother. 13th*; Spitzey, K. H., Karrer, K., eds.; H. Egermann: Vienna, Austria, 1983, Vol. 5, p. 108/50.
5. Berner, H.; Hildebrandt, J.; Schuster, I. In *Proc. Int. Congr. Chemother. 13th*; Spitzey, K. H., Karrer, K., Eds.; H. Egermann: Vienna, Austria, 1983; Vol. 5, p. 108/20, and references cited.
6. (a) An experimental description of the efforts to produce **4a** and **4b** is absent from the literature; however, the approach is alluded to in the following reports: Berner, H.; Vyplel, H.; Schulz, G.; Stuchlik, P. *Tetrahedron* **1983**, *39*, 1317. (b) Berner, H.; Vyplel, H.; Schulz, G. *Tetrahedron* **1987**, *43*, 765.
7. Berner, H.; Vyplel, H.; Schulz, G.; Stuchlik, P. *Monatsh. Chem.* **1983**, *114*, 1125.
8. Hanson, R. L.; Matson, J. A.; Brzozowski, D. B.; LaPorte, T. L.; Springer, D. M.; Patel, R. N. Organic Process Research and Development **2002**, *6*, 482.
9. Brooks, G.; Burgess, W.; Colthurst, D.; Hinks, J. D.; Hunt, E.; Pearson, M. J.; Shea, B.; Takle, A. K.; Wilson, J. M.; Woodnutt, G. Bioorg. *Med. Chem. Lett.* **2001**, *9*, 1221.
10. Berner, H.; Schulz, G.; Schneider, H. *Tetrahedron* **1980**, *36*, 1807.
11. ^{1}H NMR data for **10**: (500 MHz, $CDCl_3$, partial) δ 5.91 (dd, 1H, $J=11$, 17 Hz), 4.92 (d, 1H, $J=17$ Hz), 4.84 (d, 1H, $J=11$ Hz), 3.89 (dd, 1H, $J_1=J_2=8$ Hz), 3.86 (d, 1H, $J=10$ Hz), 3.23–3.16 (m, 1H), 3.22 (s, 3H), 2.50–2.47 (m, 1H), 2.45–2.38 (m, 1H), 2.09 (d, 1H, $J=2$ Hz), 2.05–2.00 (m, 1H), 1.84–1.68 (m, 3H), 1.48–1.44 (m, 1H), 1.23 (s, 3H), 1.04 (d, 3H, $J=7$ Hz), 0.99 (s, 3H), 0.92 (d, 3H, $J=8$ Hz).
12. ^{1}H NMR data for **11**: (300 MHz, $CDCl_3$) δ 7.36–7.22 (m, 2H), 6.94–6.82 (m, 2H), 6.55 (dd, 1H, $J=11$, 18 Hz), 5.27 (d, 1H, $J=18$ Hz), 3.80 (s, 3H), 3.45–3.35 (m, 1H), 2.91 (dd, 1H, $J_1=J_2=14$ Hz), 2.65–2.50 (m, 2H), 2.42 (br s, 1H), 2.31–2.17 (m, 3H), 2.17–1.93 (m, 2H), 1.69–1.15 (m, 8H), 1.24 (s, 3H), 0.95–0.86 (m, 3H), 0.80 (d, 3H, $J=7$ Hz).