Analgetics. Esters of 3-Pyrrolidinemethanols

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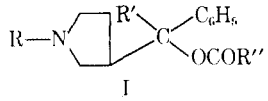
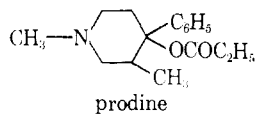
A series of esters of substituted 3-pyrrolidinemethanols has been prepared and tested for analgetic activity. The intermediate alcohols were prepared by the reaction of Grignard reagents with 3-aroylpyrrolidines or the reaction of 2-pyrrolidinones with alkyl phenyl ketones and $LiNH_2$ in liquid NH_3 and subsequent reductions. The more potent analgetics were those in which the N substituent was methyl, phenethyl, or phenylpropyl.

In the course of our search for new analgetics we have synthesized a group of esters of 1,3-disubstituted pyrrolidinemethanols (I). The basic elements of the hypothetical analgetic are apparent as illustrated by a comparison of their structure with that of prodine.



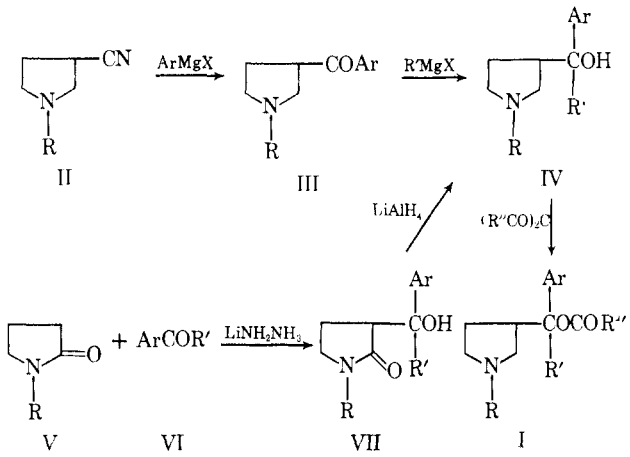
I



prodine

The preparation of these compounds is illustrated in Chart I. Reaction of 3-cyanopyrrolidine[1] (II) with an arylmagnesium bromide gave the 3-aroylpyrrolidine

CHART I



(III) and further reaction with the same or different Grignard reagent yielded the carbinols (IV). The ketones (III) yielded predominately one of the two possible diastereoisomeric racemates where R′ is different from Ar. For example, 3-benzoyl-1-methylpyrrolidine gave, on reaction with ethylmagnesium bromide, 47% of the α-carbinol and only 10% of the β isomer separated by fractional crystallization.

Alternatively, the carbinols (IV) were prepared by the reaction of a 2-pyrrolidinone (V) with an alkyl phenyl ketone (VI) and $LiNH_2$ in liquid ammonia[2] followed by reduction of the intermediate (VII) with $LiAlH_4$. The isolated over-all yield of the α- and β-carbinols (IV) by this method was 40–60 and 10–15%, respectively. The carbinols were acylated with acetic or propionic anhydride in benzene.

(1) J. Swidinsky, J. Kervenski, and B. B. Brown, *J. Pharm. Sci.*, **56**, 192 (1967).

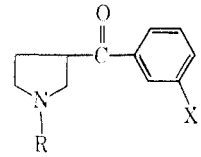
(2) Sandoz, S. A., Belgian Patent 663,377 (May 11, 1965).

The N-substituent of I was conveniently varied by starting with the appropriate 1-substituted 3-cyanopyrrolidine (II) or by catalytically hydrogenating the 1-benzylpyrrolidine (IV, R = benzyl) to the corresponding secondary amine and alkylating with the appropriate alkyl halide.

Details are given in Tables I–V and in the Experimental Section.

TABLE I

3-AROYLPYRROLIDINES

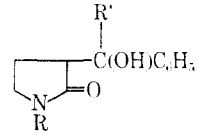


R	X	Yield, %	Mp or bp (mm), °C	Formula[c]
CH_3	H	41	93–95 (0.05)	$C_{12}H_{15}NO$
C_2H_5	H	44	97–99 (0.05)	$C_{13}H_{17}NO$
$CH(CH_3)_2$	H	38	125–127	$C_{14}H_{19}NO \cdot C_4H_4O_4$[a]
			98–100 (0.003)	
$CH_2C_6H_5$	H	53	116–118.5	$C_{19}H_{19}NO \cdot HCl \cdot H_2O$[b]
			172–175 (0.08)	
CH_3	OCH_3	25	121–124 (0.07)	$C_{13}H_{17}NO_2$

[a] Fumarate from *i*-PrOH. [b] From H_2O. [c] All compounds were analyzed for N.

TABLE II

3-SUBSTITUTED 2-PYRROLIDINONES



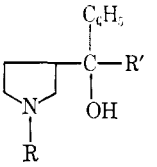
R	R′	Isomer	Yield, %	Mp, °C	Formula[c]
C_2H_5	C_6H_5	···	95	98–99[a]	$C_{19}H_{21}NO_2$
CH_3	C_6H_5	α	45	95–97.5[a]	$C_{14}H_{19}NO_2$
CH_3	CH_3	α	68	79.5–83.5[a]	$C_8H_{17}NO_2$
$(CH_2)_2C_6H_5$	C_6H_5	···	83	110–111[b]	$C_{25}H_{25}NO_2$
C_2H_5	C_2H_5	α	60	95–97[a]	$C_{15}H_{21}NO_2$

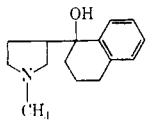
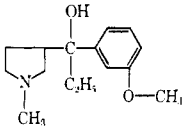
[a] Recrystallized from *i*-Pr_2O. [b] Recrystallized from *i*-Pr_2O isooctane. [c] All compounds were analyzed for C, H, N.

Pharmacological Methods.—Compounds were tested for analgetic activity in female mice (ICR strain) using a modification of the method of Nilsen.[3] A pair of 23-gauge stainless steel needles, inserted through the base of the tail, served as the electrodes. Each animal was stimulated with pulses having a falling phase of 2-msec duration at a frequency of one/sec and progressively increasing voltage, beginning at 6 v. After every fifth stimulus the voltage was increased by 2 v,

(3) P. Nilsen, *Acta Pharmacol. Toxicol.*, **18**, 10 (1961).

TABLE III
PYRROLIDINEMETHANOLS



R	R′	Isomer[a]	Prepn method[b]	Yield, %	Mp or bp (mm), °C	Formula[g]
CH_3	H	α,β	I	76	102–104 (0.05)	$C_{12}H_{17}NO$
CH_3	C_3H_7	α	II	50	98–100 (0.04)	$C_{15}H_{23}NO$[h]
CH_3	C_2H_5	α	I, II	47, 90	78–80[c]	$C_{14}H_{21}NO$
CH_3	C_2H_5	β	I	10	73–75 (0.02)	$C_{14}H_{21}NO$[i]
C_2H_5	H	α,β	III	78	108–109 (0.005)	$C_{13}H_{19}NO$
C_2H_5	C_2H_5	α,β	I, II	51, 71	94–96 (0.01)	$C_{15}H_{23}NO$
$CH(CH_3)_2$	H	α,β	III	84	98–100 (0.02)	$C_{14}H_{21}NO$
C_2H_5	$CH_2C_6H_5$	α,β	I	75	147–149 (0.01)	$C_{20}H_{25}NO$
$CH(CH_3)_2$	C_2H_5	α,β	I	72	91–93 (0.03)	$C_{16}H_{25}NO$
$CH_2C_6H_5$	H	α,β	III	43	149–150 (0.05)	$C_{18}H_{21}NO$
$C_6H_5CH_2$	H	α	III	24	127–128[d]	$C_{18}H_{21}NO$
$C_6H_5CH_2$	CH_3	α,β	I	63	148–150 (0.02)	$C_{19}H_{23}NO$
$C_6H_5CH_2$	C_2H_5	α,β	I	82	155–157 (0.01)	$C_{20}H_{25}NO$
$C_6H_5CH_2$	C_2H_5	α	I	42	72–74[e]	$C_{20}H_{25}NO$
H	C_2H_5	α	IV	49	118–120[c]	$C_{13}H_{19}NO$
H	C_2H_5	β	IV	9	101–105[c]	$C_{13}H_{19}NO$
$C_6H_5CH_2CH_2$	C_2H_5	α	V	76	167–169[f]	$C_{21}H_{27}NO \cdot HCl$
$C_6H_5CH_2CH_2CH_2$	C_2H_5	α	V	78	140.5–142.5[f]	$C_{22}H_{29}NO \cdot HCl$
$C_6H_5OCH_2CH_2$	C_2H_5	α	V	90	138–140[f]	$C_{21}H_{27}NO_2 \cdot HCl$
		α	II	10	118.5–121[k]	$C_{15}H_{21}NO$
		α,β	I	56	111–113 (0.10)	$C_{15}H_{23}NO$

[a] α and β designate the two possible diasteroisomeric racemates; α is arbitrarily assigned to the first racemate isolated. [b] See Experimental Section. [c] Recrystallized from *i*-Pr_2O. [d] Recrystallized from isooctane–C_6H_6. [e] Recrystallized from ligroin. [f] Recrystallized from *i*-Pr_2O–*i*-PrOH. [g] All compounds were analyzed for C, H, N. [h] C: calcd, 77.21; found, 76.64. [i] C: calcd, 76.66; found, 76.24.

until the animal's "pain threshold" was reached. This end point is defined as that voltage which produces vocalization (squeaks) to each of three consecutive stimuli. Only those animals whose end points were constant at 8 v on two predrug trials (approximately 1 hr apart) were utilized.

A minimum of five mice was used at an initial dose of 20 mg of free base/kg. If analgesia (defined as an increase in the pain threshold by at least 2 v) was demonstrated in 80% of the animals 15 min after intraperitoneal administration, an ED_{50} was obtained, using a minimum of four dose levels of 20 animals. Calculations were based on the method of Litchfield and Wilcoxin.[4]

Toxicity was estimated in female mice of the same strain, using two animals per dose level.

A few compounds were further investigated for analgetic activity using the method of Randall and Selitto.[5] The results were comparable to those obtained using the simpler Nilson method.

Pharmacological Results.—In general those compounds (Table VI) in which the N-substituent was methyl, phenethyl, or phenylpropyl were the most active analgetics. With the exception of the phenoxyethyl analog (**18**), these results agree with those obtained on similarly N-substituted meperidine and prodine analogs.[6] Compounds in which the substituent on the quaternary carbon (R′) was ethyl or propyl showed higher analgetic activity than those in which this substituent was hydrogen, methyl, phenyl, or benzyl. No observable difference in activity between the corresponding acetates (**22**, **23**) and propionates (**4**, **9**) was demonstrated. The alcohols were inactive as analgetics. In the two instances where the diastereoisomeric racemates were separated and tested (**4**, **5**, and **9**, **10**) no significant difference in activity was observed.

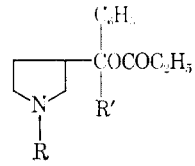
At effective analgetic doses these compounds produced neither marked sedation nor stimulation. Ataxia, piloerection, or other easily observable signs were not seen. At the higher dose levels used in the toxicity study, however, some of the animals convulsed prior to death. The effective potency range, coupled with an absence of respiratory depression and a lack of nalorphine antagonism in those compounds tested,

(4) J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).

(5) L. O. Randall and J. J. Selitto, *Arch. Intern. Pharmacodyn.*, **111**, 409 (1957).

(6) R. A. Hardy, Jr., and M. G. Howell in "Analgetics," G. deStevens Ed., Academic Press Inc., New York, 1965, Chapter V.

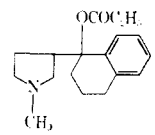
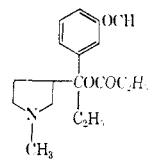
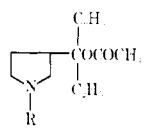
TABLE IV
PYRROLIDINEMETHANOL ESTERS



No.	R	R′	Isomer	Yield, %	Mp or bp (mm), °C	Formula[b]
1	CH_3	H	α,β	58	91–93 (0.10)	$C_{15}H_{21}NO_2$
2	CH_3	CH_3	α,β	38	90–92 (0.02)	$C_{16}H_{23}NO_2$
3	CH_3	C_2H_5	α,β	65	95–96 (0.02)	$C_{17}H_{25}NO_2$
4	CH_3	C_2H_5	α	66	112–114[a]	$C_{21}H_{29}NO_6$[c]
5	CH_3	C_2H_5	β	67	94–96 (0.02)	$C_{17}H_{25}NO_2$
6	CH_3	n-C_3H_7	α	90	108–111 (0.03)	$C_{18}H_{27}NO_2$
7	C_2H_5	H	α,β	67	107–108 (0.005)	$C_{16}H_{23}NO_2$
8	C_2H_5	C_2H_5	α,β	68	122–124 (0.01)	$C_{18}H_{27}NO_2$
9	C_2H_5	C_2H_5	α	57	108–110[b]	$C_{22}H_{31}NO_6$[c]
10	C_2H_5	C_2H_5	β	71	143.5–145[c]	$C_{22}H_{31}NO_6$[c]
11	C_2H_5	C_6H_5	...	42	168–169[d]	$C_{22}H_{28}ClNO_2$[f]
12	C_2H_5	$CH_2C_6H_5$	α	69	144–145.5[a]	$C_{27}H_{33}NO_6$[c]
13	$CH(CH_3)_2$	H	α,β	70	96–98 (0.02)	$C_{17}H_{25}NO_2$
14	$CH(CH_3)_2$	C_2H_5	α,β	51	107–109 (0.005)	$C_{19}H_{29}NO_2$
15	$C_6H_5CH_2$	H	α,β	60	155–156 (0.05)	$C_{21}H_{25}NO_2$
16	$C_6H_5CH_2$	C_2H_5	α,β	55	145–148[c]	$C_{25}H_{31}NO_6$[g]
17	$C_6H_5CH_2CH_2$	C_2H_5	α	66	137–139[c]	$C_{26}H_{33}NO_6$[g]
18	$C_6H_5OCH_2CH_2$	C_2H_5	α	57	143–147[a]	$C_{26}H_{33}NO_7$[g]
19	$C_6H_5(CH_3)_3$	C_2H_5	α	62	119–122[a]	$C_{27}H_{35}NO_6$[g]

[a] Recrystallized from *i*-PrOH–*i*-Pr_2O. [b] Methyl isobutyl ketone. [c] *i*-PrOH. [d] Et_2O–Me_2CO. [e] Fumarate salt. [f] HCl salt. [g] Oxalate salt. [h] All compounds were analyzed for C, H, N.

TABLE V
PYRROLIDINECARBINOL ESTERS

No.	Structure	Isomer	Yield, %	Mp or bp (mm), °C	Formula[c]
20		α	87	103–104 (0.05)	$C_{18}H_{25}NO_2$
21		α,β	54	120–122 (0.07)	$C_{18}H_{27}NO_3$
22	 R = CH_3	α	69	169–172.5	$C_{20}H_{27}NO_6$[a,b]
23	R = C_2H_5	α	62	100–102 (0.05)	$C_{17}H_{25}NO_2$

[a] Fumarate salt; recrystallized from MeOH–MeCOEt. [b] Potentiometric titration. [c] C, H, N analyses.

TABLE VI
ANALGETIC ACTIVITY AND RELATIVE TOXICITY OF TEST COMPOUNDS IN FEMALE MICE

No.	No. analgetic/no. tested[a]	ED_{50} (95% confid limits), mg/kg	Toxic range,[b] mg/kg
1	4/10		160–320
2	1/5		80–160
3	12/15	10.0 (6.3–18.8)	160–320
4	4/5	12.3 (7.0–21.8)	80–320
5	3/5		80–160
6	4/5	9.8 (4.5–21.3)	120–240
7	0/5		80–160
8	2/5		80–160
9	2/5		80–160
10	2/5		80–160
11	2/5		40–160
12	0/10		80–160
13	5/10		80–160
14	1/10		40–160
15	0/5		160–320
16	0/5		80–160
17	6/10	13.7 (8.3–22.7)	80–160
18	2/10		80–160
19	16/20	7.2 (2.9–18.0)	80–160
20	3/5		80–160
21	3/5		80–160
22	11/15		160–320
23	3/5		80–160
d-Propoxyphene	8/10	11.2 (6.9–18.3)	80–160
Meperidine	5/5	6.4 (4.3–9.6)	80–180

[a] 20 mg/kg ip, 15 min after injection. [b] Two doses which were toxic in 0 and 100% of the animals.

suggests no, or very low, addiction liability. Based on these and other pharmacological tests certain of the compounds have been selected for study in human subjects.

Experimental Section

General procedures are given below for the preparation of the compounds described in this paper. Analyses, yields, and physical properties are recorded in the tables and significant variations in the procedure are noted in the table footnotes. Temperatures are uncorrected. Microanalyses were done by Micro-Tech Laboratories Inc., Skokie, Ill., and Spang Microanalytical Laboratory, Ann Arbor, Mich. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

3-Aroylpyrrolidine Derivatives (Table I).—To a stirred Grignard solution (2.6 moles in 2 l. of ether) was added slowly at a rate which maintained gentle refluxing a solution of a 1-substituted 3-cyanopyrrolidine (2.0 moles) in an equal volume of ether. Stirring was continued for 2 hr after the addition was complete. To the cooled mixture was added slowly a solution of 2.5 moles of NH_4Cl in 1 l. of H_2O. The ether layer was evaporated and the

resulting suspension was heated for 1 hr on a steam bath to ensure hydrolysis of the ketimine. The product was then extracted (Et_2O), carried through an acid–base extraction, and finally purified by vacuum distillation.

3-Substituted 2-Pyrrolidinones (Table II).—To 1.5 l. of liquid NH_3 was added 1.5 moles of Li in small pieces followed by a catalytic amount of $FeCl_3$. Stirring was commenced and when the blue color had changed to gray, 1 mole of the 1-substituted 2-pyrrolidinone was added slowly. Stirring was continued for 1 hr and then an ether solution of 1 mole of the ketone was added slowly. The mixture was stirred for 1 hr and then treated with 1.5 moles of solid NH_4Cl. Ether was added to replace the evaporated NH_3. The ether solution was washed (H_2O), dried ($MgSO_4$), and evaporated. The product which crystallized on standing was purified by recrystallization.

Pyrrolidinecarbinols (Table III). Procedure I. By Reaction of 3-Aroylpyrrolidine with Alkylmagnesium Halide.—To a stirred solution of 0.3 mole of alkylmagnesium halide in 200 ml of ether maintained at 10° was added slowly a solution of 0.15 mole of 1-substituted 3-aroylpyrrolidine in 50 ml of dry ether. After the addition was complete, the mixture was stirred for 1 hr with no external cooling and then treated slowly with a solution of 0.3 mole of NH_4Cl in 300 ml of H_2O. The ether layer was separated and the aqueous suspension was extracted (Et_2O). The combined extracts were washed (H_2O) and the solvent was evaporated. The crude product was purified by distillation or recrystallization. In some cases where solid free bases were obtained the isomers were separated by fractional crystallization.

Procedure II. By Reduction of 3-Substituted 2-Pyrrolidinones.—To a suspension of $LiAlH_4$ (0.90 mole) in 500 ml of THF at gentle reflux was added a solution of 0.60 mole of the 3-substituted 2-pyrrolidinone in 200 ml of THF. The mixture was stirred at gentle reflux for 2 hr, cooled, and poured slowly onto a stirred 25% NaOH (1 l.). The solution was separated and the solvent was evaporated at reduced pressure. The crude product was purified by recrystallization.

Procedure III. By $LiAlH_4$ Reduction of 1-Substituted 3-Aroylpyrrolidine.—To a stirred suspension of 0.20 mole of $LiAlH_4$ in 300 ml of ether was added slowly at a rate which maintained gentle refluxing 0.19 mole of 1-substituted 3-aroylpyrrolidine in 100 ml of ether. Stirring and refluxing were continued for 1 hr after the addition was complete. The mixture was cooled and treated successively with 25 ml of H_2O and 200 ml of 25% NaOH. The ether layer was separated and the aqueous layer was extracted with ether. The combined extracts were washed (H_2O) and evaporated. The carbinol was purified by distillation or recrystallization.

Procedure IV. By Catalytic Reduction of the N-Benzylcarbinol.—A solution of 0.46 mole of the N-benzylcarbinol in 150 ml of 95% EtOH was reduced catalytically with 6 g of 10% Pd–C. The mixture was heated at 70° and shaken with H_2 until 1 equiv of H_2 was absorbed (about 2 hr). After cooling, the suspension was filtered and the solvent evaporated. The residual oil was purified by distillation or recrystallization.

Procedure V. By Alkylation of 3-Substituted Pyrrolidines.—A mixture of 0.09 mole of 3-substituted pyrrolidine, 0.10 mole of arylalkyl bromide, 40 g of K_2CO_3, and 200 ml of toluene was heated at reflux for 16 hr, cooled, and treated with 100 ml of H_2O. The organic layer was separated and washed (cold H_2O), and the solvent was evaporated at reduced pressure. The residual oil was purified by distillation or converted to a solid salt.

Pyrrolidinecarbinol Esters (Tables IV and V).—A mixture of 0.05 mole of the pyrrolidinemethanol, 0.07 mole of propionic or acetic anhydride, 5 ml of pyridine, and 150 ml of C_6H_6 was heated at reflux for 2–5 days under N_2. After cooling, the solution was washed (10% $NaHCO_3$, H_2O). The solvent was evaporated and the crude product was purified by distillation or conversion to a salt.

Acknowledgment.—The authors thank E. K. Rose, John Eyler, and Dr. William J. Welstead, Jr., for technical assistance.

Irreversible Enzyme Inhibitors. CXVI.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase Derived from 6-Substituted 2,4-Diamino-5-phenylpyrimidines. III[3]

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Ten candidate irreversible inhibitors derived from 5-(*p*-chlorophenyl)-2,4-diaminopyrimidine bearing a leaving group on a chain at the 6 position have been evaluated on dihydrofolic reductase from Walker 256 rat tumor and L1210/FR8 mouse leukemia; three had a chloromethyl, four had a sulfonyl fluoride, and three had a bromoacetamido leaving group. Strong evidence was obtained that the diaminopyrimidine could complex as one of two rotomers depending upon the hydrophobicity of the group at the 6 position; 6-phenoxymethyl- and 6-phenethylpyrimidines were bound in a conformation giving a hydrophobic interaction of the 6 group with the enzyme, but the more polar 6-anilinomethylpyrimidines were bound in a "flipped-over" conformation. Three of the sulfonyl fluorides, 6-[*m*-(*m*-fluorosulfonylphenylureido)phenoxymethyl]-2,4-diamino-5-(*p*-chlorophenyl)-pyrimidine (**8**), the 5-(3,4-dichlorophenyl) analog (**10**) of **8**, and the phenethyl analog (**9**) of **8** were good active-site-directed irreversible inhibitors of dihydrofolic reductase.

Recently[3b] the 6-(*p*-chloroacetylanilinomethyl)pyrimidine (**1**) was found to be an active-site-directed irreversible inhibitor[4,5] of dihydrofolic reductase[6] from several sources; that **1** probably was an irreversible inhibitor of the endo type[7] was indicated by the fact that the inactivation was slowed in the presence of the coenzyme, TPNH. The rate of inactivation of an enzyme by an active-site-directed irreversible inhibitor is dependent first upon the concentration of reversible complex between enzyme and inhibitor, which in turn is dependent upon the concentration of inhibitor and the dissociation constant of the reversible enzyme–

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(2) For the previous papers of this series see B. R. Baker and E. H Erickson, *J. Med. Chem.*, **11**, 245 (1968).

(3) For the previous papers on this type of irreversible inhibitor see (a) B. R. Baker and J. H. Jordaan, *J. Pharm. Sci.*, **56**, 660 (1967), paper LXXXVIII of this series; (b) B. R. Baker, P. C. Huang, and A. L. Pogolotti, Jr., *J. Med. Chem.*, **10**, 1134 (1967), paper CVIII of this series.

(4) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(5) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964), a review.

(6) For a review on the mode of binding of inhibitors to dihydrofolic reductase see ref 4, Chapter X.

(7) The endo type of irreversible inhibitor is defined as one that forms a covalent bond within the enzymic active-site, whereas the exo type forms a covalent bond outside of the active site;[5] see also ref 4, Chapter I.