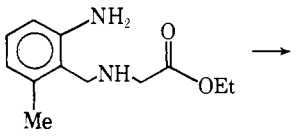
function, significant side effects are observed, thereby limiting their clinical use as antithrombotic agents.[7]

Dipyridamole, pyrimidopyrimidines, and thienopyrimidines also inhibit ADP-induced platelet aggregation[8] and have been widely studied. At concentrations which inhibit experimentally induced platelet thrombi *in vivo*, no significant effect of platelet function has been observed clinically. Clinical studies with increasing doses of the thienopyrimidines were discontinued because of serious side effects.[9]

We wish to report that 6-methyl-1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-one (BL-3459) exhibits potent activity against ADP-induced platelet aggregation *in vitro* in rabbit, dog, and human platelet rich plasma as well as in several *ex vivo* and *in vivo* models.

The synthesis was achieved by reaction of *N*-(2-amino-6-methylbenzyl)glycine ethyl ester (**1**) with cyanogen bromide presumably *via* ring closure of the 2-amino-3-(carbethoxymethyl)-3,4-dihydroquinazoline (**2**).



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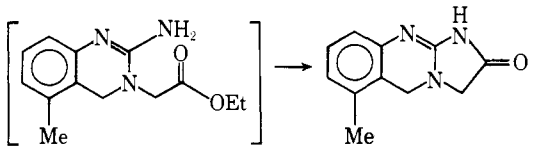

2 3

Reduction of 2-methyl-6-nitrobenzoic acid with diborane in tetrahydrofuran resulted in 2-methyl-6-nitrobenzyl alcohol which was subsequently heated with thionyl chloride in benzene. Isolation and crystallization from cyclohexane afforded 2-methyl-6-nitrobenzyl chloride: yield 75% (based on 2-methyl-6-nitrobenzoic acid); nmr ($CDCl_3$) $\tau$ 7.45 (s, $CH_3$), 5.20 (s, $CH_2$). *Anal.* ($C_8H_8ClNO_2$) C, Cl, H, N.

Condensation of the 2-methyl-6-nitrobenzyl chloride with glycine ethyl ester in the presence of triethylamine followed by catalytic hydrogenation employing 10% Pd on carbon as catalyst afforded *N*-(2-amino-6-methylbenzyl)-glycine ethyl ester: yield 85% (based on 2-methyl-6-nitrobenzyl chloride); the material was of sufficient purity to use as such; bp 128–131° (0.07 mm); ir (film) 1745 $cm^{-1}$ (C=O); nmr ($CDCl_3$) $\tau$ 7.70 (s, $CH_3$, benzyl $CH_2$), 6.20 (s, benzyl $CH_2$), 6.61 (s, glycine $CH_2$). *Anal.* ($C_{12}H_{18}N_2O_2$) C, H, N.

Equimolar quantities of cyanogen bromide and *N*-(2-amino-6-methylbenzyl)glycine ethyl ester were refluxed for 18 hr in ethyl alcohol and the solvent was removed *in vacuo*. Treatment of the resulting solid with aqueous base followed by crystallization from 1 *N* hydrochloric acid yielded 6-methyl-1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-one hydrochloride: yield 55%; mp >250° dec; ir (KBr) 1805, 1690, 1605, 1590 $cm^{-1}$; nmr (TFA) $\tau$ 7.70 (s, $CH_3$), 5.45 (s, 3-$CH_2$), 5.10 (s, 4-$CH_2$). *Anal.* ($C_{11}H_{11}N_3O \cdot HCl \cdot H_2O$) C, H, N (Fischer).

Marked activity was exhibited by compound **3** on platelet function (Table I) *in vitro* and *ex vivo* in rabbits (ip) and dogs (po) with no significant increases in bleeding times at doses exceeding the $ED_{50}$ values. Oral activity was established in several modified *in vivo* models[10] including the biolaser induced thrombosis in the rabbit ear chamber[11] (ED = 10 mg/kg), endotoxin shock in anesthetized beagle dogs[12] (ED = 10 mg/kg), hemorrhagic shock in anesthetized beagle dogs[13] (ED = 1 mg/kg), and electrically induced carotid artery thrombosis in the dog[14] (ED = 0.5 mg/kg).

These results show that 6-methyl-1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-one significantly affects platelet function and may be of value in the treatment of platelet disorders.

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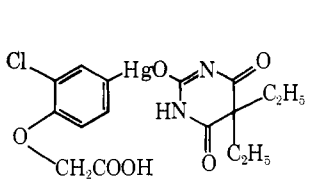
*Received August 8, 1974*

## (1-Oxo-2-substituted-5-indanyloxy)acetic Acids, a New Class of Potent Renal Agents Possessing Both Uricosuric and Saluretic Activity. A Reexamination of the Role of Sulfhydryl Binding in the Mode of Action of Acylphenoxyacetic Acid Saluretics

*Sir:*

Because of their many desirable pharmacodynamic attributes, including potent saluresis, proper urinary $Na^+/Cl^-$ balance, and uricosuric activity, the mercurial diuretics, particularly the phenoxyacetic acids, *e.g.*, merbaphen (**1**)[1] and mersalyl,[2,3] served as models which led to the discovery of the family of (acryloylphenoxy)acetic acids,[4] typified by ethacrynic acid (**2a**). These mercurials and ethacrynic acid exhibit biological similarities in that they induce potent saluresis in dogs[5] and in man[6] but not in rats;[7] however, they differ in that while the mercurials are



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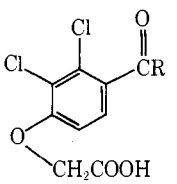


**2a**, R = $-C(=CH_2)C_2H_5$
**b**, R = $-CH(CH_3)C_2H_5$
**c**, R = $-C(=CHCH_3)C_2H_5$

**Table I.** Oral Activity in Rats[a]

| Compd | Enantiomer | No. of cages | Dose, mg/kg | Milliequivalents × 100/cage Na+ | K+ | Cl- |
|---|---|---|---|---|---|---|
| 5a | ± | 3 | 9 | 46 | 14 | 55 |
| | | 3 | 27 | 124 | 46 | 176 |
| | | 3 | 81 | 237 | 75 | 309 |
| 5b | ± | 6 | 9 | 40 | 21 | 44 |
| | | 6 | 27 | 67 | 33 | 80 |
| | | 6 | 81 | 134 | 53 | 189 |
| 5b | + | 3 | 81 | 181 | 53 | 265 |
| 5b | − | 3 | 81 | 90 | 44 | 161 |
| 5c | ± | 9 | 1 | 86 | 31 | 106 |
| | | 12 | 3 | 97 | 51 | 163 |
| | | 12 | 9 | 125 | 49 | 190 |
| | | 12 | 27 | 212 | 72 | 311 |
| | | 12 | 81 | 261 | 78 | 370 |
| 5c | + | 3 | 3 | 123 | 23 | 119 |
| | | 3 | 9 | 128 | 75 | 203 |
| | | 3 | 27 | 195 | 80 | 279 |
| | | 3 | 81 | 260 | 76 | 362 |
| 5c | − | 3 | 3 | 148 | 90 | 248 |
| | | 3 | 9 | 200 | 83 | 296 |
| | | 3 | 27 | 237 | 79 | 338 |
| | | 3 | 81 | 294 | 102 | 428 |
| 5d | ± | 12 | 3 | 65 | 18 | 70 |
| | | 12 | 9 | 92 | 27 | 110 |
| | | 12 | 27 | 167 | 47 | 218 |
| | | 12 | 81 | 223 | 75 | 316 |
| 5d | + | 3 | 3 | 67 | 23 | 78 |
| | | 3 | 9 | 95 | 33 | 124 |
| | | 3 | 27 | 136 | 48 | 184 |
| | | 3 | 81 | 258 | 87 | 370 |
| 5d | − | 3 | 3 | 64 | 27 | 79 |
| | | 3 | 9 | 52 | 26 | 75 |
| | | 3 | 27 | 82 | 36 | 123 |
| | | 3 | 81 | 143 | 60 | 220 |
| Furosemide | | 6 | 9 | 7 | 14 | 22 |
| | | 6 | 27 | 125 | 55 | 213 |
| | | 6 | 81 | 244 | 77 | 390 |
| Hydrochlorothiazide | | 6 | 3 | 123 | 34 | 154 |
| | | 6 | 9 | 112 | 38 | 137 |
| | | 6 | 27 | 131 | 34 | 156 |
| | | 6 | 81 | 128 | 37 | 143 |
| Placebo (vehicle) | | 9 | | 8 | 22 | 8 |

[a] Female rats (Charles River, 150–170 g) were maintained overnight on a sugar diet with water *ad libitum*. The test substance was dissolved in pure DMF and subsequently diluted with water (which contained 3 drops of Tween-80 per 100 ml) such that the final vehicle was 4% DMF. At the time of the test, animals were given the vehicle (as placebo) or test substance suspended in a final volume of 5.0 ml po. Rats were housed in groups of three in metabolism cages. Urine was collected for the 0–5-hr interval in graduated cylinders and was analyzed for sodium, potassium, and chloride content. Animals that received placebo were run concurrently. Results are reported as milliequivalents × 100 per cage and are the geometric means of the indicated number of cages per dose level. Standard methodology was used for determination of electrolyte levels.

**Table II.** Oral Activity in Dogs[a]

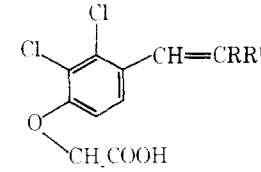
| Compd | Enantiomer | No. of Animals | Dose, mg/kg | Milliequivalents per 6 hr Na+ | K+ | Cl- |
|---|---|---|---|---|---|---|
| 5a | ± | 8 | 2.5 | 11 | 4 | 16 |
| | | 8 | 5 | 21 | 7 | 29 |
| 5b | ± | 9 | 5 | 10 | 3 | 13 |
| | | 6 | 10 | 13 | 3 | 17 |
| | | 6 | 20 | 13 | 4 | 15 |
| 5c | ± | 4 | 5 | 25 | 5 | 36 |
| 5d | ± | 3 | 2.5 | 9 | 2 | 11 |
| | | 3 | 5 | 24 | 5 | 29 |
| | | 3 | 10 | 26 | 5 | 35 |
| Hydrochlorothiazide | | 24 | 1 | 16 | 5 | 22 |
| | | 25 | 5 | 22 | 12 | 30 |
| | | 23 | 10 | 27 | 13 | 32 |
| Furosemide | | 24 | 5 | 28 | 7 | 37 |
| | | 24 | 25 | 35 | 8 | 43 |
| 2a | | 10 | 1 | 21 | 5 | 26 |
| | | 8 | 3 | 37 | 7 | 42 |
| 2b | | 4 | 20 | 9 | 2 | 12 |
| Placebo (vehicle) | | 24 | | 1 | 1 | 1 |

[a] Oral tests were carried out on a colony of trained female mongrel dogs weighing 8–10 kg. All dogs received 100 ml of water the previous day and were fasted overnight. On the day of the test, 250 ml of water was administered orally, followed by 500 ml of water (orally) 1 hr later. One hour after the last oral priming dose of water, bladders were emptied by catheterization and the study was commenced by administration of compound or placebo. Compounds were given in gelatin capsules and the animals were maintained in metabolism cages for collection of spontaneously voided urine. Spontaneous urine was combined with bladder urine collected by catheterization at the end of 6 hr. Urine volumes were measured, and aliquots were analyzed for sodium, potassium, and chloride content by standard methodology. Values are reported as geometric means.

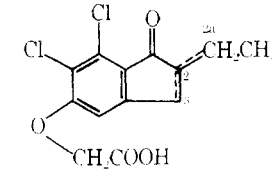
uricosuric,[2,3,8] **2a** causes uric acid retention,[9,10] an undesirable attribute of all nonmercurial diuretics.

The mercurial diuretics and the (acryloylphenoxy)acetic acids also exhibit marked similarities in their *in vitro*[4,11] and *in vivo*[12] reaction with sulfhydryl groups. The concept that mercurials and **2a** have the same or a similar mode of action at the cellular level[13] is supported by the fact that **2a** competes with mercurials for the same receptors.[14] Furthermore, the concentration of protein-bound sulfhydryl groups in renal cells is minimal when **2a** diuresis is maximal.[15]

We have observed similar sulfhydryl binding activity and biological properties for the styrene[16] (*e.g.*, **3a–c**), indene[17,18] (*e.g.*, **4a**), and α-alkylideneindan[19] (*e.g.*, **4b**) congeners as those reported for **2a**.




**3a.** R = $COCH_3$; $R^1$ = $COCH_3$
**b.** R = $COCH_3$; $R^1$ = $CH_3$
**c.** R = $NO_2$; $R^1$ = $CH_3$




**4a**, 2,3 double bond
**b**, 2,2a double bond

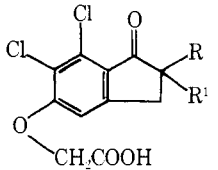
It was recognized, however, that *in vitro* reaction[11] with sulfhydryl-containing compounds (whether considered in terms of reaction rates or equilibrium constants) did not correlate well with *in vivo* diuretic data in any of the phenoxyacetic acid series (**2a**, **3**, and **4**). Furthermore, we have observed that when the double bond in **2a** is reduced to give **2b**, which precludes a 1,4-addition reaction with sulfhydryl groups, the saluretic response is markedly reduced but significant saluretic activity still remains. We

**Table III.** Oral Data Obtained in Chimpanzees (Δ Drug-Control)[a,b]

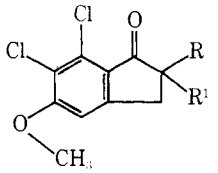
| Compd No. | Isomer | Dose, mg/kg | Δ urine vol, ml/min | Δ $C_{urate}/C_{inulin}$ | Δ μ equiv/min $Na^+$ | Δ μ equiv/min $K^+$ | Δ μ equiv/min $Cl^-$ |
|---|---|---|---|---|---|---|---|
| 5a | ± | 5 | 6.2 | 0.18 | 649 | 146 | 748 |
| 5b | ± | 5 | 1.6 | 0.18 | 123 | 51 | 326 |
| | | 10 | 4.6 | 0.26 | 568 | 131 | 715 |
| 5b | + | 2.5 | 0.3 | 0.31 | 224 | 46 | 283 |
| | | 5 | 3.1 | 0.26 | 300 | 102 | 370 |
| 5b | − | 5 | 2.9 | 0.10 | 292 | 74 | 365 |
| 5c | ± | 5 | 4.5 | 0.02 | 506 | 84 | 612 |
| 5c | + | 0.5 | −2.1 | 0.04 | 214 | 48 | 264 |
| | | 5 | 10.9 | 0.41 | 1593 | 156 | 1906 |
| 5c | − | 5 | 8.3 | 0.06 | 992 | 145 | 1271 |
| 5d | ± | 0.25 | 1.4 | 0.01 | 225 | 21 | 331 |
| | | 5 | 2.5 | 0.20 | 425 | 64 | 367 |
| | | 10 | 4.6 | 0.36 | 735 | 133 | 985 |
| 5d | + | 5 | 0.3 | 0.10 | 325 | 110 | 477 |
| 5d | − | 5 | 1.4 | 0.17 | 268 | 44 | 308 |
| 2b | | 10 | −1.6 | 0.15 | 236 | 35 | 337 |
| Furosemide | | 5 | 8.8 | −0.02 | 1035 | 55 | 1073 |
| Hydrochlorothiazide | | 5 | 1.0 | −0.02 | 144 | 73 | 198 |
| Probenecid | | 5 | 0.1 | 0.05 | | | |
| | | 10 | | 0.29 | | | |

[a] Fasted, male chimpanzees weighing 21–77 kg were immobilized with phencyclidine (which was shown not to affect the results) (1.0–1.5 mg/kg im plus 0.25 mg/kg iv as needed) and were prepared by catheterization for standard renal clearance studies using routine clinical asceptic procedures. Pyrogen-free inulin (iv) was used to measure glomerular filtration rate (GFR). Clearance of inulin, urate, and the excretion rates of $Na^+$, $K^+$, and $Cl^-$ was determined by standard Auto Analyzer techniques. (Inulin and urate in chimpanzee plasma are freely filterable.) Average control clearances were calculated from three 20-min consecutive periods. Drug-response values were derived as the average of eight 15–20 min clearance periods after oral administration of an aqueous solution of the compound through an indwelling nasal catheter. All data are reported as the difference between (average) treatment and control values obtained from single experiments. [b] Mersalyl, since it is inactive po, was given iv (1 mg/kg calculated as Hg) and produced a Δ urine volume of 13.5, a Δ $C_{urate}/C_{inulin}$ of 0.70, and a Δμ equiv/min of $Na^+$, $K^+$, $Cl^-$ of 1781, 81, and 1799. For comparative purposes, it should be noted that a given dose of **5a–d** produced a significantly greater diuretic, saluretic, and uricosuric response when given iv as compared to the po data in Table III.

now wish to report that compounds, such as **5a–d**, which are inert to reaction with sulfhydryl-containing compounds, exhibit marked saluresis and diuresis in several species and, therefore, the significance of sulfhydryl binding in the mechanism of action of the known phenoxyacetic diuretics appears to be secondary. The saluretic effects of these compounds are observed not only in dogs and chimpanzees but, surprisingly, also in rats. Even more unexpected was the finding that these compounds possess significant uricosuric activity in chimpanzees. Thus, we have obtained for the first time potent nonmercurial diuretic agents which have the desirable property of being uricosuric.



**5a**, R = $-CH(CH_3)_2$; $R^1$ = H
**b**, R = c-$C_5H_9$-; $R^1$ = H
**c**, R = $-CH(CH_3)_2$; $R^1$ = $CH_3$
**d**, R = c-$C_5H_9$-; $R^1$ = $CH_3$



**6a**, R = $-CH(CH_3)_2$; $R^1$ = H
**b**, R = c-$C_5H_9$-; $R^1$ = H

A systematic structure–activity (S–A) study of these indans **5** indicates that structural requirements for saluretic–diuretic activity are generally similar to those seen for the related series **2–4**. On the other hand, the S–A requirements for uricosuric activity are rather different, but there are structural features that are common to each requirement. Thus, it has been possible to design and synthesize compounds in which either saluretic or uricosuric effects predominate or where both activities are optimal. Eventually, uricosuric saluretics were synthesized which were comparable to probenecid and the loop diuretics in their respective intrinsic pharmacodynamic responses with dose potencies equivalent to the more active of the diuretics in current use. The representative compounds shown in Tables I–III illustrate some of these generalizations that were observed in appropriate studies in rats, dogs, and chimpanzees.

**Table IV**

| Compd | Enantiomer | Chiral base used | Solvent | $[\alpha]^{25}_D$ (*c* 3, $Me_2CO$) |
|---|---|---|---|---|
| 5b | + | 1-(−)-α-Methylbenzylamine | *i*-PrOH | +38.2 |
| 5b | − | Cinchonine | EtOH | −38.2 |
| 5c | + | Cinchonine | MeCN | +48.4 |
| 5c | − | (−)-Cinchonidine | MeCN | −48.4 |
| 5d | + | (−)-Cinchonidine | EtOH–$H_2O$ | +34 |
| 5d | − | 1-(−)-α-Methylbenzylamine | EtOH–$H_2O$ | −34 |

It is worthy of note that the nature of R in formula **5** is important for both saluretic and uricosuric activity and that introduction of a second substituent, such as methyl, at $R^1$ significantly enhances the activity; thus, the activity of **5c** > **5a** and **5d** > **5b**. Resolution of three racemic pairs (**5b–d**) permitted the demonstration of appreciable differences in the relative activities of the enantiomorphs.

Clinical studies[20] with **5a,b,d** have confirmed the marked diuretic-saluretic and uricosuric activities as well as relative potencies seen in chimpanzees.[21]

The syntheses of **5a** and **5b** have been disclosed;[17] compounds **5c** and **5d** were prepared from **6a** and **6b**[25] which were treated with KO-*t*-Bu and $CH_3I$ in *t*-BuOH-benzene (1:1) to give the analogous compounds where $R^1 = CH_3$. Cleavage of the ether group with pyridine hydrochloride, followed by reaction with $BrCH_2COOEt$ and $K_2CO_3$ in DMF, then basic hydrolysis, and acidification gave **5c** and **5d**.[25]

Resolution of **5b,c,d** was carried out by recrystallization of appropriate salts of chiral bases as seen in Table IV.

**Acknowledgment.** The authors are indebted to Drs. K. H. Beyer, Jr., R. F. Hirschmann, J. M. Sprague, and C. A. Stone for their guidance and advice in this work.

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*Received July 9, 1974*

# *Book Reviews*

**Metal Ions in Biological Systems. Volume 1. Simple Complexes.** Edited by Helmut Sigel. Marcel Dekker, New York, N.Y. 1974. 267 pp. $21.75.

The editor's objective in this carefully outlined series of six volumes is "to focus attention on the connection between the chemistry of metal ions and their role for life," and "to break down the barriers between the historically independent spheres of chemistry, biochemistry, biology, medicine, and physics." Volumes 2 and 3 of this series were published ahead of Volume 1 and have been reviewed [*J. Med. Chem.*, 17, 910 (1974)].

Volume 1 contains six chapters devoted chiefly to complexes of transition metals with simple ligands such as nucleosides, nucleotides, amino acids, and oligopeptides. Each chapter provides selective rather than exhausting coverage of the literature. The terminology, abbreviations, and symbolisms used throughout are consistent with Volumes 2 and 3, for which the editor should be complimented.

Chapter 1 covers nucleoside and nucleotide complexes. It reviews the several methods used in their study and their limitations, tabulates much stability constant data, and points out that, as expected, hard metal ions bind at the phosphates while soft metals bind at the heterocyclic base. It also stresses that the various methods used to measure binding give results which compare qualitatively but not quantitatively, and possible causes are discussed.

Chapter 2 provides a kinetic and mechanistic background for nucleotide–metal complexation. Chelation of the metal by nucleotide groups is much like any other chelation process, but special factors such as base-stacking introduce new complications only recently appreciated; hence, the discussion is divided into pre- and post-1967 periods. Considerable caution is needed in interpretation of or extrapolation from binding site and complexation studies to more complex systems, however, because the extremes of metal–ligand ratios and concentrations used in the simpler systems may not reflect biological conditions. The fine points of kinetics and mechanism of complexation are reviewed in detail and there is a succinct summary with examples of the biological significance of the results. The latter includes a discussion of the interesting Eigen–Hammes explanation of the often antagonistic effects of calcium and magnesium ions in biological systems involving phosphates.