one tumor liver was used for all assays.

Protein was determined by the method of Lowry et al.[24] using bovine serum albumin as a standard.

Enzyme Assay. The enzyme was assayed using [*methyl*-^{14}C]-*S*-adenosyl-L-methionine and *E. coli* B tRNA as substrates. The incubation mixture (0.5 ml) contained 50 μmol of Tris–HCl buffer (pH 8.2), 5 μmol of $MgCl_2$, 2.5 μmol of β-mercaptoethanol, 0.25 M ammonium acetate, 250 μg of *E. coli* B tRNA, various amounts of [$^{14}CH_3$]-SAM, and enzyme as indicated. Incubation was carried out at 37°. In the inhibition assays various amounts of the synthesized sulfonium compounds were added. Methylation was determined by subtracting a blank containing no tRNA from the assay value.

At completion of the reaction a 0.1-ml aliquot was withdrawn, pipetted onto a Whatman 3 MM filter disk, dried, and immersed immediately in cold 5% TCA. After 30 min the disks were washed further with 500 ml of cold TCA, ethanol–ether, and ether in 50-ml portions.[25] The disks were dried, placed in 5 ml of scintillation solution containing PPO, POPOP, and toluene, and counted.

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(9) Abbreviations used are SAM, *S*-adenosyl-L-methionine; SAH, *S*-adenosyl-L-homocysteine; MPAS, 5′-methylpropyl(5′-adenosyl)sulfonium iodide; EPAS, 5′-ethylpropyl(5′-adenosyl)sulfonium iodide; MEAS, 5′-ethylmethyl(5′-adenosyl)sulfonium iodide; MTA, methyl 5′-adenosyl sulfide; ETA, ethyl 5′-adenosyl sulfide; PTA, propyl 5′-adenosyl sulfide; DAB, 3′-methyl-4-dimethylaminoazobenzene; PPO, 2,5′-diphenyloxazole; POPOP; 1,4-bis[2-(5-phenyloxazolyl)]benzene.
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(2-Piperidine)- and (2-Pyrrolidine)ethanones and -ethanols as Inhibitors of Blood Platelet Aggregation

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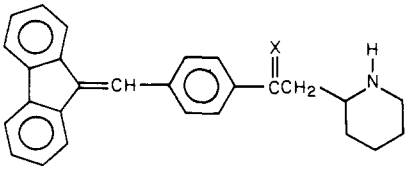
(*E*)-4-[4-(Methylthio)phenyl]-1-(2-piperidinyl)-3-buten-2-one hydrochloride (**44**, RMI 14133A) was found to inhibit ADP-induced aggregation of blood platelets. It was selected from a large series of (2-piperidinyl)- and (2-pyrrolidinyl)ethanones synthesized by a modified Schopf reaction from enolate magnesium salts of β-keto acids and 2,3,4,5-tetrahydropyridine trimer or 3,4-dihydro-2*H*-pyrrole trimer, respectively. Evaluation of the compounds was carried out in vitro on human blood platelets. Structure–activity relationships are discussed. **44** also inhibited platelet aggregation ex vivo in guinea pigs. Subacute toxicity evaluation in dogs and guinea pigs showed it to have an unfavorable therapeutic ratio. 1-[4′-Chloro(1,1′-biphenyl)-4-yl]-2-(2-piperidinyl)ethanone hydrochloride (**18**, RMI 12436A) was found to lower serum cholesterol levels in rats with concurrent accumulation of (3β)-cholesta-5,7-dien-3-ol, suggesting inhibition of 7-dehydrocholesterol Δ^7-reductase.

We reported earlier on the blood platelet aggregation inhibitory activity of α-[*p*-(fluoren-9-ylidenemethyl)-phenyl]-2-piperidineethanol (**1**).[1] The synthesis of **1** was accomplished by a novel modification of the Schopf reaction via **2**.[2,3] This piperidinemethyl ketone **2** was found to also inhibit blood platelet aggregation.[2] Since relatively few such ketones had been prepared prior to the development of our new synthetic method, we set out to synthesize additional analogues and to evaluate their effects on adenosine diphosphate (ADP) induced aggregation of human blood platelets. Physiologically, platelet aggregation precedes blood clot formation, and it is therefore felt that inhibition of platelet aggregation may also inhibit arterial thrombosis.[4] Inhibitors of platelet aggregation may



1, X = H, OH
2, X = O

Scheme I

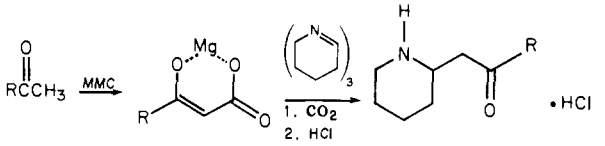
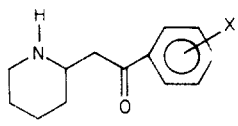
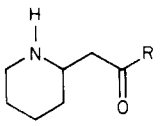


Table I. 2-Piperidinemethyl and 2-Pyrrolidinemethyl Ketones as Inhibitors of Platelet Aggregation in Human Plasma



3-25



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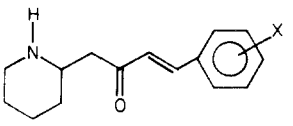
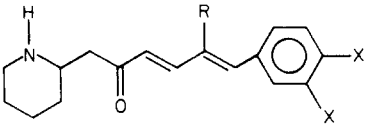
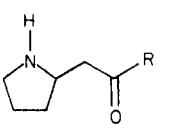


40-44



45-47



48-51

No.	X	Mp, °C	% yield[a]	Formula	Effect on human blood platelets[b] % inhibn of aggregation, μg/ml 100	30	10	3	% PF3 release, μg/ml 300	100
3[c]	H	167-169		$C_{13}H_{17}NO\cdot HCl$	1					
4	4-Me	172-173	38	$C_{14}H_{19}NO\cdot HCl$	14					
5	4-*t*-Bu	184-186 dec	16	$C_{17}H_{25}NO\cdot HCl$	63	16 (2)	0			
6	4-*n*-C_8H_{17}	130-131	31[d]	$C_{21}H_{33}NO\cdot HCl$	92	41				
7	4-*n*-$C_{12}H_{25}$	126-127 dec	80[d]	$C_{25}H_{41}NO\cdot HCl$	95	51				
8	4-c-C_6H_{12}	213-215 dec	38	$C_{19}H_{27}NO\cdot HCl$	100	7				
9	4-Cl	190-192 dec	9	$C_{13}H_{16}ClNO\cdot HCl$	14					
10	2-CF_3	182-184	21	$C_{14}H_{16}F_3NO\cdot HCl$	32					
11	4-CN	190-191	12	$C_{14}H_{16}N_2O\cdot HCl$	0					
12	4-SCH_3	184-186	60	$C_{14}H_{19}NOS\cdot HCl$	54					
13	4-SO_2NMe_2	204-206	10	$C_{15}H_{22}N_2O_3S\cdot HCl$	7					
14	4-OBu	157-158	35	$C_{17}H_{25}NO_2\cdot HCl$	92	27				
15	4-*n*-$OC_{12}H_{25}$	126-127	67[e]	$C_{25}H_{41}NO_2\cdot HCl$	100	71	0		0.09	0.01
16	2-CH=CHCH=CH-3[f]	222-223 dec	14	$C_{17}H_{19}NO\cdot HCl$	86	29			0.31 (3)	0.01 (3)
17	4-C_6H_5	208-210 dec	18	$C_{19}H_{21}NO\cdot HCl$	91	35	7		0.14 (3)	0.01 (3)
18	4-C_6H_4-*p*-Cl	195-196	66[g]	$C_{19}H_{20}ClNO\cdot HCl$	98	70	10 (2)		0.20 (2)	0.01 (2)
19	4-C_6H_4-*p*-OMe	205-206 dec	10[h]	$C_{20}H_{23}NO_2\cdot HCl$	100	33	0			
20	4-OC_6H_5	161-164 dec	26	$C_{19}H_{21}NO_2\cdot HCl$	98	11	2			
21	4-OC_6H_4-*p*-Br	227-228 dec	49	$C_{19}H_{20}BrNO_2\cdot HCl$	60 (2)	4				
22	4-OC_6H_4-*p*-OMe	177-179 dec	72	$C_{20}H_{23}NO_3\cdot HCl$	24					
23[i]	4-SC_6H_5	149-151	50	$C_{19}H_{21}NOS\cdot HCl$	60					
24	4-$CH_2C_6H_5$	206-207	58	$C_{20}H_{23}NO\cdot HCl$	60					
25[i]	4-*trans*-CH=CHC_6H_5	227-229	57	$C_{21}H_{23}NO\cdot HCl$	100	18				
	R									
26	-*n*-$C_{11}H_{23}$	119-121	52[d]	$C_{18}H_{35}NO\cdot HCl$	87 (2)	12	6			
27	-$CH_2CH_2CH=CMe_2$	114-116 dec	33	$C_{13}H_{23}NO\cdot HCl$	12					
28[i]	-(1-Adamantyl)	240-241	37[i]	$C_{17}H_{27}NO\cdot HCl$	31					
29[i]	-$CH(C_6H_5)_2$	197-199 dec	37	$C_{20}H_{23}NO\cdot HCl$	68	16	0			
30	-$CH(C_6H_5)C_6H_3$-2,5-Me_2	190-192	6	$C_{22}H_{27}NO\cdot HCl$	92 (2)	29 (2)	0			
31	-$CH(C_6H_5)C_6H_2$-2,4,6-Me_3	242-244 dec	24	$C_{23}H_{29}NO\cdot HCl$						
32	-$CH(C_6H_5)C_6H_4$-4-Cl	191-193	27	$C_{20}H_{22}ClNO\cdot HCl$						
33	-$C(C_6H_5)_2CH_3$	198-200	44	$C_{21}H_{25}NO\cdot HCl$	100	19 (2)	0			
34	-$C(C_6H_5)_2C_3H_7$	174-176	27	$C_{23}H_{29}NO\cdot HCl$						
35	2-Fluorenyl	222-223	10	$C_{20}H_{21}NO\cdot HCl$	100	38				
36	2-Dibenzofuranyl	218-220	32	$C_{19}H_{19}NO_2\cdot HCl$	97	14				
37	2-Dibenzothiophenyl	210-212	28	$C_{19}H_{19}NOS\cdot HCl$	100	32	0		0.09	0.00
38	3-Phenanthryl	190-192	38[g]	$C_{21}H_{21}NO\cdot HCl$	100 (2)	33	0			
39	9-Phenanthryl	215-216	24	$C_{21}H_{21}NO\cdot HCl$	91	12				
	X									
40	H	183-185	31[g]	$C_{15}H_{19}NO\cdot HCl$	95	33	9			
41	4-*t*-Bu	198-200 dec	56	$C_{19}H_{27}NO\cdot HCl$	98	73	25	14	1.70	0.09
42[i]	4-OMe	188-189	43	$C_{16}H_{21}NO_2\cdot HCl$	42					
43	4-OBu	172-174	29	$C_{19}H_{27}NO_2\cdot HCl$	84	23	13 (2)		0.13	0.01
44[j]	4-SMe	192-194 dec	68	$C_{16}H_{21}NOS\cdot HCl$	80	55 (2)	42 (2)	22 (2)	0.16 (2)	0.01

Table I (*Continued*)

No.	R	X	Mp, °C	% yield[a]	Formula	Effect on human blood platelets[b]					
						% inhibn of aggregation, μg/ml				% PF3 release, μg/ml	
						100	30	10	3	300	100
45	H	H	170-180	40	$C_{17}H_{21}NO\cdot HCl$						
46	H	OMe	193-195	46[k]	$C_{19}H_{25}NO_3\cdot HCl$	60	11				
47	Me	H	179-180	48	$C_{18}H_{23}NO\cdot HCl$	74	25				
		R									
48[i]	$-C_6H_4CH_2$-*p*-C_6H_5		200-201 dec	63	$C_{20}H_{23}NO\cdot HCl$	61	17	0			
49	$-C_6H_4$-O-*p*-C_6H_5		161-162	57[g]	$C_{18}H_{19}NO_2\cdot HCl$	34					
50	$-C_6H_4$-*p*-CH=(fluoren-9-ylidene)		219-220	27[g]	$C_{26}H_{23}NO\cdot HCl$	0					
51[i]	2-Fluorenyl		243-244 dec	65[g]	$C_{19}H_{19}NO\cdot HCl$	55	29	9			
2[l]	(See text)					68	2	4		0.40	0.01

[a] Compounds were recrystallized from *i*-PrOH and a small amount of H_2O, unless otherwise indicated. [b] In vitro effect of test compound on the inhibition of platelet aggregation caused by ADP in human platelet-rich plasma. PF3 activity is given as percent of maximum. When more than one determination was made this is indicated by the number in parentheses. [c] C. H. Tilford and M. C. Van Campen, Jr., *J. Am. Chem. Soc.*, 76, 2431 (1954). [d] Recrystallized from CH_2Cl_2-Et_2O. [e] Recrystallized from benzene. [f] 1-Naphthyl. [g] Yield includes a second crop. [h] Recrystallized from MeCN-H_2O. [i] Reference 3. [j] RMI 14 133A. [k] Recrystallized from MeOH-MeCOEt. [l] Reference 2.

find therapeutic use, particularly in disease states frequently associated with hypersensitivity of platelets to circulating aggregating agents (e.g., atherosclerosis and diabetes).[5,6]

Chemistry. The compounds shown in Table I were prepared by our modification of the Schopf reaction[2,3] as shown in Scheme I. Methyl ketones were treated with magnesium methyl carbonate (MMC) in dimethylformamide[7] and the resulting internal enolate magnesium salts of β-keto acids were allowed to react at room temperature with 2,3,4,5-tetrahydropyridine, generated in situ from the trimer,[3,8] in the presence of CO_2. The scope and limitations of this reaction have been reported earlier.[3] The reaction has also been applied successfully in another laboratory.[9] Representative examples are described in the Experimental Section.

With the group of compounds **3–15**, simple substitution on the phenyl ring was explored. The substituents vary in inductive and resonance effects as well as in lipophilicity. The group of compounds **17–25** has in common two phenyl groups linked either directly or through an ether, thioether, methylene, or vinylene bridge. Compounds **26** and **27** represent aliphatic examples; **28** is the adamantyl analogue. The group of compounds **29–34** represents diphenylmethyl analogues and **35–39** tricyclic aryl derivatives. Compounds **40–47** are phenylethenyl and -butadienyl ketones, which, to our knowledge, were not available through previously known synthetic methods. This is also true of the pyrrolidinemethyl ketones **48–51**.

The methyl ketones required as starting materials for preparation of the compounds of Table I were either commercially available or were prepared by known synthetic procedures. Preparation of the novel *trans*-4-(*p*-methylthiophenyl)-3-buten-2-one required for synthesis of **44** is described in the Experimental Section. The alcohols listed in Table II were prepared by $NaBH_4$ reduction of the corresponding ketones. In some instances, the diastereomeric isomers were separated (e.g., **53**, **53a**); in others a mixture of isomers was obtained. Ir, uv, and NMR spectra were obtained for all compounds and were consistent with the assigned structures.

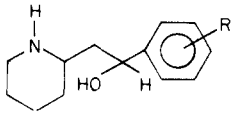
Inhibition of Platelet Aggregation. The compounds listed in Tables I and II were evaluated for inhibition of ADP-induced aggregation of human blood platelets by the method of Mustard et al.[10] and for release of platelet factor 3 (PF3) by the method of MacKenzie et al.[11] PF3 is a procoagulant factor and its release is an undesired property.[12] Because MacKenzie et al.[11] showed that PF3 release or PF3-like activity caused by a normal breakfast in volunteers is in the order of 0.1–0.3% of the maximum PF3 activity released from platelets on sonication, we adopted these values as our limit of acceptability.

Of all compounds listed in Tables I and II, **44** was the most active by far. Other compounds that showed high in vitro activity were the ketones **7**, **14–16**, **18**, **30**, **37**, and **41** and the carbinols **54–56**, **61**, **64**, **66**, and **72**.

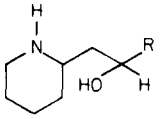
It is evident that aromatic substituents that affect inductive forces and resonance do not greatly enhance inhibitory activity but that large lipophilic substituents do (**3–15**). This pattern extends into the groups of biphenyl (**17–25**), diphenylmethylene (**29–34**), tricyclic aryl (**35–39**), and phenylvinylene analogues (**40–47**) and is mirrored in the piperidineethanols (**52–72**). In fact, SAR can most readily be summarized by the conclusion that platelet aggregation inhibitory activity of piperidinemethyl ketones and piperidineethanols requires a large lipophilic substituent. A similar conclusion was reached in other series.[12,13] A notable exception to this finding is the adamantanyl analogue **28** that showed little activity. Once the requirement for a large lipophilic substituent is met, aromatic substituents do affect the degree of inhibitory activity. This is particularly evident in the phenylethenyl ketone series (**40–44**), in which the thiomethyl substituent of **44** markedly enhances the activity of that compound over the activity of differently substituted analogues. The pyrrolidine analogues (**48–51**, **73–75**) were less active in every instance in which direct comparison with the corresponding piperidine analogue could be made. Comparison of ketones with the corresponding alcohols gives a mixed pattern. In many instances the analogues are nearly equally active (e.g., **16** vs. **54**, **18** vs. **56**, **22** vs. **59**, **29** vs. **62**, **38** vs. **67**); in some the alcohols are more active (**53** vs. **9**, **61** vs. **25**, **66** vs. **37**); in others the ketones are more active (**5** vs. **52**, **40** vs. **68**, **44** vs. **70**). Thus by a number of SAR criteria, **44** stands out for its high activity.

A number of compounds were evaluated in an ex vivo system in which the degree of platelet aggregation induced by small amounts of ADP is determined on platelet-rich plasma obtained from guinea pigs given test compound orally for 4 days. These data are shown in Table III.

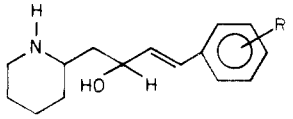
Table II. 2-Piperidineethanols and 2-Pyrrolidineethanols as Inhibitors of Platelet Aggregation in Human Plasma



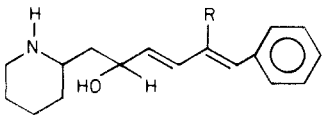
52-61



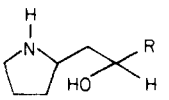
62-67, 67a



68-70



71, 72



73-75

No.	R	Mp, °C	% yield[a]	Formula	Effect on human blood platelets[b]: % inhibn of aggregation, μg/ml: 100	30	10	3	% PF3 release, μg/ml: 300	100
52[c]	4-*t*-Bu	223-224 dec	34[d]	$(C_{17}H_{27}NO)_2 \cdot C_4H_4O_4$[e]	57	0				
53	4-Cl	188-190	24[d]	$C_{13}H_{18}ClNO \cdot HCl$	62 (2)	17 (2)	11 (2)		0.09	0.04
53a[f]		126-129	28[g]	$C_{13}H_{18}ClNO$	50 (2)	10	0			
54	2-CH=CHCH=CH-3[h]	152-155	94[i]	$C_{17}H_{21}NO \cdot C_2H_4O_3$[j]	65	22			0.07	0.00
54a[f]		150-155 dec	76[i]	$C_{17}H_{21}NO \cdot C_2H_4O_3$[j]	64	13			0.12	0.00
55[c]	4-C_6H_5	152-170	39[d]	$C_{19}H_{23}NO \cdot C_2H_4O_3$[j]	89	41			0.21 (3)	0.01 (3)
56[c]	4-C_6H_4-*p*-Cl	164-166 dec	73	$C_{19}H_{22}ClNO \cdot C_4H_4O_4$[k]	97	46	21			
57[c]	4-C_6H_4-*p*-OMe	153-156 dec	87	$C_{20}H_{25}NO_2 \cdot C_2H_4O_3$[j]	92	17 (2)	14			
58	4-OC_6H_5	138-140 dec	90	$C_{19}H_{23}NO_2 \cdot C_2H_4O_3$[j]	68	6				
58a[f]		103-105 dec	45	$C_{19}H_{23}NO_2 \cdot C_2H_4O_3$[j]	50	12				
59[c]	4-O-C_6H_4-*p*-OMe	177-184 dec	83	$(C_{20}H_{25}NO_3)_2 \cdot C_4H_4O_4$[e]	42					
60[c]	4-S-C_6H_5	113-115 dec	75	$C_{19}H_{23}NOS \cdot C_4H_4O_4$[k]	67	0				
61[c]	4-*trans*-CH=CHC_6H_5	160-184 dec	78	$C_{21}H_{25}NO \cdot C_2H_4O_5$[j]	97	54 (2)	9 (2)			
62[c]	-$CH(C_6H_5)_2$	155-187 dec	12	$C_{20}H_{25}NO \cdot C_2H_4O_3$[j]	59					
63[c]	-$CH(C_6H_5)C_6H_2$-2,4,6-Me_3	215-219 dec	63	$C_{23}H_{31}NO \cdot C_2H_4O_3$[j]	69	33				
64	-$CH(C_6H_5)C_6H_4$-4-Cl	176-180 dec	51	$C_{20}H_{24}ClNO \cdot C_2H_4O_3$[j]	94	23	4			
64a[f]		149-152 dec	38	$C_{20}H_{24}ClNO \cdot C_2H_4O_3$[j]	91	9				
65[c]	-$C(C_6H_5)_2C_3H_7$	172-173 dec	62	$C_{23}H_{31}NO \cdot C_2H_4O_3$[j]	93	42	0			
66[c]	2-Dibenzothiophenyl	192-194 dec	68	$C_{19}H_{21}NOS \cdot C_2H_4O_3$[j]	95	61	26 (2)			
67	3-Phenanthryl	172-176 dec	73	$C_{21}H_{23}NO \cdot C_2H_4O_3$[j]	99	38	17			
67a[f]		177-179 dec	88	$C_{21}H_{23}NO \cdot C_2H_5O_3$[j]	97	53	6			
68[c]	H	174-178 dec	43	$(C_{15}H_{21}NO)_2 \cdot C_4H_4O_4$[e]	47					
69[c]	4-OMe	148-150 dec	35	$(C_{16}H_{23}NO_2)_2 \cdot C_4H_4O_4$[e]	50					
70[c]	4-SMe	178-184 dec	40	$(C_{16}H_{23}NOS)_2 \cdot C_4H_4O_4$[e]	79	29 (2)	10			
71[c]	H	168-170 dec	45	$(C_{17}H_{23}NO)_2 \cdot C_4H_4O_4$[e]	86	42	0			
72[c]	Me	161-174 dec	32	$(C_{18}H_{25}NO)_2 \cdot C_4H_4O_4$[e]	75	26	24	14		
73[c]	-$C_6H_4CH_2CH_2$-*p*-C_6H_5	191-192 dec	50	$(C_{20}H_{25}NO)_2 \cdot C_4H_4O_4$[e]	66	13 (2)	4			
74[c]	-C_6H_4O-*p*-C_6H_5	96-117	62	$C_{18}H_{21}NO_2 \cdot C_4H_4O_4$[k]	12					
75[c]	2-Fluorenyl	147-149	47	$C_{19}H_{21}NO \cdot C_4H_4O_4$[k]	97	26 (3)	15 (2)			
1[l]	(See text)	172-174 dec		$C_{27}H_{27}NO \cdot C_2H_4O_3$[j]	99 (4)	28 (6)	1 (6)		0.75 (6)	0.16 (6)
1a[f,l]	(See text)	142-144		$C_{27}H_{27}NO$	100	4				

[a] See Table I, footnote *a*. [b] See Table I, footnote *b*. [c] Probably a mixture of diastereoisomers. [d] Recrystallized from Me_2CO-MeOH. [e] Neutral fumarate salt. [f] Pair of diastereoisomers. [g] Recrystallized from hexane. [h] α-Naphthyl. [i] Recrystallized from Me_2CO. [j] Glycolate. [k] Maleate. [l] Reference 2. For data on other reference compounds, see ref 12.

Table III. Effect of Oral Administration to Guinea Pigs on in Vitro ADP-Induced Platelet Aggregation[a]

No.	Daily dose (days), mg/kg po	No. of animals treated (control)	Concn of ADP, μg/ml PRP	Inhibition of aggregation: Av ΔT (% ± SEM) Control	Av ΔT (% ± SEM) Treated	Av total response (cm² ± SEM) Control	Av total response (cm² ± SEM) Treated	% inhibn	p value
7	30 (4)	5 (6)	0.45	19.5 ± 2.1	19.4 ± 2.8	4.6 ± 1.7	2.9 ± 1.2	37	N.S.
	30 (4)	5 (6)	0.80	38.5 ± 3.9	41.8 ± 5.5	8.3 ± 0.5	8.5 ± 1.2	0	
14	30 (4)	7 (7)	0.45	16.4 ± 2.8	16.1 ± 2.1	2.4 ± 0.8	3.0 ± 1.0	0	
	30 (4)	7 (7)	0.80	34.8 ± 1.7	31.0 ± 2.1	8.0 ± 0.6	7.8 ± 0.5	3	N.S.
15	30 (4)	6 (8)	0.45	40.9 ± 7.5	31.8 ± 4.8	6.5 ± 2.5	3.7 ± 1.7	44	N.S.
	30 (4)	6 (7)	0.80	69.1 ± 6.7	55.4 ± 5.0	16.3 ± 1.8	11.0 ± 1.7	32	0.05
	30 (4)	6 (8)	0.45	40.3 ± 6.1	41.5 ± 4.2	6.2 ± 1.7	5.6 ± 1.4	10	N.S.
	30 (4)	6 (7)	0.80	67.8 ± 5.3	65.5 ± 4.5	14.8 ± 1.7	14.8 ± 1.1	0	
	30 (4)	7 (7)	0.45	28.8 ± 4.5	29.8 ± 4.3	3.3 ± 1.2	3.6 ± 0.9	0	
	30 (4)	7 (7)	0.80	47.0 ± 4.4	52.3 ± 4.3	9.2 ± 1.6	11.4 ± 1.3	0	
18	30 (4)	6 (6)	0.45	17.0 ± 6.1	25.0 ± 3.1	12.2 ± 5.2	16.6 ± 3.7	0	
	30 (4)	6 (6)	0.80	47.8 ± 4.6	47.2 ± 2.3	8.9 ± 1.9	9.6 ± 1.0	0	
41	30 (4)	7 (7)	0.45	19.4 ± 4.9	14.9 ± 4.8	2.4 ± 0.9	2.1 ± 1.3	11	N.S.
	30 (4)	7 (7)	0.80	40.1 ± 6.7	35.1 ± 5.6	8.4 ± 1.8	7.4 ± 1.8	11	N.S.
44[b]	10 (4)	7 (8)	0.55	23.7 ± 3.0	29.3 ± 4.0	3.2 ± 0.7	6.4 ± 1.8	0	
	10 (4)	7 (8)	0.80	33.3 ± 3.6	42.4 ± 6.2	7.5 ± 1.2	8.8 ± 1.7	0	
	30 (4)	6 (6)	0.45	21.4 ± 2.8	15.3 ± 2.6	29.0 ± 11.5	10.2 ± 2.4	65	$0.05 > p < 0.1$
	30 (4)	6 (6)	0.80	36.2 ± 3.7	36.1 ± 2.8	7.6 ± 1.2	6.7 ± 0.9	12	N.S.
	30 (4)	7 (7)	0.55	19.2 ± 5.4	6.0 ± 1.8	32.9 ± 13.8	4.2 ± 2.3	87	0.05
	30 (4)	7 (7)	0.80	39.0 ± 6.7	18.7 ± 2.8	8.2 ± 1.9	2.5 ± 0.8	70	<0.02
	100 (4)	6 (6)	0.45	19.3 ± 7.2	7.0 ± 4.4	3.1 ± 1.4	0.4 ± 0.3	87	$0.05 > p < 0.1$
	100 (4)	6 (6)	0.80	47.6 ± 6.3	33.7 ± 7.1	10.1 ± 2.3	6.1 ± 2.7	40	N.S.
	100 (4)	6 (7)	0.55	28.0 ± 4.9	10.9 ± 3.4	5.3 ± 1.6	1.0 ± 0.7	83	<0.05
	100 (4)	6 (7)	0.80	45.0 ± 6.8	23.0 ± 6.3	10.0 ± 2.1	3.5 ± 2.1	65	0.05
	100 (1)	5 (8)	0.55	31.3 ± 10.1	11.7 ± 3.6	5.6 ± 2.6	0.7 ± 0.4	88	0.05
	100 (1)	5 (8)	0.80	57.5 ± 7.0	39.7 ± 6.3	12.8 ± 2.0	9.1 ± 2.3	28	$0.2 > p < 0.3$
	300 (4)	8 of 9 animals died within 4 days							
55	30 (4)	6 (5)	0.45	6.8 ± 2.8	7.6 ± 2.9	2.5 ± 1.0	3.3 ± 1.5	0	
	30 (4)	6 (5)	0.80	25.6 ± 4.4	31.7 ± 3.9	5.3 ± 1.4	5.6 ± 1.7	0	
64	30 (4)	7 (7)	0.45	30.3 ± 2.9	32.7 ± 2.7	4.7 ± 0.8	4.6 ± 0.9	2	N.S.
	30 (4)	7 (7)	0.80	38.3 ± 2.6	39.2 ± 2.7	8.6 ± 1.2	6.8 ± 1.0	20	N.S.

[a] Compound administered orally for 4 days. Blood taken 2 h after the last dose. See Experimental Section. For ex vivo data on compound **1**, see ref 1. [b] RMI 14 133A.

Compound **44** (RMI 14 133A) consistently showed activity at 100 and 30 mg/kg/day. At 300 mg/kg/day guinea pigs did not survive the 4-day test period. **44** was given orally to dogs for 4 weeks at 50 and 100 mg/kg/day. The compound caused death within 9 days at the higher dose. Both levels caused local irritation to the upper gastrointestinal tract.[14] It was concluded from these studies that while **44** inhibits blood platelet aggregation it has an unfavorable therapeutic ratio.

Accumulation of 3β-Cholesta-5,7-dien-3-ol. Several compounds were evaluated for effects on plasma lipids in rats. Compounds were administered by admixture to the diet and feeding for 10 days. Serum cholesterol concentrations were determined and compared to those of an untreated control group observed simultaneously. The results are shown in Table IV. Compound **18** (RMI 12 346A) reduced serum cholesterol levels by 85 and 68% at doses of 56 and 22 mg/kg, respectively. Examination of plasma sterols of rats treated with **18** showed accumulation of 3β-cholesta-5,7-dien-3-ol. This suggests that **18** inhibits the enzyme 7-dehydrocholesterol Δ^7-reductase. A number of such inhibitors are known,[15] particularly *trans*-1,4-bis(2-dichlorobenzylaminomethyl)cyclohexane dihydrochloride (AY-9944)[16] and analogues,[17,18] boxidine [1-[2-[4'-(trifluoromethyl)-4-biphenylyl]oxy]pyrrolidine] and related basic ether-substituted biphenyls,[19,20] trifluperidol,[21] and basic ether-substituted phenylbenzimidazoles and related diamines.[22,23] Of these, only boxidine resembles compound **18** in that it also contains a biphenyl group. Otherwise, **18** appears to represent a novel structure type among 7-dehydrocholesterol Δ^7-reductase inhibitors. It is apparent from the large number of inactive compounds in Table IV that structural requirements for this activity are highly specific.

Table IV. Effect on Plasma Cholesterol in Rats[a]

No.	Daily dose, mg/kg dd[b]	% redn vs. control	No.	Daily dose, mg/kg dd[b]	% redn vs. control
2	29	0	**27**	32	0
6	32	10[c]	**33**	32	8[c]
7	90	27[d]	**36**	34	14[c]
14	31	16[c]	**37**	42	6[c]
15	45	7[c]	40	31	8[c]
17	28	16[d]	44	61	1[c]
18[e]	56	85[d]	**48**	32	0
	22	68[d]	**50**	30	12[c]
19	26	0	51	30	0
23	50	9[c]	56	47	72[d]
24	136	27[d]	57	56	10[c]
25	20	75[d]			

[a] Groups of six animals were treated for 10 days; plasma cholesterol was determined and compared to that of an untreated control group. [b] Daily dose administered by admixture to food. Dose calculated from food consumption measurement. [c] Not statistically significant ($p > 0.05$). [d] Statistically significant at $p < 0.05$. [e] RMI 12 436A.

Experimental Section

Melting points were determined in open capillaries in a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were taken on a Perkin-Elmer 521 instrument. NMR spectra were taken in a Varian Model A-60 instrument (Me_4Si as internal standard). Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of theoretical values.

1-(4-Dodecylphenyl)-2-(2-piperidinyl)ethanone Hydrochloride (7). Magnesium methyl carbonate[7] (0.5 mol, 1 M in

DMF) was heated to 120° under CO_2, 28.9 g (0.1 mol) of 1-(4-dodecylphenyl)ethanone was added, and the mixture was stirred at 120° for 4 h under a stream of N_2; MeOH that was formed was allowed to escape. The mixture was cooled to room temperature, 10.1 g (0.12 mol) of 2,3,4,5-tetrahydropyridine trimer (α-tripiperidein)[3] was added, and the mixture was stirred for 66 h in an atmosphere of CO_2. The reaction mixture was poured into 800 ml of 2 N HCl, and the resulting precipitate was collected, dissolved in CH_2Cl_2, dried (Na_2SO_4), and azeotroped with Et_2O until product crystallized. It was recrystallized from CH_2Cl_2–Et_2O to give 32.5 g (80%) of 7 (Table I): ir (KBr) 1673 cm^{-1}.

1-[4′-Chloro(1,1′-biphenyl)-4-yl]-2-(2-piperidinyl)ethanone Hydrochloride (18). Magnesium methyl carbonate[7] (0.5 mol, 1 M in DMF) was heated to 120° under CO_2, 23.1 g (0.1 mol) of 1-[4′-chloro(1,1′-biphenyl)-4-yl]ethanone was added, and the mixture was stirred at 120° for 4 h under a stream of N_2; MeOH that was formed was allowed to escape. The mixture was cooled to room temperature, 10.1 g (0.12 mol) of 2,3,4,5-tetrahydropyridine trimer[3] was added, and stirring was continued for 42 h in an atmosphere of CO_2. The mixture was poured into concentrated HCl–ice (1:1), and the precipitated solid was collected, washed with 2 N HCl and Et_2O, and recrystallized twice from *i*-PrOH to give 10.6 g of 18 (Table I): ir (KBr) 1685 cm^{-1}. Collection of a second crop of 12.5 g, mp 180–184°, raised the yield to 66%.

(*E*)-4-[4-(Methylthio)phenyl]-1-(2-piperidinyl)-3-buten-2-one Hydrochloride (44). To a cold (0°) solution of 400 g (2.63 mol) of 4-(methylthio)benzaldehyde in 600 ml of acetone 240 ml of 10% NaOH solution was added dropwise over 45 min. The mixture was stirred at 0° for 4 h and acidified with 2 N HCl, and the resulting precipitate was collected. Recrystallization from 95% EtOH gave 370.6 g (73%) of (*E*)-4-[4-(methylthio)-phenyl]-3-buten-2-one, mp 85–90°. A sample was recrystallized twice from MeCN to mp 99–102°: ir (KBr) 1660 cm^{-1}; uv (MeOH) 336 nm (ϵ 24 500), 244 (9870); NMR ($CDCl_3$) δ 7.51 (d, 1, J = 16.5 Hz), 7.50 (d, 2, J = 8 Hz), 7.25 (d, 2, J = 8 Hz), 6.68 (d, 1, J = 16.5 Hz), 2.50 (s, 3), 2.35 (s, 3). Anal. ($C_{11}H_{12}OS$) C, H.

Magnesium methyl carbonate (0.21 mol, 1 M in DMF) was heated to 120° under CO_2, 10.0 g (0.052 mol) of (*E*)-4-[4-(methylthio)phenyl]-3-buten-2-one was added, and the mixture was stirred at 120° for 4 h under a stream of N_2; MeOH that was formed was allowed to escape. The mixture was cooled to 0°, 4.3 g (0.052 mol) of 2,3,4,5-tetrahydropyridine trimer[3] was added, and stirring was continued for 6 days at 0° in an atmosphere of CO_2. Two additional portions of 2.2 g (0.026 mol) of 2,3,4,5-tetrahydropyridine trimer were added after 24 and 48 h, respectively. The reaction mixture was poured into concentrated HCl–ice (1:1), the product was extracted with CH_2Cl_2, the extract was dried (Na_2SO_4), and the solvent was evaporated. The residue was washed with Et_2O and recrystallized from *i*-PrOH–H_2O to give 11.1 g (68%) of 44 (Table I): ir (KBr) 1685 cm^{-1}; uv (MeOH) λ max 344 nm (ϵ 24 900) 247 (9800); NMR ($CDCl_3$, F_3CCO_2H) δ 7.65 (d, 1 J = 17 Hz), 7.48 (d, 2, J = 8.5 Hz), 7.20 (d, 2, J = 8.5 Hz), 6.70 (d, 1, J = 17 Hz), 3.2–3.8 (broad, 5), 2.50 (s, 3), 1.65–1.95 (broad, 6).

1-[4-(2-Phenylethyl)phenyl]-2-(2-pyrrolidinyl)ethanone Hydrochloride (48). Magnesium methyl carbonate[7] (0.4 mol, 2 M in DMF) was heated to 120° under CO_2, 22.4 g (0.1 mol) of 1-[4-(2-phenylethyl)phenyl]ethanone was added, and the mixture was stirred at 120° for 4 h under a stream of N_2; MeOH that was formed was allowed to escape. The mixture was allowed to cool under CO_2, 8.3 g (0.12 mol) of 3,4-dihydro-2*H*-pyrrole trimer[3] was added, and stirring at room temperature under CO_2 was continued for 40 h. The mixture was poured into 200 ml of concentrated HCl and 800 g of ice, and the resulting precipitate was collected and recrystallized twice from *i*-PrOH–H_2O to give 20.1 g (63%) of 48 (Table I): ir (KBr) 1670 cm^{-1}.

α-(4-Phenoxyphenyl)-2-piperidineethanol Glycolate Diastereoisomers (58, 58a). To 4.75 g (0.126 mol) of $NaBH_4$ in 200 ml of anhydrous EtOH was added 13.9 g (0.042 mol) of 20 and the mixture was stirred at room temperature for 4 h. The mixture was poured into water, acidified with 10% aqueous HOAc to destroy boron complexes, made basic with 2 N NaOH, and extracted into Et_2O. The extract was washed (H_2O) and dried (Na_2SO_4) and the solvent was evaporated to give 13.4 g of oil. To a portion (5.8 g) of this material was added 1.54 g of glycolic acid and two crystallizations from *i*-PrOH gave 3.2 g (90%) of one diastereoisomer of 58, mp 138–140°. From the mother liquors 1.6 g (45%) of the other diastereoisomer 58a, mp 103–105°, was obtained. A mixture melting point showed depression and the fingerprint region of the ir (KBr) spectra of the two diastereoisomers differed.

***trans*-α-(4-Methoxystyryl)-2-piperidineethanol Fumarate (2:1) (69).** To 6.15 g (0.16 mol) of $NaBH_4$ in 250 ml of anhydrous EtOH was added 15.7 g (0.0532 mol) of 42 and the mixture was stirred at room temperature for 4 h. The mixture was poured into water and was extracted with Et_2O. The extract was treated with 10% aqueous HOAc to decompose boron complexes, and the aqueous phase was separated, made alkaline, and reextracted with Et_2O. The extract was dried (Na_2SO_4) and the solvent was evaporated. To the resulting oil (14.0 g) was added 6.5 g (0.056 mol) of fumaric acid in *i*-PrOH and the product that crystallized was recrystallized twice from *i*-PrOH–H_2O and gave 5.2 g (35%) of 69 (Table II): uv (95% EtOH) 263 nm (ϵ 48 200), shoulders at 270, 293 and 305 nm; NMR (Me_2SO-d_6) δ 7.33 (d, 2, J = 8.5 Hz), 6.84 (d, 2, J = 8.5 Hz), 6.51 (d, 1, J = 15 Hz), 6.38 (s, 1, fumaric acid), 6.04 (2 d, 1, J = 15 Hz, J' = 5 Hz), 4.35 (broad, 1), 3.75 (s, 3), 2.9–3.2 (broad, 3), 1.4–1.9 (broad, 8).

Biological Methods. Blood Collection and Isolation of Plasma. Whole blood was obtained from voluntary, experienced donors before breakfast. Donors were instructed to take no drugs, particularly aspirin,[24] for 5 days before giving blood. No plasma was used that was lipemic or, in a preliminary aggregation experiment, showed no second-phase aggregation (aspirin-like effect). Blood was collected by the two-syringe technique and was decalcified with 3.8% Na citrate solution, one part to nine parts of blood. The citrated blood was centrifuged at 100g for 10 min and citrated platelet-rich plasma (PRP) was isolated. Platelet-poor plasma (PPP) was isolated by recentrifuging the blood residue at 1500g for 15 min.

Inhibition of ADP-Induced Platelet Aggregation. Compounds were tested for inhibition of ADP-induced aggregation in a Bryston platelet aggregometer by the procedure of Mustard et al.[10] Human PRP was diluted with autologous PPP to 400 000 platelets/mm^3. Saline was added to another aliquot of the same plasma sample to serve as control. After incubation for 20 min at 37°, ADP (2 μg/ml final concentration) was added to induce aggregation. The increase in light transmittance (ΔT) through the plasma sample in the aggregometer, produced by platelet aggregating, was recorded. The maxima of the ΔT responses for control and test samples were then used to calculate percent inhibition of platelet aggregation by the test compound. More detail on the method is discussed elsewhere.[25]

Platelet Factor 3 Activation. Test compound solution was added to human citrated PRP and incubated at 37° for 20 min; a modified Stypven test was then performed. Plasma was diluted 1:10 for this modified test.[11]

Effect of in Vivo Treatment on in Vitro ADP-Induced Aggregation.[25] Test compound was given to guinea pigs by a stomach tube at the indicated dose for 4 days. An untreated control group was maintained in the same room for the same period of time. Blood was removed by heart puncture 2 h after the last dose and citrated PRP was isolated and adjusted for in vitro ADP-induced platelet aggregation. ADP was added at the concentration indicated in Table III. Max ΔT were obtained as described. The total response was obtained by measuring the area between the aggregation curve and baseline transmittance for the 5-min period following ADP addition. Percent inhibition was calculated from the average total response of treated vs. control group.

Effect on Plasma Cholesterol in Rats. Young male rats of the Wistar strain, obtained from Royalhart Laboratory Animals, Inc., New Hampton, N.Y., weighing 170–190 g initially were used in these tests. The compounds to be tested were mixed thoroughly with Purina Lab Chow (Ralston Purina Co., St. Louis, Mo.), and the diet was fed ad libitum to groups of six animals for 10 days. An untreated control group was included in each experiment. Food consumption and body weights were routinely recorded and these data were used to calculate the average daily dose of the test compounds. At the end of the treatment period, the rats were bled by cardiac puncture. Plasma cholesterol was determined by automated procedures.[26] Values for plasma cholesterol in

treated animals were compared with the values obtained for untreated control rats run simultaneously. Significance of the difference between the values was calculated by Student's t test. The data are expressed as percent reduction from control levels. Plasma cholesterol concentrations for typical control groups were 58 mg/100 ml by this method.

7-Dehydrocholesterol Determination. Nonsaponifiable lipids were extracted into petroleum ether (bp 40–60°) by the method of Abell et al.[27] Tentative identification of 3β-cholesta-5,7-dien-3-ol was indicated by immediate color development in the Lieberman–Burchard test. A silylated sample was gas chromatographed on 5% ECNSS-S on Chromosorb W DMCS at 203°. A peak at 14.8-min retention time was identical with one obtained with an authentic sample of 3β-cholesta-5,7-dien-3-ol. The retention time for cholesterol was 10.8 min on this column.

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Catechol *O*-Methyltransferase. 9. Mechanism of Inactivation by 6-Hydroxydopamine

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A series of methylated analogues of 6-hydroxydopamine (6-OHDA) has been synthesized and evaluated as irreversible inhibitors of catechol *O*-methyltransferase (COMT). These analogues have been prepared in an effort to elucidate the mechanism involved in the inactivation of this enzyme by 6-OHDA. The analogues prepared had methyl groups incorporated in the 2 and/or 5 positions of 6-OHDA so as to block nucleophilic attack at these positions in the corresponding oxidation products [6-hydroxydopamine-*p*-quinone (6-OHDAQ), aminochromes I and II]. Such 2- and/or 5-methylated 6-OHDA analogues were found to be inhibitors of COMT with the inactivation apparently resulting from modification of an essential amino acid residue at the active site of the enzyme. The activity of these analogues as inhibitors of COMT argues against a mechanism involving a 1,4 Michael addition reaction by a protein nucleophile at the 2 or 5 positions on 6-OHDAQ or on the corresponding aminochromes. Instead, an alternative mechanism is proposed to explain these data, which involves attack of a protein nucleophile at the carbonyl group in the 6 position of 6-OHDAQ or at the imine functionality on aminochromes I and II. The results of the present experiments have provided insight into the mechanism involved in inactivation of COMT by 6-OHDA. In addition, this study has provided considerable insight into the chemical reactivity of the electrophilic species generated after oxidation of 6-OHDA.

6-Hydroxydopamine (6-OHDA, 1)[2] has become a widely utilized pharmacological tool, because of its ability to produce selective destruction of norepinephrine- or dopamine-containing nerve terminals.[3,4] The mechanism by which 6-OHDA produces its degenerative effects remains a matter of speculation; however, in part its specificity