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Diuretics. 1. 1-Imidoyl-2-(2- and 3-indolyl)indolines

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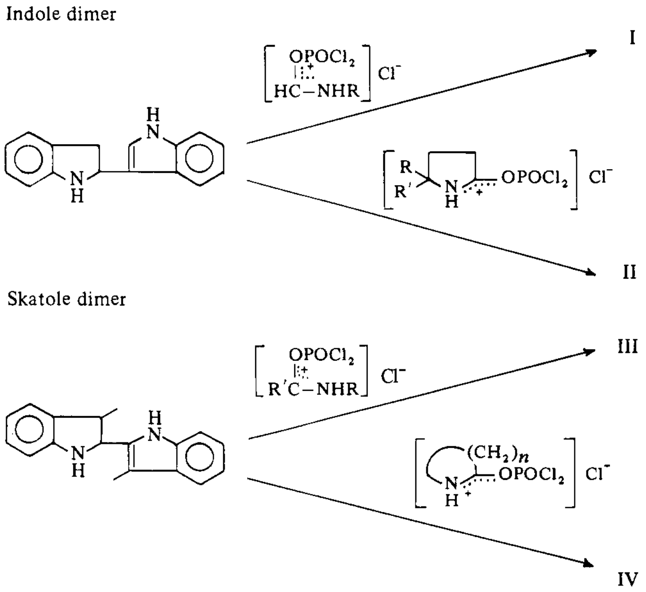
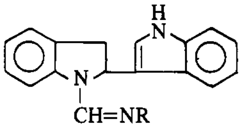
A series of 1-imidoyl-2-(2- and 3-indolyl)indolines was synthesized and evaluated for diuretic activity. The compounds were generally prepared by treating various dimers of indoles with carboxamide–$POCl_3$ adducts. Several 2,3,5,6-tetrahydro-5-(3-indolyl)-1*H*-pyrrolo[2,1-*b*][1,3]benzodiazepines were obtained by a rearrangement reaction. A discussion of structure–activity relationships is presented.

Intensive synthetic work on oral diuretics in the past 2 decades has resulted in several efficient agents exemplified by chlorothiazide,[1] ethacrynic acid,[2] furosemide,[3] spironolactone,[4] and triamterene.[5] In spite of the apparent commercial success of these agents, a totally satisfactory diuretic agent has not been attained. In his address for the Third Award in Medicinal Chemistry, Sprague[6] clearly indicated the necessity for continuous effort of medicinal chemists in this field.

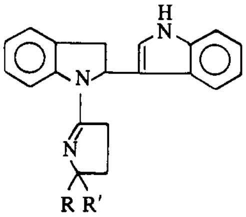
We wish to report a new chemical class of oral diuretics represented by structures I-V.† Preliminary pharmacological evaluation has been encouraging in view of selective saluretic, K^+ sparing responses elicited by these compounds. This paper deals with chemical syntheses, biological screening results, and structure–activity relationships of the series.

Chemistry. Compounds I-IV were prepared by treating various dimers of indoles with appropriate carboxamide-$POCl_3$ adducts.[7]

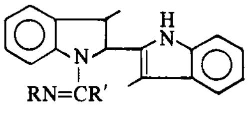




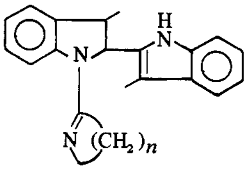
I



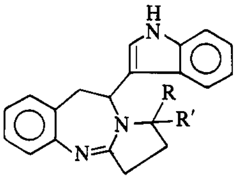
II



III



IV



V

36, R, R' = H

37, R = H; R' = CH_3

38, R, R' = CH_3

Dimerization of Indoles. Indoles are known to dimerize and trimerize in the presence of a protonic acid. The chemistry of this polymerization has been reviewed.[8]

By choosing an appropriate solvent as the reaction medium, we were able to dimerize several indoles with minimal concurrent trimerization. An ideal solvent would dissolve the particular indole to be dimerized, but immediately precipitate the dimer hydrochloride when the solution is treated with gaseous HCl. Purification of an indole dimer hydrochloride by a recrystallization method is not feasible, because in solution the dimer hydrochloride equilibrates with its monomer and trimer, resulting in the formation of a mixture (indole monomer ⇌ indole dimer ⇌ indole trimer).

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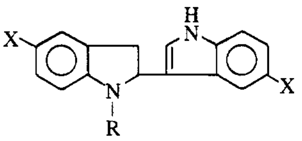
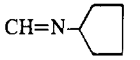
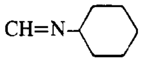
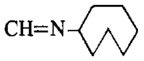
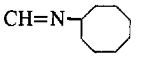
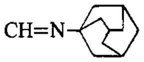
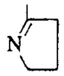
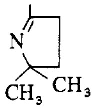
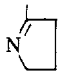
†Roman numerals refer to classes of compounds mentioned only in the text. Arabic numerals refer to individual compounds and compounds in the tables.

Table I. 1-Imidoyl-2-(3-indolyl)indolines



No.	X	R	Mp, °C	Recryst solvent	% yield[a]	Formula	Anal.	Chemical shifts δ, Hz, *J* value, cps: H-2 (*J* value)	H-7	Solvent
1	H	CH=NCH$_3$	213.5-214.5	EtOH-Et$_2$O	23	$C_{18}H_{17}N_3 \cdot HCl$	C, H, N	5.77 (5.0, 9.2)		CHCl$_3$
2	H	CH=NC$_2$H$_5$	215.5-216.5	EtOH-Et$_2$O	25	$C_{19}H_{19}N_3 \cdot HCl$	C, H, N	5.74 (5.2, 9.6)	7.57	CDCl$_3$
3	H	CH=NCH(CH$_3$)$_2$	215.5-217.5	EtOH-Et$_2$O	40	$C_{20}H_{21}N_3 \cdot HCl$	C, H, N	5.65 (5.3, 10.0)	7.65	CDCl$_3$
4	H	CH=NC(CH$_3$)$_3$	233.5-234.5	EtOH	36	$C_{21}H_{23}N_3 \cdot HCl$	C, H, N	5.71 (5.4, 9.6)	7.71	CDCl$_3$
5	H		215.5-217.5	EtOH	52	$C_{22}H_{23}N_3 \cdot HCl$	C, H, N	5.66 (5.6, 9.5)	7.67	CDCl$_3$
6	H		201.5-203.5	EtOH-Et$_2$O	18	$C_{23}H_{25}N_3 \cdot HCl$	C, H, N	5.75 (5.5, 9.7)	7.68	CDCl$_3$
7	H		182.5-184.5	EtOH	20	$C_{24}H_{27}N_3 \cdot HCl$	C, H, N	5.72 (5.4, 9.5)	7.61	CDCl$_3$
8	H		214-216.5	EtOH-Et$_2$O	33	$C_{25}H_{29}N_3 \cdot HCl \cdot 0.5H_2O$	C, H, N	Obscured	7.67	CDCl$_3$
9	H		230-232.5	EtOH-Et$_2$O	34	$C_{27}H_{29}N_3 \cdot HCl$	C, H, N	5.99 (multiplet)	Obscured	CF$_3$CO$_2$H
10	H	CH=NC$_6$H$_5$	179.5-181	EtOH-Et$_2$O	13	$C_{23}H_{19}N_3 \cdot HCl$	C, H, N	6.26 (6.5, 9.5)	Obscured	CF$_3$CO$_2$H
11	H	CH=NC$_6$H$_4$OCH$_3$-*p*	178-180	EtOH	20	$C_{24}H_{21}N_3O \cdot HCl \cdot 0.5H_2O$	C, H, N	~6.8 (*ϕ* region was 6.7-8.1)	~8.0	(CD$_3$)$_2$SO
12	H	CH=NC$_6$H$_4$CH$_3$-*p*	185-186	EtOH	15	$C_{24}H_{21}N_3 \cdot HCl \cdot 0.5H_2O$	C,[b] H, N	6.26 (6.5, 9.3)	Obscured	CF$_3$ CO$_2$H
13	H	CH=NC$_6$H$_4$Cl-*p*	177-178.5	EtOH-Et$_2$O	12	$C_{23}H_{18}ClN_3 \cdot 0.5H_2O$	C, H, N	(*ϕ* region 6.7-8.1)		(CD$_3$)$_2$SO
14	H		213.5-215.5	EtOH-Et$_2$O	28	$C_{20}H_{19}N_3 \cdot HCl$	C, H, N	5.71 (3.0, 9.5)	8.20	CDCl$_3$
15	H		194.5-199.5	EtOH-Et$_2$O	24	$C_{21}H_{21}N_3 \cdot HCl$	C, H, N	5.75 (3.5, 10.0)	8.32	(CD$_3$)$_2$SO
16	H		260-261	EtOH-Et$_2$O	44	$C_{22}H_{23}N_3 \cdot HCl$	C, H, N	5.72 (3.0, 10.0)	8.27	(CD$_3$)$_2$SO
17	NO$_2$		234.5-237.5	EtOH-heptane	37	$C_{20}H_{17}N_5O_4 \cdot HCl$	C, H, N	6.55 (broadened)	Obscured	(CD$_3$)$_2$SO
18	NO$_2$	CH=NCH(CH$_3$)$_2$	215.5-216.5	EtOH-Me$_2$CO	16	$C_{20}H_{19}N_5O_4$	C, H, N	6.01 (4.6, 10.2)	7.80	(CD$_3$)$_2$SO
19	Br	CH=NCH(CH$_3$)$_2$	189.5-191.5	EtOH-Et$_2$O	46	$C_{20}H_{19}Br_2N_3 \cdot HCl \cdot 0.5 C_2H_5OH$	C,[c] H, N	6.39 (3.0, 9.0)	Obscured	(CD$_3$)$_2$SO
20	OMe	CH=NCH(CH$_3$)$_2$	204.5-206.5	EtOH	45	$C_{22}H_{25}N_3O_2 \cdot HCl$	C,[d] H, N	6.40 (3.0, 7.5)	7.79	(CD$_3$)$_2$SO
21	CN	CH=NCH(CH$_3$)$_2$	223.5-225.5	EtOH-CHCl$_3$	32	$C_{22}H_{19}N_5$	C, H, N	5.84 (5.0, 10.0)	7.85	(CD$_3$)$_2$SO
22	OCH$_2$Ph	CH=NCH(CH$_3$)$_2$	222-223	EtOH	30	$C_{34}H_{33}N_3O_2 \cdot HCl$	C, H, N	6.42 (*ϕ* overlap)	~7.8	(CD$_3$)$_2$SO

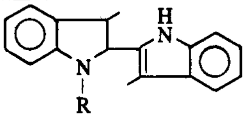
[a] Based on analytically pure sample. Many of these experiments were conducted only once and the optimal condns were not established. [b] C: calcd, 72.62; found, 73.09. [c] C: calcd, 48.44; found, 47.90. [d] C: calcd, 66.07; found, 65.43.

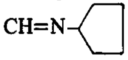
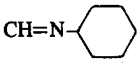
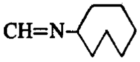
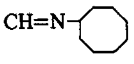
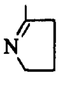
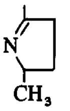
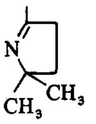
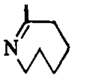
However, the free base obtained by neutralizing the dimer hydrochloride can be recrystallized without complication. Dimers of indole,[9] skatole,[10] 3-ethyl-,[11] 5-bromo-, 5-methyl-, 5-methoxy-, 5-nitro-, 5-acetyl-, 5-benzyloxy-, 5-cyano-, 6-nitro-, and 7-methylindoles[12] were obtained in this manner. Efforts to obtain a pure 5-methanesulfonamidoindole dimer have not been successful. Attempted recrystallization of the crude dimer of 5-methanesulfonamidoindole, which contains an acidic function, in a variety of solvents gave a 1:1 (monomer:dimer) mixture.

Imidoylation. a. Indole Dimers. Imidoylation of indole dimer is markedly subject to steric hindrance. While indole dimer reacted normally with 2-pyrrolidinone-POCl$_3$ adduct, it failed to react with valerolactam-POCl$_3$, 3-thiomorpholinone-POCl$_3$, and caprolactam-POCl$_3$ adducts. 7-Methylindole dimer, which has an added group at the ortho position, did not react with 2-pyrrolidinone-POCl$_3$ complex.

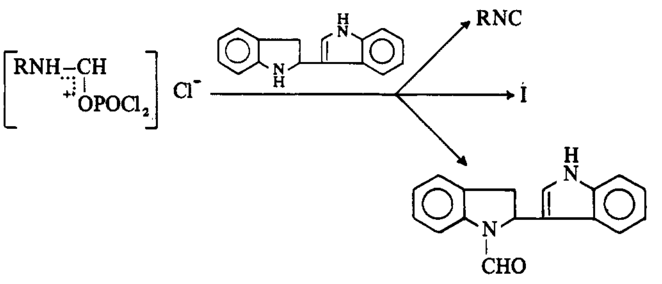
In contrast to 2-pyrrolidinone-POCl$_3$ imidoylation, the reaction with a formamide-POCl$_3$ reagent was complicated with hydrolysis and elimination side reactions[13] with the formation of the formyl derivative and an isocyanide as byproducts. The yields of desired I depend on the nature of the R groups. Secondary and tertiary alkyl groups appear to be more favorable than primary alkyl groups.

Table II. 1-Imidoyl-3-methyl-2-(3-methyl-2-indolyl)indolines



No.	R	Mp, °C	Recryst solvent	% yield[a]	Formula	Anal.	Chemical shift, H-7, δ, Hz
23	CH=NCH$_3$	229.5-230.5	EtOH-Et$_2$O	24	$C_{20}H_{21}N_3 \cdot HCl$	C, H, N	7.68
24	CH=NCH(CH$_3$)$_2$	208.5-210.5	EtOH-Et$_2$O	22	$C_{22}H_{25}N_3 \cdot HCl$	C, H, N	8.02
25	CH=NC(CH$_3$)$_3$	226.5-228.5	EtOH-Et$_2$O	25	$C_{23}H_{27}N_3 \cdot HCl$	C, H, N	8.09
26	C(CH$_3$)=NCH$_3$	203.5-206.5	EtOH	37	$C_{12}H_{23}N_3 \cdot HCl$	C, H, N	7.63
27		200-201	EtOH-Et$_2$O	36	$C_{24}H_{27}N_3 \cdot HCl$	C, H, N	7.55
28		211.5-213.5	EtOH-Et$_2$O	34	$C_{25}H_{29}N_3 \cdot HCl$	C, H, N	7.65
29		211.5-213.5	EtOH-Et$_2$O	32	$C_{26}H_{31}N_3 \cdot HCl$	C, H, N	Not examined
30		206-207	EtOH-Et$_2$O	55	$C_{27}H_{33}N_3 \cdot HCl$	C, H, N	7.58
31		261.5-263.5	EtOH	24	$C_{22}H_{23}N_3 \cdot HCl$	C, H, N	8.30
32		202-204	EtOH-Et$_2$O	14	$C_{23}H_{25}N_3 \cdot HCl \cdot 0.25H_2O$	C, H, N	8.32
33		234-236.5	EtOH-Et$_2$O	20	$C_{23}H_{25}N_3 \cdot HCl$	C, H, N	8.31
34		225.5-227.5	EtOH-Et$_2$O	29	$C_{24}H_{27}N_3 \cdot HCl$	C, H, N	8.22
35		182.5-184.5	EtOH-Et$_2$O	29	$C_{24}H_{27}N_3 \cdot HCl \cdot 0.5C_2H_5OH$	C, H, N	7.81

[a] See footnote *a* in Table I.



The physical constants of 18 1-(formimidoyl)- and four 1-[2-(1-pyrrolinyl)]-2-(3-indolyl)indolines are listed in Table I.

b. Skatole Dimer. Apparently, imidoylation with skatole dimer is much less sterically hindered than the corresponding indole dimer reactions. Skatole dimer reacted normally with various carboxamide-POCl$_3$ adducts. The resulting 13 1-imidoyl-3-methyl-2-(3-methyl-2-indolyl)indolines are listed in Table II.

Rearrangement of II to V. In neutral or basic solutions, 2-(3-indolyl)-1-[2-(1-pyrrolinyl)]indolines (II) rearrange to 2,3,5,6-tetrahydro-5-(3-indolyl)-1*H*-pyrrolo[2,1-*b*][1,3]-benzodiazepines (V). For **14**, the rearrangement can also be effected thermally.

II	→	V
14, R,R′ = H		36
15, R = H; R′ = CH$_3$		37
16, R,R′ = CH$_3$		38

The structural studies, investigation of the rate and reaction conditions, and a proposed mechanistic rationale for the rearrangement have been reported in a previous paper.[14]

Spectral Data. a. Indole Dimer Series (I and II). The molecular models show that the indoline C-2 proton is nearly eclipsed with one of the C-3 protons of the fairly rigid indoline ring. This nonequivalence of the two C-3 protons leads to an ABX system[15] which displays the C-2 proton as a doublet of doublets at δ 5.6-5.7 Hz in pmr spectra. The deshielding effect of the imido C=N group on the indoline C-7 proton is also interesting. While the more flexible linear imidoyl I exhibits the C-7 proton at δ 7.6-7.7 Hz, the more constrained cyclic imidoyl II exhibits it at δ 8.1-8.3 Hz.

The mass spectra of I and II show high relative abundances of indole dimer fragments (*m/e* 233 and *m/e* 232) in the high-to-medium mass region.

b. Skatole Dimer Series (III and IV). Compounds III with linear imidoyl groups display the C-7 protons at δ 7.55-7.65 Hz in their pmr spectra. The chemical shift of the C-7 proton of structure IV is in the δ 7.8-8.3 Hz region.

In mass spectra, the dimer fragment (*m/e* 260) has the highest relative abundance above *m/e* 132.

c. 1*H*-Pyrrolo[2,1-*b*][1,3]benzodiazepines (V). The more flexible diazepine ring gives rise to an AXX′ pattern in the pmr spectra for the C-5 proton (a "triplet" at δ 5.3-5.4 Hz) and an unshifted C-10 proton.

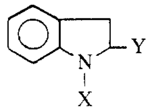
Biological Data. Diuretic activity was evaluated in the conscious rat by a method similar to that of Lipschitz, *et al.*[16] The results are tabulated in Table III, wherein the relative potencies of test compounds are expressed in terms of *T/U* values for urinary volume and electrolyte (Na$^+$ and K$^+$)

Table III

No.	Diuretic activity (*T*/*U* ratio) Volume excretion	Na$^+$	K$^+$
1	1.69	*a*	*a*
2	1.86	1.63	0.74
3	2.42	1.64	0.84
4	2.76	2.43	0.56
5	1.94	1.74	0.82
6	2.33	2.21	1.02
7	2.14	2.35	1.29
8	2.00	1.26	0.93
9	2.66	2.44	1.13
10	0.47	*a*	*a*
11	0.91	0.89	1.14
12	0.78	0.89	0.77
13	0.83	0.90	0.75
14	2.68	2.56	1.22
15	2.78	2.52	0.94
16	3.09	2.79	1.67
17	0.59	0.80	0.47
18	1.59	1.55	1.04
19	1.28	1.54	0.92
20	2.53	2.44	1.08
21	1.23	1.12	0.81
22	0.94	0.96	0.79
23	1.51	*a*	*a*
24	1.61	1.83	1.31
25	2.25	2.33	1.34
26	1.96	2.47	1.04
27	1.54	1.49	0.89
28	1.21	1.39	0.76
29	1.00	1.11	.74
30	0.93	0.87	1.02
31	1.25	*a*	*a*
32	1.96	1.74	1.16
33	2.42	1.64	0.84
34	2.50	2.71	0.77
35	2.44	3.80	1.34
26	3.11	2.78	1.31
37	2.76	1.81	0.86
38	3.09	2.79	1.67
Trichlormethiazide	1.83	1.92	1.42

[a] Electrolyte data not available.

Table IV



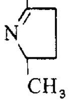
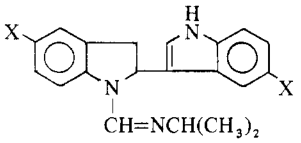
X	Y	Diuretic activity, *T*/*U* ratio Volume excretion	Na$^+$	K$^+$
H	3-Indolyl	0.73	0.77	0.71
CHO	3-Indolyl	0.79	0.79	0.67
H	2-Skatyl	0.71	0.67	0.62
CHO	2-Skatyl	0.78	0.62	0.61
$CH_3N{=}C{-}CH_3$	H	0.80	0.83	0.97
	H	0.68	0.88	1.15

Table V



X	σ	*T*/*U* (volume excretion)
NO_2	0.778	1.59
CN	0.660	1.23
Br	0.232	1.28
H	0	2.42
OCH_3	−0.268	2.53

excretion. A *T*/*U* value for a response is a ratio of the response evoked by a test compound to that evoked by urea as the reference standard.

In the indole dimer series (I and II), 8 compounds (**3, 4, 6, 9, 14, 15, 16, 20**) produced significant volume diuresis with $T/U > 2.25$. For **3, 4,** and **15**, the general patterns of relative electrolyte excretion were optimal; *i.e.*, augmented Na$^+$ loss with decreased or minimally altered K$^+$ outflow. Compounds **6, 9, 14,** and **20** also showed favorable patterns. Compound **5** evoked favorable electrolyte excretion but promoted a low volume response.

In the skatole dimer series, **25, 33, 34,** and **35** promoted substantial *T*/*U* volume responses. Compds **25, 26, 34,** and **35** increased Na$^+$ excretion significantly above reference levels. However, only **33** and **34** decreased relative excretion of K$^+$.

All compounds (**36, 37, 38**) of structure V elevated the volume diuretic and Na$^+$ excretion responses above reference standard levels. Compound **37** had $T/U > 1$ for K$^+$ excretion.

Trichlormethiazide, a reference agent, produced only moderate increases in urinary volume and Na$^+$ excretion, accompanied by substantial elevation in K$^+$ outflow. This pattern is characteristic of the responses evoked by other diuretic benzothiadiazines.

Structure-Activity Relationships. Diuretic properties are shown in both the indole dimer (I, II) and the skatole dimer (III, IV) series. The diuretic potency of a particular member of the indole dimer series is usually greater than that of the corresponding member of the skatole dimer series (compare the pairs: **1, 23**; **3, 24**; **4, 25**; **5, 27**; **6, 28**; **7, 29**; **8, 30**; **14, 31**; **15, 32** and **35**; **16, 34**).

The imidoyl moiety (RN=C) at the 1 position of the indoline ring seems to be an essential feature for the diuretic effect. This moiety may be a part of a cyclic system (II and IV). The presence of an indolyl group at the 2 position of the indoline ring is also important for biological activity. Table IV illustrates the general lack of diuretic potency of several related compounds.

In a homologous series with the imidoyl group RN=C, wherein R represents a primary, secondary, or tertiary alkyl group, the diuretic potency decreases in the order: tertiary alkyl > secondary alkyl > primary alkyl. (Compare the sets: **1, 3, 4**; **6, 9**; **14, 15, 16**; **23, 24, 25**; **31, 32, 34**.) The diuretic activity vanishes if R represents a phenyl group (compounds **12-15**).

In a subseries of 5-substituted indole dimers, no linear relationship (see Table V) exists between *T*/*U* values for volume excretion and Hammett σ values,[17] indicating that other factors besides the electronic effect may also be involved.

Based upon the screening results, we have selected 8 compounds (**2, 3, 4, 5, 14, 26, 34, 36**) for more extensive evaluation as diuretic agents. There are indications that the kaliuretic and other undesirable responses promoted by the presently available drugs may be absent in these new structures.

Experimental Section

Diuretic Screening. Fasted, intact female Harlan rats (150-200 g) were dosed orally with 25 mg/kg of 0.9% saline contg a std

Table VI. Dimers of Indoles

Substituent	Reaction medium	Recrystn solvent	Mp, °C	% yield[a]	Formula[d]
None	$ClCH_2CH_2Cl$	*i*-Pr_2O–Skelly B	103.5–105.5	79	$C_{16}H_{14}N_2$
3-Me	Et_2O	*i*-Pr_2O	126–126.5	84	$C_{18}H_{18}N_2$
3-Et	C_6H_6	*a*	155–157	57	$C_{20}H_{22}N_2 \cdot HCl$
5-Br	C_6H_6	Heptane–C_6H_6	124–126	62	$C_{16}H_{12}Br_2N_2$
5-Me	C_6H_6	Hexane–C_6H_6	129–131	32	$C_{18}H_{18}N_2$
5-OMe	C_6H_6	EtOH–C_6H_6	140–141	88	$C_{18}H_{18}N_2O_2$ [b]
5-NO_2	Et_2O	EtOH	200–202	49	$C_{16}H_{12}N_4O_4$
5-Ac	C_6H_6	C_6H_6	133–135	52	$C_{20}H_{18}N_2O_2 \cdot 0.5C_6H_6$
5-OCH_2Ph	C_6H_6	Hexane–C_6H_6	103–105	43	$C_{30}H_{26}N_2O_2$
5-CN	C_6H_6	EtOH	214–215	35	$C_{18}H_{12}N_4$
6-NO_2	Et_2O–C_6H_6	MeOH	186.5–188.5	13	$C_{16}H_{12}N_4O_4$ [c]
7-Me	C_6H_6	Cyclohexane	84–87	94	$C_{18}H_{18}N_2$

[a] See footnote *a* in Table I. [b] C: calcd, 73.45; found, 72.99. [c] C: calcd, 59.26; found, 58.81. [d] All compds were analyzed for C, H, N.

dose (960 mg/kg) of urea as the ref std. Other groups of 6–14 animals were treated orally with the test compd dissolved or suspended in a 25 ml/kg saline load vol at a standardized dose of 25 mg/kg. The rats were placed in metabolism cages (2 per cage) and pooled samples of urine from each pair were collected over a 5-hr period. The pooled samples representing a given group treatment were measured for total vol and Na^+ and K^+ (mequiv). A response index, T/U, where T is the response to the test compd and U the pooled average response in 3–5 pairs of rats treated with urea, was computed for vol, Na^+ and K^+ excretion. The vol of urine excreted by each group was expressed as % fluid administered; % excretion by the std ref group was taken as a base of 100% or 1.0 for calcg relative diuretic activity of test compd. T/U ratios, with regard to electrolyte outflow following test drugs, were similarly computed based upon Na^+ and K^+ excretion associated with urea diuresis. Trichlormethiazide, administered orally at a dose of 8 mg/kg in a 25 ml/kg saline load vol, served as a ref agent.

Dimerization of Indoles. General Procedure. The particular indole (0.05 mole) was dissolved in 250 ml of an anhyd solvent, usually PhH. The soln was chilled with an ice bath and satd with dry HCl. The resulting ppt was isolated by filtration. The crude dimer·HCl was basified in $CHCl_3$ (100–150 ml) with dil aqueous NH_4OH. The organic layer was sepd, washed, and concd under reduced pressure to the residual crude base which was recrystd. Freedom of the dimer from monomer or trimer was confirmed by the pmr spectrum.

The physical constants of 12 indole dimers are listed in Table VI.

Indole Monomer-Polymer Equilibration. Indole was dimerized as described in the previous section. The crude HCl salt was purified by washing it well with $CHCl_3$ and then twice with Me_2CO. After drying in a vacuum desiccator, the product melted at 178–181°. Pmr analysis in DMSO-d_6 did not detect any monomer or trimer·HCl. *Anal.* ($C_{16}H_{14}N_2 \cdot HCl$), C, H, N, Cl.

A sample (0.5 g) of the pure dimer·HCl was dissolved in MeOH with heating. No pptn occurred on chilling. Removal of the solvent under reduced pressure gave a residual oil which solidified on drying in a vacuum desiccator. The solid was pulverized and a pmr spectrum in DMSO-d_6 was taken. Comparison of the spectrum with reference spectra of the monomer, dimer·HCl, and trimer·HCl gave an estimate of the composition of the mixture: monomer, 40%; dimer, 30%; trimer, 30%.

1-Formyl-2-(3-indolyl)indoline. $POCl_3$ (7.7 g, 0.05 mole) was added dropwise to a mixt of indole dimer (11.7 g, 0.05 mole), *N*-ethylformamide (7.3 g, 0.1 mole), and 1,2-dichloroethane (125 ml). The temp of the reaction mixt was kept below 30–35°. After stirring for 15 hr at room temp, the mixt was treated with 100 ml of 29% NH_4OH. The organic layer was sepd, washed with 100 ml of 1.5 *N* HCl followed by 100 ml of H_2O, dried ($MgSO_4$), and concd to a solid residue, which was recrystd twice from EtOH, yield 2.6 g (20%), mp 192–194°. *Anal.* ($C_{17}H_{14}N_2O$) C, H, N.

3-Methyl-1-formyl-2-(3-methylindol-2-yl)indoline. $POCl_3$ (4.6 g, 0.03 mole) was added dropwise to a mixt of skatole dimer (7.9 g, 0.03 mole), *N*-cyclohexylformamide (3.8 g, 0.03 mole), and 1,2-dichloroethane (120 ml) over a period of 30 min. The mixt was stirred at room temp for 15 hr, followed by treatment with 80 ml of 29% of NH_4OH. The organic layer was sepd, dried ($MgSO_4$), and concd. The residue was recrystd twice from EtOH (60–70 ml), yield 4.8 g (55%), mp 181–183°. *Anal.* ($C_{19}H_{18}N_2O$) C, H, N.

1-(*N*-Methylacetimidoyl)indoline and 1-(5-methyl-1-pyrrolin-2-yl)indoline were prepd[18] from appropriate carboxamide-$POCl_3$ reagents on indoline.

1-Imidoyl-2-(2- and 3-indolyl)indolines. General Procedure. An equimolar mixt of dimer of indole, carboxamide (or lactam), and Et_3N in 1,2-dichloroethane (375 ml per 0.1 mole) was cooled to −20° and treated with an equimolar soln of $POCl_3$ in 1,2-dichloroethane (300 ml per 0.1 mole) over a period of 45 min. The temp of the mixt was maintd at about −20°. The mixt was allowed to warm to room temp with stirring over a 2.5-hr interval. The product was recovered by pouring the reaction mixt into an ice-chilled aqueous NaOAc soln (78 g of NaOAc in 250 ml of H_2O per 0.1 mole run), and adjusting the pH to 14 with aq KOH. The solid was collected on a filter, washed, dried, and recrystd from an appropriate solvent. The free base was converted to the corresponding HCl salt by treatment of ethanolic HCl.

Rearrangement of 14 to 36. The rearrangement in polar solvents has been described.[14] The thermal rearrangement was effected as follows. A 100-mg sample of 2-(3-indolyl)-1-[2-(1-pyrrolinyl)]indoline (**14**) was heated at 180 ±5° for 10 min. The material melted first and resolidified. Recrystn from 10 ml of EtOH gave 53 mg of pure 2,3,5,6-tetrahydro-5-(3-indolyl)-1*H*-pyrrolo[2,1-*b*][1,3]benzodiazepine (**36**), mp 237–238°. Its ir and pmr spectra were identical with that of an authentic sample.[14] *Anal.* ($C_{20}H_{19}N_3$) C, H, N.

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Receptor Site Labeling Through Functional Groups. 2. Reactivity of Maleimide Groups[1]

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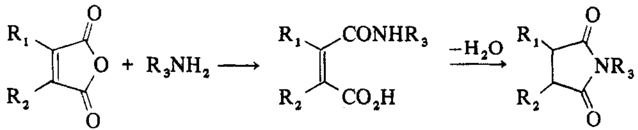
N-Alkylmaleimides are reactive groups suitable for binding drug fragments (D) linked to them *via* a chain (C_n) to receptor sites which bear suitable functional groups (*e.g.*, thiol groups). To evaluate the theiotropic (thiol-seeking) character of maleimides, rates of reaction with glutathione (GSH) in aq buffer have been examined. GSH adds to *N*-ethyl-2-methylmaleimide at the same atom as that which carries the Me group, demonstrating that electronic effects are far more important than steric effects in this reaction. Addition of GSH to *N*-ethyl-2,3-dimethylmaleimide reaches equilibrium in a solution containing 90–95% adduct. Based on the kinetic results, several 2-methylmaleimidylbarbiturate derivatives have been prepared and tested; modest signs of biological activity appeared for one compound. The rate constants permit rational choice of appropriate functional groups, provide insight into the biological effects of *N*-alkyl (or aryl-)-2,3-dichloromaleimides, and lead to a logical prediction for molecular modification of the maleimide antibiotic, showdomycin.

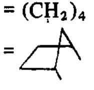
We have described an approach to the labeling of receptor sites, having a suitable functional group in proximity to the site, through the synthesis of molecules incorporating a drug fragment, D, a connection, C_n, and a reactive moiety, Y. The Y moiety was chosen for its theiotropic (thiol-seeking) properties and in our first effort, limited to two maleimidyl groups of extremely different reactivities.[1,‡]

In order to provide a graded range of Y reactivity, we have now evaluated a number of additional N-substituted maleimides, with respect to the rate of reaction with the tripeptide thiol, glutathione (GSH), in aq buffer. On the basis of the rate constants for the reaction between maleimides and GSH, several barbiturate derivatives were selected and synthesized.

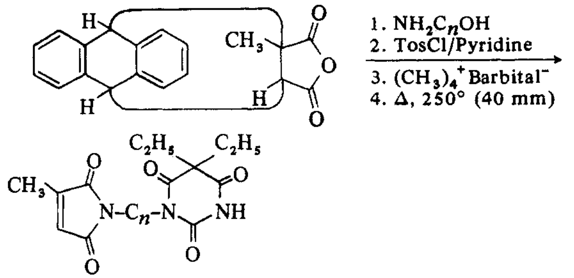
Results

Synthesis. N-Substituted maleimides are prepared from the corresponding maleic anhydride *via* the intermediate maleamic acids (eq 1).



1, $R_1 = CH_3$; $R_2 = H$; $R_3 = Et$
2, $R_1 = CH_3$; $R_2 = CH_3$; $R_3 = Et$ (1)
2H, $R_1 = CH_3$; $R_2 = CH_3$; $R_3 = HOCH_2CH_2$
3, R_1–$R_2 = (CH_2)_4$
4, R_1–R_2 = 

Two barbiturate derivatives of *N*-ethyl-2-methylmaleimide (**1**), with either CH_2CH_2 (**5**) or $(CH_2)_2O(CH_2)_2$ (**6**) connecting the drug fragment to reactive group, were synthesized by the general route used previously.[1] The additional steric hindrance introduced by the 2-Me group necessitated the use of anthracene as a protecting group for the double bond of the maleic acid anhydride. A summary equation indicated the pathway (eq 2).



(2)

5, $C_n = CH_2CH_2$
6, $C_n = CH_2CH_2OCH_2CH_2$

Kinetic Studies. The rate of the disappearance of uv absorption due to the *N*-ethylmaleimide or maleimide was followed after dissolving GSH in a deoxygenated solution of maleimide in aq buffer. Strict second-order kinetics were followed in all cases except that of the 2,3-dimethylmaleimide which followed a rate equation for a bimolecular reaction to an equilibrium between products and reactants. The rate constants derived from the studies of the kinetics of the reaction are listed in Table I.

Biological Tests. According to the idea that maleimides function *via* reaction with SH groups within biological systems (either GSH or protein SH groups, with the former reacting much faster), maleimides should vary in biological effect in a fashion parallel to their rates of reaction with GSH. Indeed, by ip injection, NEM (**7**) was much more toxic than NEC (**1**) which, in turn, was far more toxic than DEM (**2**). A barbiturate linked to NEC by a C_2 chain (**5**) was similar to NEC in toxicity. At usual barbiturate levels, one linked through a C_2OC_2 chain to NEC (**6**) (*i.e.*, **202C**) was considerably more toxic, indicating that the depressant action of the barbiturate may have added to the toxic character of the NEC portion of the molecule. At low dose levels, the biological effects produced by **202C** were definitely more marked than those produced by the same levels of NEC. The biological activity of **6** is consistent with the activity found for a C_2OC_2-linked barbiturate derivative of dimethylmaleimide.[1]

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[‡] The term "thiophilic" refers to nucleophilicity *toward* sulfur[2] whereas "theiotropic" activity depends on the nucleophilic activity *of* sulfur as a thiol.