Cholecystokinin Antagonists. Synthesis and Biological Evaluation of 4-Substituted 4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepines

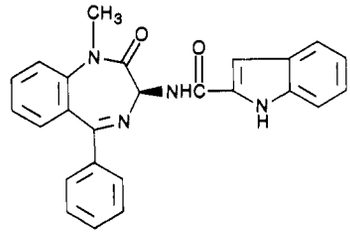
Mark G. Bock,* Robert M. DiPardo, Ben E. Evans,* Kenneth E. Rittle, Daniel F. Veber, Roger M. Freidinger, Raymond S. L. Chang,† and Victor J. Lotti†

Departments of Medicinal Chemistry and Microbial Pharmacometrics, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486. *Received June 29, 1987*

A series of 4-substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepines was prepared by standard methodology. These compounds were tested in vitro as antagonists of the binding of [^{125}I]cholecystokinin (CCK) to rat pancreas and guinea pig brain receptors and of the binding of [^{125}I]gastrin to guinea pig gastric glands. All compounds proved to have greater affinity for the peripheral CCK receptor with some analogues having IC_{50}'s in the subnanomolar range. In vivo activity of selected compounds in mice is presented and the structure/activity profile of this class of benzodiazepines as CCK antagonists is discussed.

The inference that cholecystokinin (CCK) is a major hormonal stimulator responsible for pancreatic and biliary exocrine secretion, gall bladder contraction, and gut motility is now widely accepted.[1-5] Evidence has accumulated that attributes a variety of additional functions to this peptide hormone, including satiety sensation,[6] sedation,[7] and antagonism of the analgesic effects of endogenous opiates.[8] The discovery and wide distribution of CCK in the brain[9-12] has aided in formulating the hypothesis that it may also function as a neurotransmitter or neuromodulator in the central nervous system.[13-16] In light of these findings, the development of effective agents that interact competitively with the CCK receptor becomes all the more essential for the clarification of the role of CCK in normal and pathological physiology.

We recently disclosed the design and synthesis of highly specific nonpeptidal antagonists of CCK[17,18] with subnanomolar potency.[19] The compound of principle significance is the 3-substituted 1,4-benzodiazepin-2-one (L-364,718; 1), which was found to be orally active and to have



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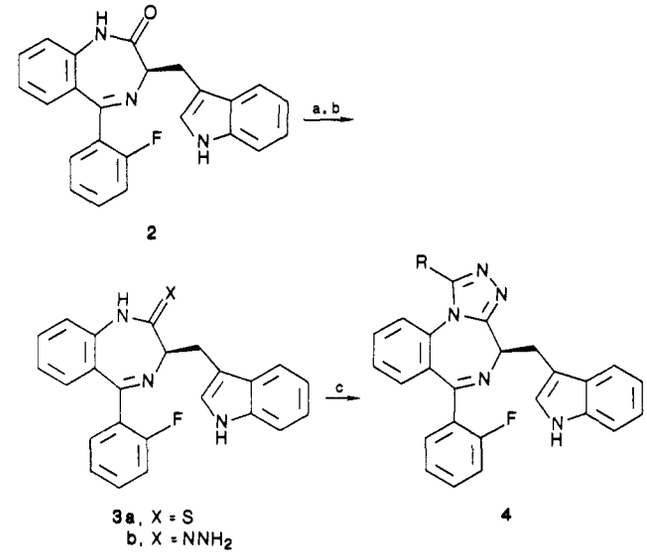
long duration of action. Its preparation represents, to our knowledge, the first example of the successful design of a nonpeptidal ligand for a peptide receptor.

The desirable biological profile of 1 prompted an examination of other classes of benzodiazepines to assess if alternate structure types also possess CCK antagonist activity. In the discussion that follows, we give an account of our work on the synthesis and biological evaluation of the series of 4-substituted triazolobenzodiazepines shown in Table I.[20]

Chemistry

The 3-(indolylmethyl)-1,4-benzodiazepine **2** (Scheme I) was prepared as described by Evans et al.,[18] in which 2-amino-2′-fluorobenzophenone was reacted with D-tryptophan acid chloride hydrochloride followed by cyclization in basic medium. This material was then elaborated to the 4-triazolobenzodiazepines **4–7** (Table I) by the following three-step sequence: conversion of **2** to the thioamide **3a** with 2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane (Lawesson's reagent),[21] treatment of **3a** with hydrazine to give the amidrazone **3b**, and formation

Scheme I[a]



[a] (a) $(CH_3OC_6H_4)_2P_2S_4$, toluene, 110 °C, 1.5 h; (b) NH_2NH_2 (95%), CH_3OH, 23 °C, 2.5 h; (c) $RC(OCH_3)_3$, C_2H_5OH, H_2SO_4 (cat.), 23 °C, 10 h.

†Department of Microbial Pharmacometrics.

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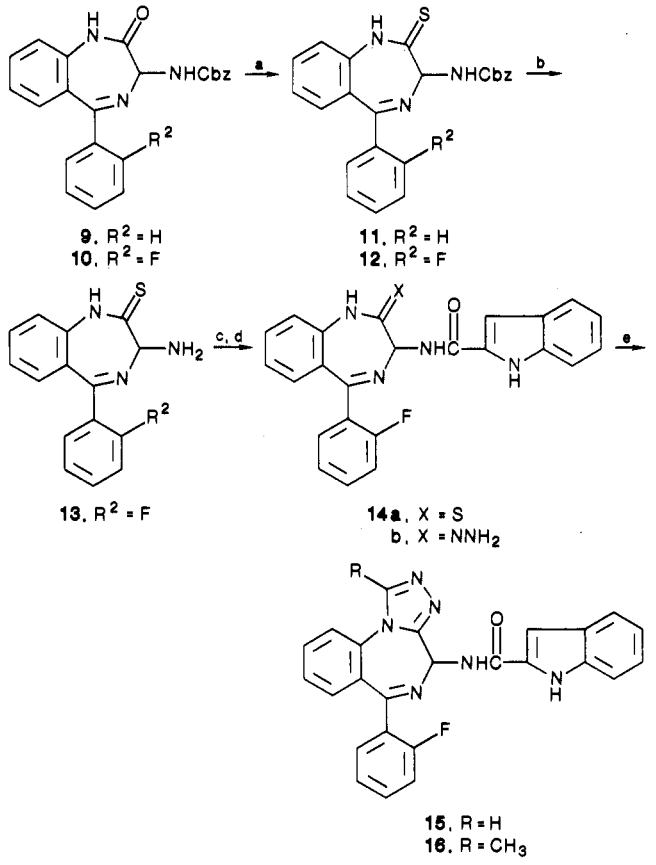
Scheme II[a]

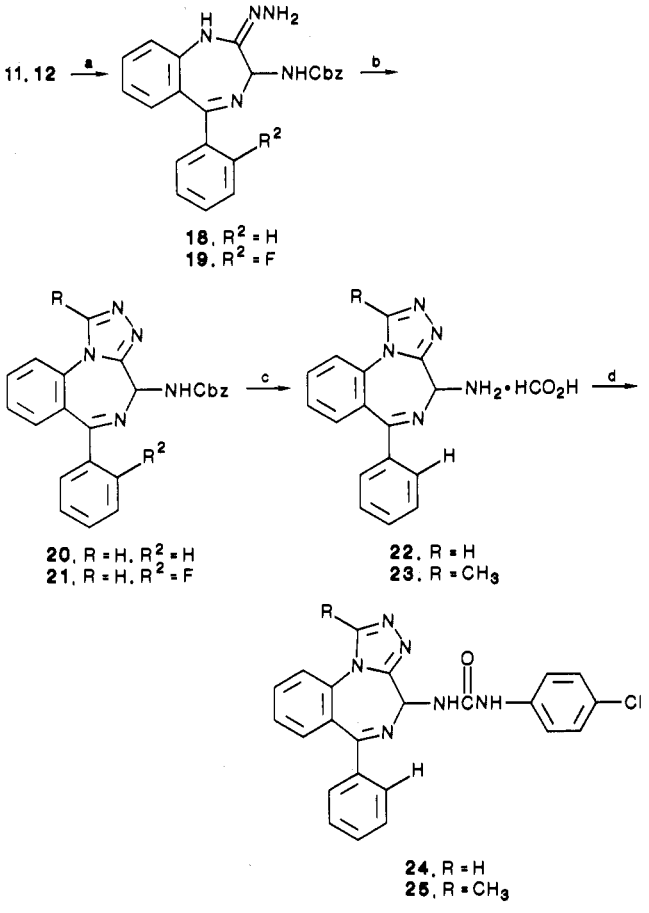


[a] (a) $(CH_3OC_6H_4)_2P_2S_4$, toluene, 110 °C, 5 h; (b) HBr (g), HOAc–CH_2Cl_2, 0 °C, 6 h, then NaOH (20%); (c) indole-2-carboxylic acid, EDC, NEt_3, DMF, 23 °C, 18 h; (d) NH_2NH_2 (95%), CH_3OH, 23 °C, 1.5 h; (e) $RC(OCH_3)_3$, C_2H_5OH, H_2SO_4 (cat.), 23 °C, 10 h.

of the triazole ring in compounds **4–7** by condensing **3b** with the requisite ortho ester in the presence of catalytic quantities of sulfuric acid.[22] Compound **8**, in turn, was obtained by heating **4** with dimethylmethyleneammonium chloride in dry, degassed *N,N*-dimethylformamide. Interestingly, two additional byproducts were detected during the course of this reaction, one of which apparently rearranged to the desired product during the workup/purification procedure. We speculate that these products could have resulted from initial attack of the reagent on the triazole nitrogen atoms[23] but are unable to present conclusive evidence for the proposal.

The 4-triazolobenzodiazepinamides **15–17** were derived in five synthetic operations, as summarized in Scheme II, from the previously described protected 3-aminobenzodiazepines **9** and **10**.[24] Thus, treatment of **9** and **10** with the Lawesson reagent (vide supra) gave **11** and **12**, respectively. Compound **12** was then most conveniently deprotected with hydrogen bromide in a methylene chloride–acetic acid solvent mixture. The 3-amino-1,4-benzodiazepine **13**, isolated as its free base, was acylated by the water-soluble carbodiimide reagent, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC). Final conversion to the triazolobenzodiazepines **15** and **16** was accomplished via the amidrazone **14b** with identical reaction conditions as described for the synthesis of **4** (Scheme I). The preparation of **17** followed a similar course as outlined in Scheme II except that 4-chlorobenzoic acid was substituted for 2-indolecarboxylic acid in the acylation step.

Scheme III[a]



[a] (a) NH_2NH_2 (95%), CH_3OH, 23 °C, 1.5 h; (b) $RC(OCH_3)_3$, C_2H_5OH, H_2SO_4 (cat.), 23 °C, 10 h; (c) 10% Pd/C, CH_3OH–90% HCO_2H (4.5% v/v); (d) 4-chlorophenyl isocyanate, NEt_3, THF, 23 °C, 10 h.

For the synthesis of the triazolobenzodiazepinylureas **24** and **25** (Scheme III), expedience dictated that the triazole ring be fashioned before the 3-amino group was functionalized. Accordingly, the benzyloxycarbonyl (Cbz) protected triazolobenzodiazepines **20** and **21** were obtained in two steps from **11** and **12**, respectively, in the standard fashion. The Cbz group was then removed under transfer hydrogenation conditions to give the corresponding amines **22** and **23** in high yield. Urea formation was then accomplished in a straightforward manner by simply treating the respective amine formate salts of **22** and **23** with 4-chlorophenyl isocyanate.

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(19) Chang, R. S. L.; Lotti, V. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4923.
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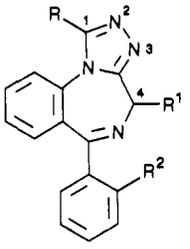
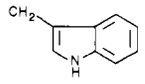
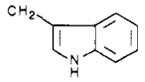
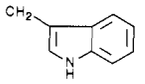
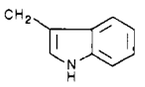
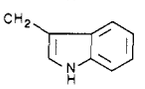
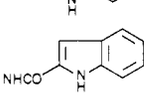
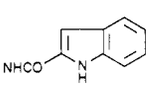
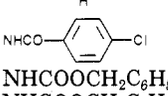
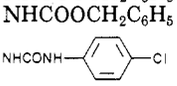
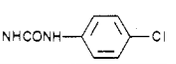
Table I. Physical and Analytical Data for the 4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepines



compd	R	R^1	R^2	conf[a]	yield,[b] %	scheme (proc)[c]	mp, °C	formula[d]	anal.[e]
4	H		F	*R*	47	1 (A)	148–150	$C_{25}H_{18}FN_5 \cdot 0.5CHCl_3$	C, H, N
5	CH_3		F	*R*	40	1 (A)	75[f]	$C_{26}H_{20}FN_5 \cdot 0.5H_2O \cdot 0.4Et_2O$	C, H, N
6	CCl_3		F	*R*	75	1 (A[g])	162 dec	$C_{26}H_{17}Cl_3FN_5 \cdot 0.5CHCl_3$	C, H, N
7	C_6H_5		F	*R*	46	1 (A)	280–283	$C_{31}H_{22}FN_5 \cdot 0.55CHCl_3$	C, H, N
8	$(CH_3)_2NCH_2$		F	*R*	35	1 (B)	134–136	$C_{28}H_{25}FN_6 \cdot 0.22CHCl_3$	C, H, N
15	H		F	*RS*	70	2 (A)	291	$C_{25}H_{17}FN_6O \cdot 0.1EtOAc$	C, H, N
16	CH_3		F	*RS*	68	2 (A)	294 dec	$C_{26}H_{19}FN_6O \cdot 0.2EtOAc$	C, H, N
17	H		F	*RS*	43	2 (A)	250–251	$C_{23}H_{15}ClFN_5O$	C, H, N
20	H	$NHCOOCH_2C_6H_5$	H	*RS*	65	3 (A)	135–138	$C_{24}H_{19}N_5O_2 \cdot 0.25EtOAc$	C, H, N
21	H	$NHCOOCH_2C_6H_5$	F	*RS*	60	3 (A)	123	$C_{24}H_{18}FN_5O_2$	C, H, N
24	H		H	*RS*	32	3 (C)	294–296	$C_{23}H_{17}ClN_6O$	C, H, N
25	CH_3		H	*RS*	17	3 (C)	292–294	$C_{24}H_{19}ClN_6O \cdot 0.35H_2O$	C, H, N

[a] 4-Position of triazalobenzodiazepine ring. [b] Yield refers to analytically pure product. [c] Refers to the scheme in which the general synthesis of the compound is outlined; procedure is detailed in the Experimental Section. [d] All compounds were fully characterized spectroscopically (^{1}H NMR, MS, IR); ^{1}H NMR confirmed the presence of a solvate where indicated. [e] Combustion analyses were within ±0.4% of the theoretical value; compound purity was further verified by HPLC, which in all cases was >97%. [f] Foams. [g] Trichloroacetonitrile was used as coreactant.

Biology

The methods employed for the determination of [^{125}I]-CCK-33 binding to rat pancreas and guinea pig cortex and [^{125}I]gastrin binding to guinea pig gastric glands were previously described.[17,19,25] Values shown are the means of triplicate determinations.

The in vivo activities of selected compounds were determined on the basis of their ability to antagonize CCK-8 inhibition of charcoal meal gastric emptying in mice as described previously.[26] The mice were administered the test compounds at one or more dose levels, ($N \geq 10$/dose) by the oral route 1 h prior to CCK-8. CCK-8 (80 μg/kg, sc) was given 5 min prior to a charcoal meal and gastric emptying determined at the indicated time intervals. ED_{50} values were based upon data obtained at a minimum of three dose levels by regression analysis. The data are presented in Table III.

Discussion

The triazolobenzodiazepines generated in this study (cf. Table I) were tested as antagonists of the binding of [^{125}I]CCK to rat pancreas and guinea pig brain receptors; gastrin antagonism at the receptor level was measured as a function of the displacement of [^{125}I]gastrin from guinea pig gastric glands. These data are summarized in Table II where they are compared with those of the anxiolytic agents alprazolam, **26**, and triazolam, **27**.

After completion of this work, certain 1,4-benzodiazepines (e.g. diazepam, lorazepam, chlordiazepoxide) were demonstrated to antagonize the effects of CCK both in the periphery[27–29] and in the central nervous system[30,31]

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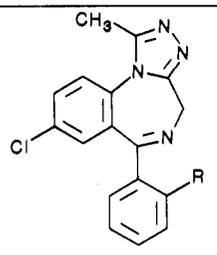
Table II. Receptor Binding Affinities for the 4H-[1,2,4]Triazolo[4,3-a][1,4]benzodiazepines

	IC_{50}, μM		
	[^{125}I]CCK		[^{125}I]gastrin,
compd	pancreas	brain	gastric glands
4	0.2	~100	25
5	0.3	39	12
6	25	>100	>100
7	22	~100	>100
8	14	~100	60
15	0.0009	0.053	0.065
16	0.0002	0.008	0.007
17	0.0044	9.3	2.4
20	0.1	7.7	4.8
21	0.22	8	3
24	0.12	0.48	0.38
25	0.082	0.12	0.38
26[a]	64	>100	>100
27[a]	39	>100	>100

[a]



26, R = H, alprazolam
27, R = Cl, triazolam

Table III. In Vivo CCK Antagonist Potencies for Selected 4H-[1,2,4]Triazolo[4,3-a][1,4]benzodiazepines in Mice

	ED_{50},[a] mg/kg, for antagonism of CCK-8 inhibition of gastric emptying	
compd	po (1 h)	po (5 h)
1 (standard)	0.04	0.06
5	76	200[b]
15	0.02	0.12
16	0.05	0.13[b]
17	0.12	

[a] Dose necessary to protect 50% of the mice from CCK inhibition of charcoal meal gastric emptying as described in ref 26. In concurrent studies, all of the control mice not given CCK exhibited gastric emptying of the charcoal meal (N = 70). 96% of the mice administered the drug vehicle (0.5% methylcellulose) and CCK-8 did not exhibit gastric emptying (N = 70). [b] 4 h.

by as yet undefined mechanisms. The observed effects, however, were weak. In accord with these results, our earlier studies showed the CCK antagonist potencies of 1,4-benzodiazepines to depend critically on the nature of the group attached to the 3-position of the benzodiazepine ring and to the linkage forming the attachment.[17] Perusal of the data presented in Table II indicates that these criteria may be extrapolated to the present study. Thus, alprazolam, **26**, and triazolam, **27**, established the benchmark for 4-unsubstituted triazolobenzodiazepines and, as anticipated, they are inactive by our criteria. The substituent attached to the 4-position of the triazolobenzodiazepine ring, which is optimum for CCK antagonist potency, was again found to be the indole moiety. Similar to previous findings,[17] an aminocarbonyl linkage from the benzodiazepine ring to the 2-position of indole is preferred to a methylene bridge to the 3-position of the indole nucleus (cf. **4** and **15**, **5** and **16**).

The synthesis of compounds **4–8** was undertaken to evaluate the influence of different C-1 substituents on the CCK antagonist activity of the 4-triazolo[4,3-a][1,4]-benzodiazepines. The configuration at the chiral center in all cases is *R* (from D-tryptophan) and was based on the considerations described in our previous disclosures.[17,18] The most potent compound in this group proved to be the C-1-unsubstituted parent compound **4**. The electronegative trichloromethyl group in **6** had a deleterious effect on activity, as did substituents with greater steric bulk than a methyl group (cf. **5** with **6–8**). Incorporation of a (dimethylamino)methyl substituent at C-1 (**8**) also gave no potency enhancement over the C-1-unsubstituted compound **4**.

In this limited study, the effects of substitution on the C-6 phenyl ring and on the 8-position of the benzodiazepine nucleus were only examined in a cursory manner. While subsequent studies will address this point in more detail, our current evidence (exemplified by structures **20** and **21**) is that the presence or absence of a fluoro substituent on the ortho position of the C-6 phenyl ring has little influence on potency and selectivity.

A comparison among the results of the three receptor binding assays in Table II reveals that all compounds exhibited selectivity for the peripheral CCK receptor. The 4-substituted triazolobenzodiazepines in this study, therefore, complement the results obtained with the 3-substituted benzodiazepines, like **1**, and provide an additional tool to examine these receptor classes. Structure **17**, for example, is a CCK receptor antagonist with 4.4 nM affinity and >2000-fold selectivity for peripheral CCK vs central CCK receptors. Interestingly, the more potent compounds **15** and **16** were less discriminating among the three receptors.

Four of the CCK receptor antagonists in Table II were evaluated in more detail and examined in vivo. These results are summarized in Table III. As these data show, compounds **15–17** are orally active in mice. The ED_{50} values are less than 1 mg/kg and are consistent with the corresponding high receptor binding affinities of these compounds. Examination of the ED_{50} values for structures **15** and **16**, determined after 5- and 4-h pretreatments, respectively, indicates that these compounds also have reasonable duration of action.

Summary and Conclusions

We have previously demonstrated that, by appropriate structural modification, benzodiazepines can be tailored to interact competitively with the peripheral CCK receptor.[17-19] The results described in this study demonstrate that our previous findings can be extended to the triazolobenzodiazepine class with similar effect. The compounds disclosed in this work are potent, selective, orally effective antagonists of cholecystokinin. As such, they represent CCK antagonists of alternate structure type with potentially different pharmacokinetic properties.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian EM 390 instrument with tetramethylsilane as an internal standard or on a Nicolet NT-360 spectrometer with an internal lock on the deuterium resonance of the solvent. Fast atom bombardment (FAB) mass spectra were run on a Finnigan-Mat 731 instrument. Infrared spectra were recorded on a Perkin-Elmer 297 spectrophotometer. HPLC was performed on a Hewlett-Packard 1084B instrument with a Waters C-18 column. Flash chromatography was performed as described

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by Still[32] on silica gel (E. Merck, 0.04–0.063 mm); thin-layer chromatography (TLC) and preparative thick-layer chromatography (PTLC) were carried out on E. Merck 60F-254 precoated silica gel plates (0.25, 0.5, and 2.0 mm).

(*R*)-5-(2-Fluorophenyl)-1,3-dihydro-3-(1*H*-indol-3-ylmethyl)-2*H*-1,4-benzodiazepine-2-thione (3a). A solution of 100 mL of toluene containing 6.98 g (18.20 mmol) of **2** and 4.41 g (10.92 mmol) of 2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane (Lawesson's reagent) was refluxed for 1.5 h. The solvent was removed under reduced pressure, and the residue was partitioned between ethyl acetate and sodium hydroxide solution (10%). The organic phase was washed with brine, dried, and rotoevaporated. Plug filtration of the residual brown oil through silica gel (hexane–ethyl acetate elution) gave a solid, which was recrystallized from ether to give the analytical product as an etherate solvate in 65% yield: mp 147–148 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (d, 2 H, 8 Hz), 4.07 (t, 1 H, 8 Hz), 7.1 (m, 5 H), 7.26 (m, 3 H), 7.4 (m, 4 H), 7.64 (d, 1 H, 9 Hz), 8.05 (br s, 1 H), 9.77 (s, 1 H).

(*R*)-[5-(2-Fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-3*H*-1,4-benzodiazepin-2-yl]hydrazine (3b). A solution of 25 mL of methanol containing 1.5 g (3.76 mmol) of **3a** was treated with 2 mL (62 mmol) of hydrazine (95%) at room temperature. The resulting solution was stirred for 2.5 h and concentrated under reduced pressure. The residual oil was dissolved in methylene chloride (100 mL) and washed with water (3 × 100 mL) and brine (1 × 50 mL). The organic phase was concentrated to give 1.4 g of **3b** as a solid, which was used directly without further purification.

General Procedure for the Synthesis of [1,2,4]Triazolo[4,3-*a*]-4-(3-indolylmethyl)-1,4-benzodiazepines. Preparation of (*R*)-6-(2-Fluorophenyl)-4-(1*H*-indol-3-ylmethyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (4). Procedure A. Trimethyl orthoformate (203 mg, 1.91 mmol) and **3b** (230 mg, 0.58 mmol) were combined in 5 mL of ethanol. This solution was treated with a catalytic amount of concentrated sulfuric acid (2 drops) at room temperature and allowed to stand for 10 h. The reaction mixture was treated with saturated sodium bicarbonate solution (2 mL) and concentrated. The residue was dissolved in methylene chloride and washed with water and brine. Rotoevaporation of the dried organic phase gave the crude product (240 mg), which was flash chromatographed on silica gel (chloroform–methanol elution, 95:5 v/v) to give the analytical product.

(*R*)-6-(2-Fluorophenyl)-4-(1*H*-indol-3-ylmethyl)-*N*,*N*-dimethyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine-1-methanamide (8). Procedure B. (*R*)-6-(2-Fluorophenyl)-4-(1*H*-indol-3-ylmethyl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (**4**) (250 mg, 0.61 mmol) and dimethylmethyleneammonium chloride[33,34] (80 mg, 0.85 mmol) were combined in 3 mL of degassed dimethylformamide. The resulting reaction mixture was protected from moisture and heated for 3 h at 80 °C. The reaction mixture was poured into 50 mL of water, rendered alkaline with aqueous sodium hydroxide solution (20%), and extracted with chloroform (3 × 75 mL). The combined organic extracts were dried (sodium sulfate) and rotoevaporated to give the crude product (300 mg). Preparative TLC on silica gel (methylene chloride–methanol elution, 95:5 v/v) afforded the analytical product.

***N*-(4-Chlorophenyl)-*N'*-(6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl)urea (24). Procedure C.** The 4-aminotriazolobenzodiazepine formate salt **22** (200 mg, 0.38 mmol) was combined with 4-chlorophenyl isocyanate (57 mg, 0.37 mmol) and triethylamine (57 μL, 0.41 mmol) in 10 mL of dry tetrahydrofuran. After the mixture stood at room temperature overnight, the product precipitated from solution in analytically pure form.

[5-(2-Fluorophenyl)-2,3-dihydro-2-thioxo-1*H*-1,4-benzodiazepin-3-yl]carbamic Acid Phenyl Methyl Ester (12). Compound **10** (6.5 g, 16.1 mmol) and Lawesson's reagent (4.9 g, 12.1 mmol) were combined in 500 mL of toluene and heated to reflux for 1.5 h. The reaction mixture was diluted to 700 mL with ethyl acetate and washed with sodium hydroxide solution (20%, 4 × 50 mL) and brine (50 mL). The dried organic phase (sodium sulfate) was concentrated in vacuo to yield the crude product as a yellow solid. Trituration with ethyl acetate afforded the analytical product (4 g). An additional 2.2 g of product was obtained after the mother liquor was processed by flash chromatography (hexane–ethyl acetate elution, 1:1 v/v) to give **12** in 92% yield: mp 190–191 °C; ^{1}H NMR ($CDCl_3$) δ 5.18 (s, 2 H), 5.55 (d, 1 H, 8 Hz), 7.03 (t, 1 H, 9 Hz), 7.07 (d, 1 H, 8 Hz), 7.20 (d, 2 H, 8 Hz), 7.3 (m, 4 H), 7.4 (m, 4 H), 7.54 (t, 1 H, 8 Hz), 7.71 (ddd, 1 H, 8, 8, 1.5 Hz), 9.65 (br s, 1 H, NH); MS, *m/e* 419 (M^+). Anal. Calcd for $C_{23}H_{18}FN_3O_2S$: C, H, N.

3-Amino-1,3-dihydro-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepine-2-thione (13). The benzyl carbamate **12** (1.0 g, 2.38 mmol) was dissolved in a 1:1 mixture of methylene chloride and glacial acetic acid. The resulting solution was cooled to 0 °C and treated with a continuous stream of hydrogen bromide gas for 6 h. The reaction mixture was concentrated under reduced pressure, and the residue was suspended in methylene chloride. The suspension was rendered alkaline with sodium hydroxide solution (20%) and then concentrated in vacuo. The residue was slurried in chloroform–methanol (9:1 v/v) and filtered, and the filtrate was flash chromatographed to give the desired product as an amorphous solid, which was used directly without further purification: ^{1}H NMR ($CDCl_3$, DMSO-d_6) δ 4.6 (s, 1 H, 5 Hz), 7.05 (t, 1 H, 5 Hz), 7.2 (m, 3 H), 7.48 (m, 3 H), 7.65 (t, 1 H, 5 Hz).

***N*-[5-(2-Fluorophenyl)-2,3-dihydro-2-thioxo-1*H*-1,4-benzodiazepin-3-yl]-1*H*-indole-2-carboxamide (14a).** The 3-aminobenzodiazepin-2-thione **13** (400 mg, 1.4 mmol) was combined with indole-2-carboxylic acid (248 mg, 1.54 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (295 mg, 1.54 mmol) in 10 mL of dry dimethylformamide. The pH of the reaction medium was adjusted to approximately 8 (moist pH paper) with triethylamine, and the reaction was stirred at room temperature for 18 h. All volatile materials were removed in vacuo. The solid residue was suspended in ethyl acetate (200 mL) and washed in succession with 10% citric acid solution, saturated sodium bicarbonate solution, and brine. The dried organic phase (sodium sulfate) was concentrated to give the title compound. The analytical material was obtained via PTLC on silica gel (hexane–ethyl acetate elution, 1:1 v/v): mp 209–211 °C; ^{1}H NMR ($CDCl_3$) δ 5.5 (d, 1 H, 8 Hz), 7.05 (t, 1 H, 9 Hz), 7.25 (m, 6 H), 7.37 (d, 1 H, 8 Hz), 7.45 (m, 2 H), 7.58 (t, 1 H, 8 Hz), 7.73 (t, 1 H, 8 Hz), 7.74 (d, 1 H, 8 Hz), 8.52 (d, 1 H, 8 Hz), 9.12 (br s, 1 H), 9.61 (br s, 1 H); MS, *m/e* 428 (M^+). Anal. Calcd for $C_{24}H_{17}FN_4OS \cdot 0.5EtOAc$: C, H, N.

***N*-[5-(2-Fluorophenyl)-2-hydrazono-3*H*-1,4-benzodiazepin-3-yl]-1*H*-indole-2-carboxamide (14b).** This material was prepared by the identical procedure as described for **3b** and isolated as a beige foam.

6-Phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-ylamine (22). A 100-mL three-necked flask fitted with a nitrogen inlet tube, stirrer, and rubber septum was carefully charged, under nitrogen, with 10 mL of methanol containing 90% aqueous formic acid (4.5% by volume) and 120 mg of palladium/carbon catalyst (10%). To this suspension was added compound **20** (150 mg, 0.37 mmol) in 5 mL of the above solvent system. The reaction mixture was stirred at ambient temperature for 1.5 h and filtered through Celite. The solvent was rotoevaporated under reduced pressure. Toluene and tetrahydrofuran were added to the residue, and this solution was concentrated to remove any vestiges of formic acid. In this way, the title compound was obtained in 90% yield as its formate salt, which was used directly in the next step without further purification.

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103195-60-4; **9**, 108895-98-3; **10**, 103373-52-0; **11**, 111113-10-1; **12**, 103373-53-1; **13**, 103373-59-7; **14a**, 103373-60-0; **14b**, 111113-23-6; **15**, 111113-11-2; **16**, 111139-85-6; **17**, 111113-12-3; **18**, 111113-13-4; **19**, 111113-14-5; **20**, 111113-15-6; **21**, 111113-16-7; **22**, 111113-18-9; **23**, 111113-20-3; **24**, 111113-21-4; **25**, 111113-22-5; **26**, 28981-97-7; **27**, 28911-01-5; EDC, 25952-53-8; CCK, 9011-97-6; 4-$ClC_6H_4CONH_2$, 619-56-7; indole-2-carboxylic acid, 1477-50-5; dimethylmethyleneammonium chloride, 30354-18-8.

Supplementary Material Available: Spectral and analytical data for products **4–8**, **15–17**, **20**, **21**, **24**, and **25** (4 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Evaluation of Poly-γ-glutamyl Metabolites of 10-Deazaaminopterin and 10-Ethyl-10-deazaaminopterin

M. G. Nair,*† N. T. Nanavati,† P. Kumar,‡ Y. Gaumont,‡ and R. L. Kisliuk*‡

Department of Biochemistry, University of South Alabama, Mobile, Alabama 36688, and Department of Biochemistry and Pharmacology, Tufts University, Health Science Campus, Boston, Massachussetts 02111. *Received May 29, 1987*

The chemical synthesis of a series of poly-γ-glutamyl metabolites of the experimental anticancer drugs 10-deazaaminopterin (10-DAAM) and 10-ethyl-10-deazaaminopterin (10-EDAAM) has been carried out by the solid-phase procedure. The synthetic products were identical with the poly-γ-glutamyl metabolites of radiolabeled 10-DAAM and 10-EDAAM produced by normal mouse tissues with regard to elution volume from [(diethylamino)ethyl]cellulose columns and susceptibility to hydrolysis by human plasma folylpolyglutamate hydrolase. Poly-γ-glutamyl metabolites with a glutamate chain length of up to four glutamate residues were detected in the tissues. The antifolate activity was evaluated with methotrexate (MTX) sensitive and MTX-resistant strains of *Lactobacillus casei* and *Streptococcus faecium*. In general, inhibitory potency decreases with increasing Glu chain length. However there are two exceptions. Addition of one Glu residue to 10-DAAM enhances its potency for MTX-resistant *L. casei* and addition of one Glu residue to 10-EDAAM enhances its potency for the MTX-sensitive *L. casei*. As shown earlier for MTX polyglutamates, polyglutamylation greatly enhances the inhibitory potency of 10-DAAM and 10-EDAAM for *L. casei* thymidylate synthase. MTX polyglutamates are 15–30 times more inhibitory than the corresponding 10-DAAM derivatives and 30–60 times more inhibitory than the corresponding 10-EDAAM derivatives. Polyglutamylation of 10-DAAM had little influence on its ability to inhibit *L. casei* dihydrofolate reductase; however, with 10-EDAAM, addition of one or two Glu residues enhanced its inhibitory potency 2.3-fold.

The coenzyme forms of folic acid exist in tissues as poly-γ-glutamyl derivatives.[1,2] In many folate-mediated enzymatic reactions, the polyglutamyl derivatives are preferred substrates.[1-3] Like folate, the well-known anticancer drug methotrexate (MTX) is metabolized to polyglutamyl derivatives in human red blood cells,[3,4] various animal tissues,[2-6] and tumors.[6,7] The chemical synthesis and preliminary biological evaluation of the polyglutamyl metabolites of MTX were reported from this laboratory in 1973.[5] MTX polyglutamates are relevant to cancer chemotherapy because: (1) their formation is related to cytotoxicity,[6,7] (2) they efflux from cells at a slower rate than MTX,[9] and (3) they are more inhibitory to thymidylate synthase[8,9] and AICAR transformylase[10,11] than the parent drug. The poly-γ-glutamyl derivatives of the antileukemic agent N^{10}-propargyl-5,8-dideazafolic acid (PDDF) were recently synthesized in this laboratory.[12] They were shown to be the most potent antifolate inhibitors of *Lactobacillus casei*,[12] L1210,[12] and human thymidylate synthase[13,14] yet described. Two analogues of aminopterin, 10-deazaaminopterin (10-DAAM) and 10-ethyl-10-deazaaminopterin (10-EDAAM),[15-19] exhibit superior antitumor activity compared to MTX in several murine tumor models. 10-EDAAM is presently undergoing advanced clinical trial at Memorial Sloan-Kettering Cancer Center. Quite recently, the polyglutamyl derivatives of both 10-DAAM and 10-EDAAM that were synthesized according to the procedures described in this paper (vide infra) were utilized to evaluate their inhibition of human thymidylate synthase.[20] In a related study, the synthetic polyglutamyl derivatives of MTX and 10-DAAM were tested as inhibitors of dihydrofolate reductase (DHFR) derived from sheep, chicken, and beef liver.[21] In this context, it was of interest to compare these synthetic compounds with the

† University of South Alabama.
‡ Tufts University.

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