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THE GIF SYSTEM AS A TOOL IN MEDICINAL CHEMISTRY: THE OXIDATION OF SCH 57726 UNDER GOAGG^{III} CONDITIONS

Darío Doller,* Samuel Chackalamannil, Andrew Stamford, Brian McKittrick, and Michael Czarniecki

Schering-Plough Research Institute, Chemical Research, CV/CNS, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033.

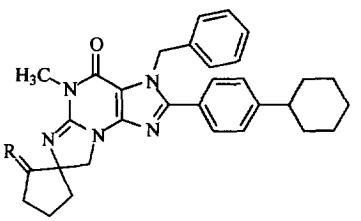
Abstract. The usefulness of the GoAgg^{III} reaction protocol in the determination of potential products of CP-450 enzyme metabolism is explored on Sch 57726, a selective c-GMP phosphodiesterase inhibitor. A single major ketone is found as product. In contrast, the reaction with *m*-CPBA affords a product of oxidative ring cleavage. © 1997 Elsevier Science Ltd.

Determining the course of in vivo metabolism of a drug candidate plays a critical role in the pharmacokinetics studies carried out during the drug discovery process. A major pathway of in vivo drug metabolism is oxidation of saturated carbon centers mediated by cytochrome P-450. Since the products of such transformations frequently undergo fast clearance, identification of primary metabolites is often difficult. In vitro liver microsome-mediated metabolism studies have been used as an alternative to in vivo studies as the first step towards identifying the metabolites. However, this method may suffer from over-metabolism and tedious isolation and identification procedures.

A chemical system capable of emulating in vivo oxidation would be of great value in determining the primary metabolic fate of drug candidates. Metalloporphyrins have been used to mimic cytochrome P-450 enzymes with varying degree of success.[1,2] Substrates under study are typically subjected to oxidation in organic solvents (dichloromethane or acetonitrile), employing tetraphenylporphin-derived ligands to modulate the redox properties of the central metal ion (generally Fe). NaClO, PhIO, or TBHP have been used as the oxidant. However, these oxidants provoke the substrate oxidation as well as the ligand oxidation, resulting in low turn-over numbers. In order to circumvent this complication, halogen atoms (F, Cl or Br) have been introduced in the porphyrin nucleus, increasing its chemical resistance to oxidation.[3] The preparation of these ligands, however, involves tedious multi-step synthetic sequences.

A simpler chemical system for the oxidation of non-activated carbon-hydrogen bonds has been reported by Barton, known as the Gif family of oxidation systems.[4] Among its most evolved members, the $GoAgg^{III}$ system is simply constituted by a solution of the substrate in pyridine/acetic acid, containing catalytic amounts of $FeCl_3 \cdot 6H_2O$ and picolinic acid; the oxidant is hydrogen peroxide 30% in water. To avoid overoxidation and to obtain high, meaningful mass balances, these reactions are typically run at low conversions (5–30%).

In the context of our efforts in the treatment of cardiovascular disorders, we discovered Sch 57726 (**I**), a type I and V selective c-GMP phosphodiesterase inhibitor.[5] We have applied the Gif oxidation protocol to Sch 57726 with the goal of determining potential sites where biological oxidation could occur. Reported herein are the results of our findings.



I: Sch 57726, R = H_2

II: R = O

Fe(III), picolinic acid, H_2O_2, pyridine, acetic acid

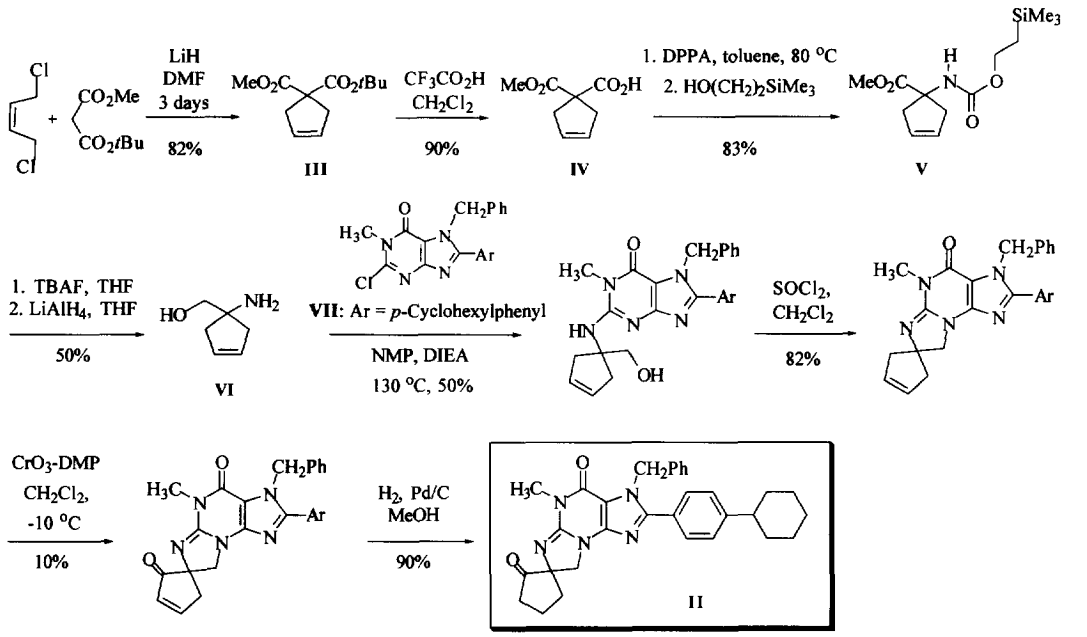
PDE Type I-Type V Inhibition by Sch 57726[a]

IC_{50} (nM)				
Type I	Type II	Type III	Type IV	Type V
110	17,000	>100,000	2,200	190

a. For IC_{50} determination procedures see ref 6.

The $GoAgg^{III}$ oxidation[7] of Sch 57726 afforded a single component after flash chromatography as the major reaction product, in 2% yield (or 30% after accounting for recovered unreacted starting material); the mass balance was good (>95%). The structure of the reaction product was initially assigned to be the ketone **II**, based upon spectroscopic data.[8] FABMS indicated a molecular ion [M+1] of 508, in agreement with the formation of a keto-derivative of Sch 57726 ([M+1] = 494). Comparison of the ^1H and ^{13}C NMR spectra of the product and the starting material showed that the *N*-benzyl and *p*-cyclohexylphenyl groups had remained unchanged. On the other hand, significant changes were seen in the cyclopentane and imidazole rings, suggesting that the carbonyl group was located nearby.

Confirmation of the structure of **II** was obtained by synthesis, which is shown in the Scheme 1. Malonic ester condensation with *cis*-1,4-dichloro-2-butene, followed by selective *tert*-butyl ester cleavage afforded the monocarboxylic acid **IV**. This was subjected to Schmidt rearrangement by treatment with diphenylphosphoryl azide to carbamate **V**. Desilylation followed by reduction with lithium aluminum hydride afforded aminoalcohol **VI**. The spiro-cyclopentene ring was built through a two-step condensation with chloride **VII**.[9] Allylic oxidation followed by catalytic reduction of the olefin afforded the corresponding saturated ketone, which showed spectral data identical to those of the Gif oxidation product, thus confirming the initial assignment.



Scheme 1. Confirmation of the structure of compound **II** by synthesis.

The regioselectivity of the Gif oxidation process could be explained considering that the putative Fe(V) = O species responsible for this particular oxidation chemistry[4] coordinates to one of the nitrogen atoms in the guanine nucleus, preceding an intramolecular insertion into the contiguous C-H bond (see Figure 1).

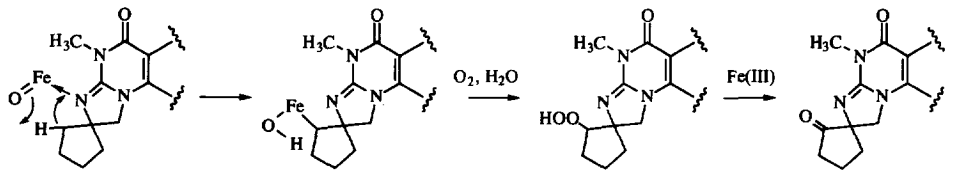
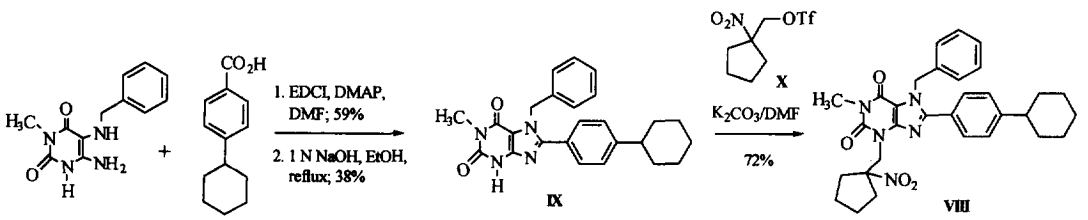


Figure 1. Postulated reaction mechanism for the formation of ketone **II**.

As a way of comparison, we also examined the reaction between Sch 57726 and *m*-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane.[10a] The reaction product (42% yield) was spectroscopically characterized as the nitro-compound **VIII**. The structure of **VIII** was confirmed by independent synthesis, through the alkylation of compound **IX** with triflate **X** (Scheme 2).[10b]



Scheme 2. Confirmation of the structure of compound **VIII** by synthesis.

Thus, it is clear that the GoAgg[III] and the *m*-CPBA oxidation pathways differ markedly. The formation of nitro-compound **VIII** can be mechanistically rationalized as shown in Figure 2, and would involve N-oxidation of Sch 57726 at the basic guanine nitrogen atom, followed by addition of a second equivalent of *m*-CPBA to the intermediate *N*-oxide, and rearrangement to a nitroso-compound, which is in turn oxidized to the final nitro-compound **VIII**.

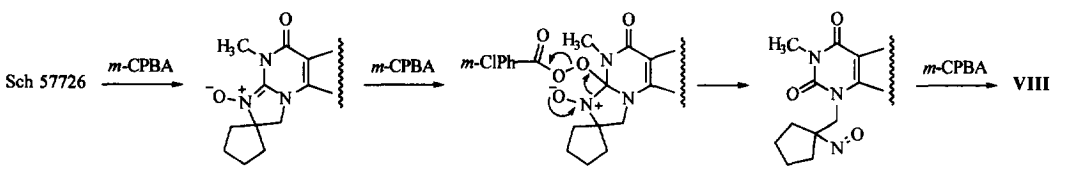


Figure 2. Proposed mechanism for the formation of compound **VIII**.

It is interesting that when in vivo oral dosing studies were carried out in rats and blood samples analyzed by HPLC-MS methods, the major metabolic pathway found for Sch 57726 as well as other spirocyclopentyl analogs was hydroxylation at the cyclopentane ring.[11] The fact that in both, the $GoAgg^{III}$ reaction as well as in vivo metabolism, the oxygenation of non-activated carbon-hydrogen bonds at the cyclopentane ring constitutes the major oxidative pathway is especially interesting when considering the existence of tertiary or benzylic C-H bonds or nitrogen atoms, which would be more susceptible to oxidation under standard oxidation conditions.

In conclusion, for the first time we have employed the Gif oxidation developed by Barton et al. for the *experimental* determination of potential in vivo metabolism sites in drug candidates. The peculiar reactivity displayed by this simple chemical system might be a viable complement to other CP450-emulating systems, which require the preparation of complex porphyrin ligands, for this type of study.

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7. Reaction conditions: In a 25-mL round bottom flask fitted with rubber septum and an oxygen-filled balloon, Sch 57726 (205 mg) was dissolved in a mixture of pyridine (10 mL) and acetic acid (1 mL), containing $FeCl_3 \cdot 6H_2O$ (10.8 mg, 0.04 mmol) and picolinic acid (15.0 mg, 0.12 mmol). The solution was stirred for 10 min at room temperature, taken to 0 °C, and H_2O_2 (30% in H_2O, 0.5 mL, 0.45 mmol) added dropwise. The reaction time was ca. 60 min. After work-up, flash chromatography (60% ethyl acetate in hexanes) afforded compound **II** (4 mg, 2%) and recovered starting material (190 mg, 93%).

8. Spectroscopic properties of compound **II**. IR (cm^{-1}): 1716, 1745. ^1H NMR (δ, $CDCl_3$, 400 MHz) 7.44 (d, 8.3Hz, 2H), 7.25 (mult, 5H, -$C_6\underline{H}_5$), 7.03 (d, 8.3Hz, 2H), 5.58 (2H, N-$C\underline{H}_2$-Ph), 4.12 (2H, C-$C\underline{H}_2$-N); 2.6–2.8 (2H, m, -CO-$C\underline{H}_2$-), 2.5 (m, 1H, tert. C-$\underline{H}$ in cyclohexyl ring). ^{13}C NMR ($CDCl_3$, δ) 29.76 (N-$\underline{C}H_3$), 49.57 (N-$\underline{C}H_2$-Ph), 44.57 (tertiary), 34.26 (double intensity), 26.77 (double intensity), and 26.08 (all in cyclohexyl ring); 36.84, 37.67, 51.84, 65.70, 210.1 (cyclopentanone ring); 56.57 (C-$\underline{C}H_2$-N). FABMS 508 (M+1).

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10. (a) *m*-CPBA oxidation of Sch 57726: To a solution of Sch 57726 (90 mg, 0.182 mmol) in dichloromethane was added $NaHCO_3$ (46 mg, 0.55 mmol) and *m*-CPBA (94 mg, 0.547 mmol) at 0 °C. After 2 h, the mixture was diluted with dichloromethane and washed with $NaHCO_3$. The organic layer was purified via flash chromatography using EtOH:dichloromethane (2:98) to elute compound **VIII** (32 mg, 43%) whose NMR spectrum was identical with that of the product prepared in part b.

    (b) Synthesis of compound **VIII**: A mixture of compound **IX**[5] (1.96 g, 4.73 mmol) and K_2CO_3 (2 g, 14.8 mmol) in NMP (15 mL) was heated to 70 °C for 15 min then cooled to room temperature, and a solution of the triflate **X** (1.8 g, 6.9 mmol, made from the corresponding alcohol by a standard procedure) in NMP (5 mL) added. The mixture was heated to 45 °C overnight, then poured into H_20, acidified with HCl to pH of 2 - 3 and partitioned with EtOAc:Hex 1:1. The organic layer was washed with 1 N NaOH, H_2O, and brine. Flash chromatography on SiO_2 using EtOAc:Hexanes 1:4 eluted compound **VIII** (1.85 g, 72%). A portion of this was crystallized from 90% EtOH to give creme-colored needles. Calcd for $C_{31}H_{35}N5O_4$ C 68.73, H 6.52, N 12.94; found C 68.85, N 6.67, N 12.83; FABMS 542 (M+H). ^1H NMR (δ, DMSO-d_6, 400 MHz) 1.16–1.5 (4H, m), 1.58–1.86 (10H, m), 2.1–2.2 (2H, m), 2.41–2.6 (3H, m), 3.21 (3H, s), 4.67 (2H, s), 5.68 (2H, s), 7.02 (2H, d, J = 8Hz), 7.28–7.37 (5H, m), 7.54 (2H, d, J = 8Hz); ^{13}CNMR (δ, DMSO-d_6) 22.6, 25.4, 26.1, 27.7, 33.5, 34.3, 40.2, 43.4, 48.4, 48.7, 98.4, 107.1, 125.5, 125.7, 127.2, 127.4, 128.6, 128.7, 136.9, 147.5, 150.1, 151.1, 151.2, 154.2.

11. The regio- and stereochemistry of the in vivo hydroxylation process could not be established as different diastereoisomers could not be resolved under the conditions of the HPLC-MS analysis.

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