Research (CNR) and Ministery of Public Education (MPI) of Italy.

Registry No. (±)-1, 115114-12-0; (±)-1 (Y = Cl), 115114-14-2; (+)-1, 115114-19-7; (-)-1, 115114-18-6; (±)-2, 115114-16-4; (+)-2, 115114-20-0; (-)-2, 115114-21-1; (±)-3, 115114-13-1; (±)-3 (Y = Cl), 115114-15-3; (+)-3, 101990-97-0; (-)-3, 115114-22-2; (±)-4, 115114-17-5; (+)-4, 101990-80-1; (-)-4, 115114-24-4; (-)-5, 115181-63-0; (+)-5, 115181-64-1; (-)-6, 115181-65-2; (+)-6, 115114-23-3; $C_6H_5COC_6H_{11}$, 712-50-5; (±)-$HSCH_2CH(OH)CH_2Cl$, 115046-74-7; L-(-)-*O,O'*-di-*p*-toluoyltartaric acid, 32634-66-5; D-(+)-*O,O'*-di-*p*-toluoyltartaric acid, 32634-68-7.

Supplementary Material Available: Tables of X-ray parameters for (+)-6 and (+)-4 (7 pages). Ordering information is given on any current masthead page.

Substituted Benzamides with Conformationally Restricted Side Chains. 2. Indolizidine Derivatives as Central Dopamine Receptor Antagonists

Frank D. King,* Michael S. Hadley, and Christine M. McClelland

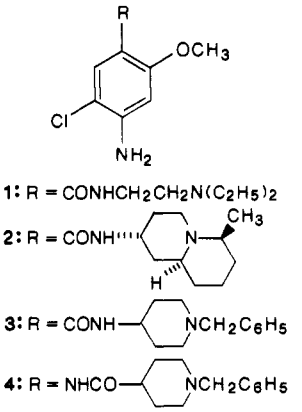
Beecham Pharmaceuticals Research Division, Medicinal Research Centre, The Pinnacles, Harlow, Essex, CM19 5AD England.
Received January 26, 1988

The substituted benzamides metoclopramide (1) and clebopride (3) are stimulants of gastric motility. They are also central dopamine receptor antagonists with 3 being the more potent. This is presumed to be due to an additional interaction of its *N*-benzyl group with the receptor. The effect of restricting the conformation of this group by replacing the *N*-benzylpiperidine side chain of 3 by phenyl-substituted quinolizidines and indolizidines has been investigated. Only the indolizidines had significant activity, the nature of which depended upon the orientation of the phenyl substituent. The 2α-phenyl isomers **5d–h** were potent central dopamine D_2 receptor antagonists with **5h** showing selectivity for the limbic system. The 2β-phenyl isomer **5c** was a gastric motility stimulant devoid of significant central dopamine receptor antagonist activity. Implications on receptor models are discussed.

Metoclopramide (1) is a substituted benzamide that is used clinically as a stimulant of upper gastrointestinal motility and as an antiemetic.[1] Its effects on the gastrointestinal tract are believed to be due to a combination of peripheral dopamine receptor antagonism and a potentiation of the cholinergic effects on gut muscle, probably mediated via 5-hydroxytryptamine.[2,3] Metoclopramide has also been shown to have antipsychotic activity at high doses. This activity is thought to be a consequence of its low potency as a central dopamine receptor antagonist.[4] The first paper in this series described how conformational restriction of the (diethylamino)ethyl side chain of 1 gave a compound **2**, which was a selective stimulant of upper gastrointestinal motility but devoid of significant dopamine receptor antagonist activity.[5]

The structurally related clebopride (3) has been marketed for the treatment of gut disorders of a psychosomatic origin.[6] It is thought to have similar dual activity to 1 but is a more potent central dopamine receptor antagonist.[7] This greater central potency, both in vivo and in vitro, is probably due to an additional binding interaction of the *N*-benzyl group with central dopamine receptors.[6]

In an earlier study, reversal of the amide linkage of 3 produced a compound, 4, which was found to be a potent central dopamine antagonist but devoid of significant upper gastrointestinal motility activity.[8] The aim of the present work was to investigate the effectiveness of the secondary binding interaction of the *N*-benzyl group by



conformationally restricting the side chain of 3 in the form of β-aryl-substituted quinolizidines and indolizidines, compounds **5a-i** (Table I). Inspection of Dreiding models indicated that β-substitution would probably incorporate the more likely binding conformations of the *N*-benzyl group of 3, with only a small increase in the N to aryl distance. The structure–activity relationships of **5a–i** will be discussed, and the results will be interpreted with reference to recent theories on the structural requirements for central dopamine antagonists.[9]

Chemistry

The synthesis of **5a–d** has been described elsewhere.[10] The indolizidines **5e–i** were prepared by an analogous procedure (Scheme I).

Reaction of the respective aminoindolizidines **10e–i** with 4-(acetylamino)-5-chloro-2-methoxybenzoyl chloride followed by selective base hydrolysis of the 4-acetylamino group gave the indolizidines **5e–i**. The equatorial amines **10e–i** were prepared stereospecifically from the ketones **9e–i** by sodium/pentanol reduction of their oxime derivatives. The ketones **9e–i** themselves were prepared by acid-catalyzed cyclization of the Michael adducts formed

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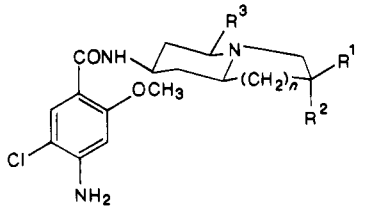
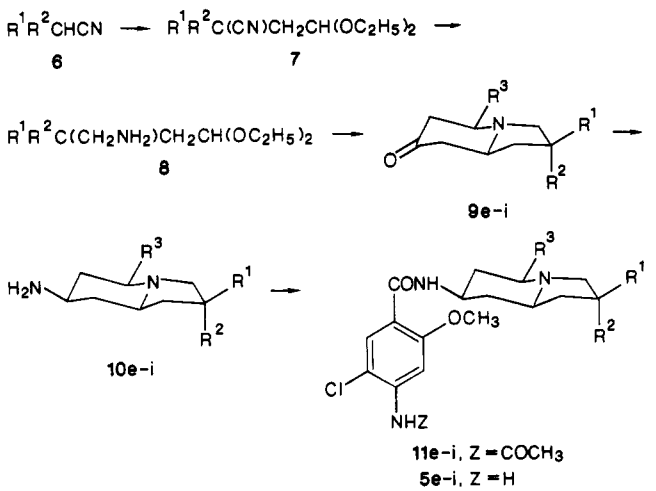
Table I. Structure of **5a–i** and Pharmacological Data



compd no.	R^1	R^2	R^3	n	antagonism of apomorphine-induced climbing[a]	stimulation of intragastric pressure[b]
1		metoclopramide			0.75 (0.62–0.90)	1.0 (0.3–2.7)
3		clebopride			0.037 (0.014–0.09)	1
5a	H	Ph	H	2	>1.0	Ia
5b	Ph	H	H	2	>1.0	Ia
5c	H	Ph	H	1	>3.0	1
5d	Ph	H	H	1	0.024 (0.009–0.066)	Ia
5e	*p*-FPh	H	H	1	0.015 (0.0094–0.024)	NT
5f	*o*-FPh	H	H	1	0.030 (0.014–0.064)	NT
5g	Ph	H	Me	1	0.40 (0.34–0.47)	Ia
5h	Ph	Me	H	1	0.078 (0.028–0.22)	Ia
5i	Ph	Ph	H	1	>1.0	Ia

[a] ED_{50}, mg/kg sc (95% confidence limits). [b] Lowest active dose, mg/kg sc; Ia = inactive (0.5–10 mg/kg sc), NT = not tested.

Scheme I



between the amines **8** and the appropriate α,β-unsaturated ketone. When a mixture of diastereoisomers was formed, separation was achieved by column chromatography, and in each case the isomer in which R_1 is aryl was the least polar.

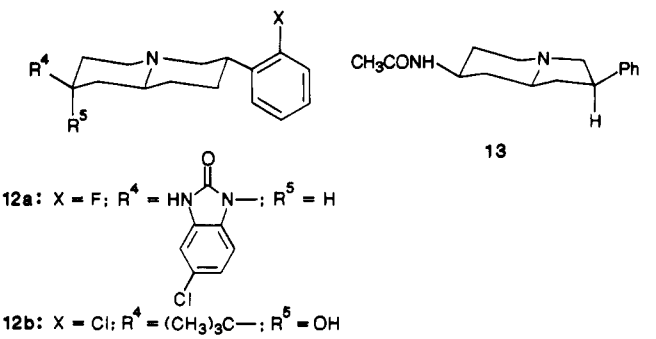
It was of interest to note that whereas the least polar isomer was normally formed as the minor product, for **9h** this isomer was the major product (isomer ratio 5:1). This reversal in stereoselectivity can be rationalized in terms of steric interactions in the transition state of the cyclization.[10]

For the majority of compounds, stereochemical assignments were made by NMR spectroscopic comparison with **5c** and **5d**. The stereochemistry of **5h** was unambiguously assigned as 2β-methyl-2α-phenyl from an X-ray crystallographic study of the *N*-acetyl derivative **11h**. This study also confirmed the presence of a hydrogen bond between the 2-methoxy group and the amidic NH as has previously been reported for related benzamides.[9] As expected from earlier conformational studies,[11] the indolizidine was trans fused with the 5-membered ring puckered such that the phenyl group occupied a pseudoequatorial position. The dihedral angle between the C_1C_2 of the indolizidine ring and the plane of the phenyl substituent was 72°.

Results

Central dopamine receptor antagonist activity was assessed by the compound's ability to antagonize apomorphine-induced climbing behavior in mice.[12] Gastric prokinetic activity was determined by their ability to increase basal intragastric pressure (IGP) in rats (Table I).[13]

Metoclopramide (**1**) and clebopride (**3**) had similar gastric prokinetic activity, but clebopride was 20-fold more potent as a central dopamine receptor antagonist. Cyclization of the *N*-benzyl group of **3** in the form of the quinolizidines **5a** and **5b** resulted in a marked reduction in potency in both test systems. The lack of central dopamine receptor antagonist potency of the equatorial isomer **5a** was somewhat surprising considering the reported activity of the related 2(3*H*)-benzimidazolone **12a**.[13]



Cyclization in the form of the indolizidines **5c** and **5d**, however, proved to be more fruitful. The 2β-phenyl compound **5c** retained the gastric prokinetic activity of **3**, but was considerably less potent as a central dopamine receptor antagonist. In contrast, the 2α-phenyl compound **5d** retained the central dopamine antagonist activity of **3** but was inactive as a gastric motility stimulant within the dose range tested. Thus, a separation of the individual

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components of the dual activity of **3** has been achieved, dependent upon the orientation of the 2-phenyl substituent.

It has been shown that the potency of both *N*-benzyl-substituted benzamides and anilides as central dopamine receptor antagonists is not significantly improved by the introduction of substituents in the *N*-benzyl aromatic ring.[8,15] Only a limited substitution program was therefore undertaken. The introduction of a para (**5e**) or ortho (**5f**) fluoro substituent did not markedly alter dopamine receptor antagonist potency. This result contrasts with that reported for a series of arylquinolizidines (e.g. **12b**) lacking a benzamide grouping in which central dopamine receptor antagonist potency is highly dependent upon the nature of the substituents in the aryl ring.[16] It thus appears likely that, for each series, the aryl substituent interacts with a different region of the central dopamine receptor.

In a related series of pyrrolidinyl benzamides it has been reported that the introduction of a methyl group α to the nitrogen atom in the side chain resulted in a slight increase in central dopamine receptor antagonist potency, presumably through conformational effects.[15] In the present series, however, α-substitution as in **5g** resulted in a marked reduction in potency. Introduction of a methyl substituent in the 2-position as in **5h** resulted in a 3-fold reduction in potency compared with that of **5d**. The reduction was much greater with the 2,2-diphenyl compound **5i**, which was inactive up to a dose of 1 mg/kg sc.

Because of the selective dopamine receptor antagonist activity found for the 2α-phenylindolizidines, a representative example was evaluated further. Although not the most potent, the 2β-methyl-2α-phenyl compound **5h** was selected as it was the most accessible synthetically.

Clinical antipsychotic potency of dopamine antagonists is highly correlated with affinity for the dopamine D_2 receptor subtype, which, unlike the D_1 receptor subtype, is not linked to the stimulation of adenylate cyclase.[17] Compound **5h** showed moderate affinity ($K_i = 2.5 \times 10^{-7}$ M) for D_2 receptors but no measurable affinity ($K_i > 10^{-5}$ M) for D_1 receptors, confirmed by the lack of an inhibitory effect, up to 10^{-3} M, on dopamine-stimulated adenylate cyclase.

Compound **5h** potently, ED_{50} = 0.02 (0.0017–0.23, 95% CL) mg/kg ip, inhibited amphetamine-induced locomotor activity in rats 4 h after dosing. This behavioral effect, like apomorphine-induced climbing in mice, is thought primarily to involve dopaminergic mechanisms in the limbic system.[18,19] Antagonism of dopaminergic transmission in the limbic system is considered to be associated with antipsychotic activity.[20] Conversely, antagonism of dopaminergic transmission in the striatum is considered to lead to catalepsy in rats and extrapyramidal effects in man.[21] In inducing catalepsy, **5h** was much less potent, ED_{50} = 3.4 (1.4–8.6, 95% CL) mg/kg ip, 4 h after dosing than in antagonizing amphetamine-induced locomotor activity. Concentrations of the dopamine metabolite, homovanillic acid (HVA), in limbic and striatal areas in rats were also indicative of limbic selectivity, ED_{200} values (see the Experimental Section) 1 h after dosing being 0.024 (0.012–0.043, 95% CL) and 0.083 (0.049–0.14, 95% CL) mg/kg sc, respectively.

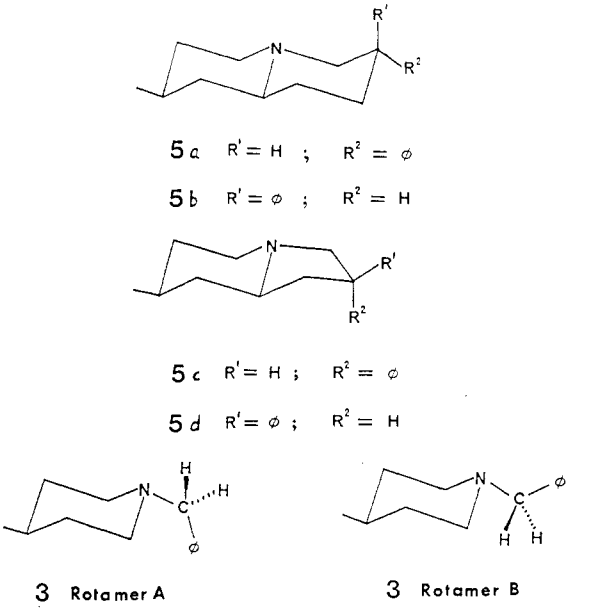

Figure 1. Side-chain conformations of **5a–d** and **3**.

Discussion

The effect of restricting the conformation of the *N*-benzyl group in the form of the quinolizidines **5a,b** and the indolizidines **5c,d** is to fix the relative position of the bridgehead nitrogen atom, the presumed interactor with the primary binding site of the receptor, and the phenyl group, as represented in Figure 1. The phenyl group effectively occupies a graded position in space going from **5b** → **5d** → **5a** → **5c**. Of these four compounds, only **5c** retained the gastric prokinetic potency of clebopride (**3**). As both metoclopramide (**1**) and clebopride (**3**) have similar potencies as gastric prokinetic agents, it is unlikely that the *N*-benzyl group has any significant binding interaction with the receptor involved. It therefore appears that **5a**, **5b**, and **5d** are much less potent as gastric prokinetic agents than **3** because of steric interference by the phenyl substituent. However, when the phenyl group is well below the plane of the azabicyclic ring as in **5c**, there is no steric interference and this activity is retained. The configuration of **5c** may therefore correspond to the active conformation of **3** at the gastric prokinetic receptor. This is equivalent to rotamer A in the figure.

In contrast, only **5d** retained the central dopamine receptor potency of clebopride (**3**). As evidence suggests that the *N*-benzyl substituent interacts with an accessory binding site on the central dopamine receptor,[9] the configuration of **5d** must correspond closely to the active conformation of **3** at this receptor, equivalent to rotamer B in the figure.

From the X-ray data on **11h**, the distance between the bridgehead nitrogen atom and the center of the phenyl ring was found to be 4.4 Å. On the basis of the X-ray data[10] for **5c**, a model compound **13** incorporating the side chain of **5d** was generated for interatomic distance calculations, by using the molecular mechanics program MM2, by phenyl transposition followed by full geometry optimization.[22] Compound **13** was calculated to have an N to center-phenyl distance of 4.6 Å, indicating that inroduction of the methyl substituent in **11h** has not significantly altered the conformation of the 5-membered ring. These N

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to center-phenyl distances are both significantly larger than the corresponding distance of 3.7 Å in **3**[9] but very close to the 4.5 Å proposed by Humber for the distance between the lipophilic site for the *tert*-butyl group and the N atom in butaclamol (**14**).[23] Thus, it appears that, in a series of phenyl-substituted benzamides, the N to center-phenyl distance can range from 3.7 to 4.6 Å without a great loss in potency.

Experimental Section

Chemistry. Melting points and boiling points are uncorrected. The elemental analyses indicated are within 0.4% of the theoretical values. ^{1}H NMR spectra were recorded on a JEOL GX 270 or Varian CFT-20 spectrometer with Me_4Si as internal standard and mass spectra were recorded on an AEI MS9 (70 eV) spectrometer. All evaporations of solvents were carried out under reduced pressure, and organic solvents were dried over K_2CO_3 unless specified otherwise. For column chromatography the silica gel used was Merck Kieselgel 60. Light petroleum refers to the fraction boiling between 60 and 80 °C. The benzamides **5a–d** were prepared by literature procedures.[10] All compounds containing chiral centers were prepared as mixtures of diastereoisomers.

General Procedure for the Preparation of Amino Acetals 8. Potassium *tert*-butoxide (12.3 g, 0.11 mol) was added to a stirred solution of the appropriate benzyl cyanide (**6**, 0.1 mol) and 1-bromo-2,2-diethoxyethane (26.7 g, 0.1 mol) in dry Et_2O (200 mL) under nitrogen. The stirring was continued at room temperature for 48 h when H_2O (10 mL) was carefully added with cooling, and the organic layer was separated, dried ($MgSO_4$), and concentrated. Distillation of the residue afforded the appropriate cyanoacetals **7**. A solution of the appropriate **7** (0.1 mol) in dry THF (25 mL) was added, dropwise, to a stirred suspension of AlH_3 (0.08 mol, prepared from 0.08 mol of $LiAlH_4$ and 0.075 mol of concentrated H_2SO_4 at 0 °C under nitrogen). The mixture was stirred at room temperature for 5 h, cooled to 0 °C, and treated with 1 M NaOH (30 mL). The precipitate was removed by filtration, and the filtrate was concentrated and distilled to give the amino acetals **8** except **8i**, which was used without further purification. **8e**: bp 108–110 °C (0.1 mm); ^{1}H NMR ($CDCl_3$) δ 7.40–6.40 (m, 4), 4.16 (dd, 1), 3.70–2.35 (m, 7), 2.20–1.60 (m, 4), 1.12–1.10 (2 t, 6). **8f**: bp 125–128 °C (0.5 mm); ^{1}H NMR ($CDCl_3$) δ 7.50–6.70 (m, 3), 4.16 (dd, 1), 3.80–2.70 (m, 7), 2.3–1.7 (m, 2), 1.12, 1.08 (2 t, 6), 1.00 (2 H, s). **8h**: bp 118–120 °C (0.1 mm); ^{1}H NMR ($CDCl_3$) δ 7.16 (s, 5), 4.18 (t, 1), 3.60–3.00 (m, 4), 2.79 (d, 2), 1.95 (d, 2), 1.33 (s, 3), 1.13, 1.06 (2 t, 6), 0.93 (s, 2). **8i**: ^{1}H NMR ($CDCl_3$) δ 7.10 (s, 10), 4.17 (dd, 1), 3.70–3.10 (m, 4), 2.80 (d, 2), 1.90 (d, 2), 1.13, 1.06 (2 t, 6), 0.90 (s, 2).

General Procedure for the Preparation of Amines 10e–i. A solution of the appropriate amino acetal **8** (0.02 mol) and the but-3-en-2-one (0.024 mol) in Et_2O (50 mL) was stirred at room temperature until the addition was complete by TLC (about 1 h). The addition product was extracted into 2.5 M HCl (50 mL), and the acid extract was heated on a steam bath for 2 h. The reaction mixture was cooled and basified with K_2CO_3, and the product was extracted into Et_2O (2 × 100 mL). The organic extracts were dried, concentrated, and purified by column chromatography on silica, eluting with Et_2O. Where applicable, the less polar isomer corresponded to the desired 2α-aryl isomer **9e–i**. A solution of the ketone **9e–i** (0.01 mol), hydroxylamine hydrochloride (1.4 g, 0.014 mol), and pyridine (1.5 mL) in EtOH (50 mL) was heated under reflux for 1 h. The solvent was evaporated, and the residue was treated with EtOAc (100 mL) and 2.5 M NaOH (10 mL). The organic layer was separated, dried, and evaporated to give the crude oxime (0.01 mol). An efficiently stirred solution of the crude oxime (0.01 mol) in pentanol (50 mL) was heated to reflux. The heat source was removed, and sodium (2.75 g, 0.12 mol) was added at such a rate as to maintain vigorous reflux. After all the sodium had dissolved, the reaction was cooled to ca. 50 °C and treated with 5 M HCl (70 mL). After further cooling to room temperature, the reaction mixture was washed with EtOAc (2 × 50 mL), the aqueous phase was basified with K_2CO_3 and the product was extracted into CH_2Cl_2 (3 × 100 mL). The organic extracts were dried and concentrated to give the amines **10e–i**, which were converted directly to the benzamides **5e–i** without further purification.

General Procedure for the Preparation of Benzamides 5e–i. A solution of 4-(acetylamino)-5-chloro-2-methoxybenzoyl chloride (2.6 g, 0.01 mol), Et_3N (4 mol), and the appropriate amine **10e–i** (0.01 mol) in dry CH_2Cl_2 (100 mL) was stirred at room temperature for 2 h. The reaction mixture was washed with saturated $NaHCO_3$ solution (50 mL), and the organic layer was separated and dried. The solvent was evaporated, and the residue was heated to reflux with 2.5 M NaOH (8 mL) in EtOH (50 mL) for 2 h. The reaction mixture was concentrated, the residue was diluted with water (50 mL), and the product was extracted into EtOAc (2 × 100 mL). Concentration of the extracts afforded an oil, which was purified by column chromatography on silica, eluting with EtOAc. Recrystallization from EtOAc/light petroleum afforded the free base. Compounds **5e**, **5g**, and **5h** were converted to the monohydrochloride salt by addition of 1 molar equiv of ethanolic HCl to an ethanol solution of the compound followed by precipitation with Et_2O.

(2α,7α,8aβ)-4-Amino-5-chloro-2-methoxy-*N*-[octahydro-2-(4-fluorophenyl)indolizin-7-yl]benzamide monohydrochloride (5e): mp 242–245 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.4 (br s, 1), 7.90–7.50 (m, 3 including 7.65, s, 1), 6.5 (s, 1), 3.84 (s, 3); MS, *m/e* 417.1620 (M^+). Anal. ($C_{22}H_{26}Cl_2FN_3O_2$) C, H, N, Cl.

(2α,7α,8aβ)-4-Amino-5-chloro-2-methoxy-*N*-[octahydro-2-(2-fluorophenyl)indolizin-7-yl]benzamide (5f): mp 98–99 °C; ^{1}H NMR ($CDCl_3$) δ 8.12 (s, 1), 7.75–6.80 (m, 4), 6.30 (s, 1), 3.88 (s, 3); MS, *m/e* 417.1618 (M^+). Anal. ($C_{22}H_{25}ClFN_3O_2$) C, H, N, Cl.

(2α,5α,7α,8aβ)-4-Amino-5-chloro-2-methoxy-*N*-(octahydro-5-methyl-2-phenylindolizin-7-yl)benzamide monohydrochloride (5g): mp 169–171 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.5 (br s, 1), 7.90–7.20 (m, 7 including 7.65, s, 1), 6.52 (s, 1), 3.84 (s, 3), 1.34 (d, 3); MS, *m/e* 413.1863 (M^+). Anal. ($C_{23}H_{28}ClN_3O_2 \cdot HCl \cdot H_2O$) C, H, N, Cl (for monohydrate).

(2β,2α,7α,8aβ)-4-Amino-5-chloro-2-methoxy-*N*-(octahydro-2-methyl-2-phenylindolizin-7-yl)benzamide monohydrochloride (5h): mp 238–239 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.80 (d, 1), 7.68 (s, 1), 7.60–7.20 (m, 5), 6.55 (s, 1), 3.84 (s, 3), 1.52 (s, 3); MS, *m/e* 413.1865 (M^+). Anal. ($C_{23}H_{28}ClN_3O_2 \cdot HCl \cdot 0.5H_2O$) C, H, N, Cl (for hemihydrate).

(7α,8aβ)-4-Amino-5-chloro-2-methoxy-*N*-(octahydro-2,2-diphenylindolizin-7-yl)benzamide (5i): mp 112–115 °C; ^{1}H NMR ($CDCl_3$) δ 8.09 (s, 1), 7.57 (d, 1), 7.22 (br s, 10), 6.26 (s, 1), 3.84 (s, 3); MS, *m/e* 475.2035 (M^+). Anal. ($C_{28}H_{30}ClN_3O_2$) C, H, N, Cl.

Pharmacology. Activity on gastric motility was determined in male Wistar rats (200–500 g) in which a chronic gastric fistula had previously been inserted.[24] The rats were fasted overnight and then individually restrained in Bollman cages for the duration of the experiment. Gastric motility was assessed from the mean amplitude of pressure waves (mean motility index of Bech et al.[25]) recorded via the gastric fistula for four 10-min periods before and after subcutaneous administration of compound. Only rats with a low pretreatment basal motility (mean amplitude <4 mmHg) were used. With groups of 8–10 animals, the lowest dose of a compound that showed a statistical increase ($p < 0.05$, Student's *t* test) in a greater number of rats than is encountered in a control (vehicle dosed) group was ascertained.

Antagonism of apomorphine-induced climbing behavior was assessed by a modification of the method of Protais et al.[24] Usually three groups of 10 male Tuck To mice (20–28 g) were treated (sc) with either a graded dose of test compound or vehicle 40 min prior to administration of a submaximal dose of apomorphine hydrochloride (1 mg/kg sc). The degree of antagonism was determined 10 and 20 min later and scored blind. For each experiment a dose (0.1 mg/kg sc) of clebopride (**3**) was also included as a positive control.

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Antagonism of amphetamine-induced locomotion was determined by dosing groups of five male rats (210–250 g) with either a graded dose of test compound or vehicle 3.75 h prior to the administration of amphetamine (3.7 mg/kg sc) and then assessing their degree of locomotion after a further 15 min.

Induction of catalepsy was determined by dosing groups of five male rats (200–250 g) with either a graded dose of test compound or vehicle, and then, at appropriate time intervals, placing each paw in turn on a 4.1 cm high rubber cork. A score of 1 was given for each paw which the rat retained on the bung for 15 s. Catalepsy was determined at 1, 2, and 4 h after dosing, and the 4 h figure is quoted.

ED_{50} values and 95% confidence limits for antagonism of apomorphine-induced climbing, amphetamine-induced locomotion, and induction of catalepsy were calculated by the method of Litchfield and Wilcoxon.[26]

Affinities for dopamine D_1 and D_2 receptors were determined in rat striatal tissue by measuring the displacement of *cis*-[^{3}H]-flupenthixol in the presence of spiperone and [^{3}H]spiperone, respectively. Increases in homovanillic acid (HVA) concentrations were determined with groups of five male rats (250–350 g) injected with either a graded dose of test compound or vehicle. After 1 h, the rats were killed swiftly by decapitation, and the corpus striatum and nucleus accumbens were removed and stored in individual sample tubes at −70 °C within 2 min. HVA content was assessed by the method of Westerink and Korf.[27] ED_{200} values (doses to elevate HVA to 200% of control value) and 95% confidence limits were calculated from regression and analysis of variance.

Acknowledgment. We thank F. Brown, D. H. Turner, B. McRitchie, and J. Hatcher for assistance with biological testing, G. Riley and D. Graves for the receptor binding assays, R. T. Martin for the computer calculations, D. J. Williams (Imperial College, London) for the X-ray analysis, and A. Dodson for preparing the manuscript.

Registry No. **5a**, 106659-14-7; **5b**, 106659-09-0; **5c**, 106757-54-4; **5d**, 106757-51-1; **5e**, 115114-02-8; **5e**·HCl, 115181-62-9; **5f**, 115114-03-9; **5g**, 115114-04-0; **5g**·HCl, 115114-10-8; **5h**, 115114-05-1; **5h**·HCl, 115114-11-9; **5i**, 115031-78-2; **6e**, 459-22-3; **6f**, 326-62-5; **6g**, 140-29-4; **6h**, 1823-91-2; **6i**, 86-29-3; **8e**, 115031-79-3; **8f**, 115031-80-6; **8g**, 106658-99-5; **8h**, 20946-90-1; **8i**, 28168-63-0; *trans*-**9e**, 115031-84-0; *cis*-**9e**, 115031-87-3; *trans*-**9f**, 115031-82-8; *cis*-**9f**, 115031-88-4; **9g**, 85358-19-6; *trans*-**9h**, 115031-83-9; *cis*-**9h**, 115031-86-2; **9i**, 115031-85-1; **10e**, 115114-06-2; **10f**, 115114-07-3; **10g**, 115114-08-4; **10h**, 115114-09-5; **10i**, 115031-81-7; **11h**, 115115-08-7; $CH_3CH{=}CHCOCH_3$, 625-33-2; 1-bromo-2,2-diethoxyethane, 2032-35-1; but-3-en-2-one, 78-94-4; 4-(acetylamino)-5-chloro-2-methoxybenzoyl chloride, 4516-32-9.

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Etodolac, a Novel Antiinflammatory Agent. The Syntheses and Biological Evaluation of Its Metabolites

Leslie G. Humber,* Eckhardt Ferdinandi, Christopher A. Demerson, Syed Ahmed, Uresh Shah, Dominick Mobilio, Joseph Sabatucci, Barbara De Lange, Francesco Labbadia, Philip Hughes, John DeVirgilio, Glenn Neuman, Thuy T. Chau, and Barry M. Weichman

Ayerst Laboratories Research, Inc., Princeton, New Jersey 08543-8000. *Received January 7, 1988*

The syntheses of five metabolites of the antiinflammatory drug etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole-1-acetic acid) are described, viz. 6-hydroxyetodolac, *N*-methyletodolac, 4-ureidoetodolac, 8-(1′-hydroxy)etodolac, and 4-oxoetodolac. These syntheses were used to confirm the identities of the metabolites. The metabolites themselves, as well as the previously reported metabolite 7-hydroxyetodolac, were tested in a rat adjuvant edema model and in vitro for their capacity to block prostaglandin production in chondrocyte cells. All either were inactive or possessed only marginal activity. The isolation of *N*-methyletodolac and 4-oxoetodolac from human and rat urine, respectively, is also described.

Etodolac,[1] 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic acid (**1**), is a clinically effective analgesic and antiinflammatory agent, which has been shown to possess an exceptional safety profile with respect to the gastrointestinal tract and renal function and to have the potential to retard the progression of skeletal changes in rheumatoid arthritis.[2] The metabolic disposition of etodolac has been studied in various species, including humans,[3-5] and several metabolites have been isolated. 6-Hydroxyetodolac (**2**), and 7-hydroxyetodolac (**3**), as their methyl esters, were identified in human urine by comparison with samples prepared via a low-yield microbial transformation, wherein the positions of the hydroxyl groups had been assigned on the basis of NMR spectral data.[4] 4-Ureidoetodolac (**4**) and 8-(1′-hydroxy)etodolac (**5**) were identified in human urine, and their structures were assigned on the basis of NMR and mass spectral data of derivatives,[4,6] while 4-oxoetodolac (**6**) was identified in rat urine (see below). *N*-Methyletodolac (**7**) was recently reported, without details, to be present in human urine,[7] and this finding is confirmed by the present study; its structure was tentatively assigned on the basis of NMR and mass spectral data.[7]

In order to confirm the identities of these metabolites by comparison with authentic samples, the syntheses of **2** and **4**–**7** have been carried out; a synthesis of metabolite **3** was recently reported.[8] We describe also the biological evaluation in vitro and in vivo of metabolites **2**–**7**, as well

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