of ferric chloride (2 g) and stirred overnight at room temperature. The resulting yellow-green precipitate was recovered by filtration and washed with water. The mother aqueous solution, neutralized with NH_4OH and extracted with EtOAc, gave an additional amount of the reaction product, which was recrystallized from ethanol: yield 0.86 g (80%); mp 261–265 °C dec; ^1H NMR ($C-D_3OD$) δ 2.00 (s, 6 H, 5-CH_3 and 6-CH_3), 2.45 (s, 3 H, 2-CH_3), 13.3 (br s, 1 H, NH, vanishes by addition of D_2O). Anal. ($C_{10}H_{10}N_4O_4$) C, H, N.

5(6-)-(Chloromethyl)-4,7-dimethoxybenzimidazole (13) and 5,6-Bis(chloromethyl)-4,7-dimethoxybenzimidazole (14). A 40% aqueous solution of formaldehyde (4 mL) was added to a suspension of 0.4 g of 4,7-dimethoxybenzimidazole[36] (2.24 mmol) in 10 mL of concentrated HCl. Then, gaseous HCl was bubbled into the suspension at room temperature, with stirring, for 1 h. The reaction mixture was then heated on a water bath for 2 h and, after cooling at room temperature, neutralized with concentrated NH_4OH. The resulting precipitate of inorganic salts was filtered, and the aqueous solution was extracted several times with EtOAc. The combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed on a silica gel column eluted with EtOAc–MeOH (95:5). The first eluted fractions were evaporated to give **14**, which was recrystallized from ethanol: yield 15 mg (2.8%); mp 105–106 °C; ^1H NMR (DMSO) δ 4.0 (6 H, s, OCH_3), 5.0 (4 H, s, 5- and 6-CH_2Cl), 8.15 (1 H, s, 2-H), 12.8 (1 H, very br signal, NH, vanishes upon addition of D_2O). Anal. ($C_{11}H_{12}N_2O_2Cl_2$) C, H, N.

By further elution of the column were obtained fractions that were evaporated to give **13** as a residue, which was recrystallized from EtOAc: yield 100 mg (21%); mp 158–160 °C; ^1H NMR (DMSO) δ 3.94 (3 H, s, OCH_3), 4.06 (3 H, s, OCH_3), 4.70 (2 H, s, CH_2Cl), 6.87 (1 H, s, 6-H), 8.15 (1 H, s, 2-H), 12.9 (1 H, very br signal, vanishes upon addition of D_2O). Anal. ($C_{10}H_{11}N_2O_2Cl$) C, H, N.

5(6-)-(Chloromethyl)benzimidazole-4,7-dione (15). A solution of 1 g of $Ce(NH_4)_2(NO_3)_6$ in 10 mL of water was added dropwise at room temperature, during a 10-min interval, to a stirred solution of 0.2 g of **13** (0.88 mmol) in 50 mL of CH_3CN. The resulting mixture was stirred at room temperature for 4 h and then, after addition of 10 mL of water, was extracted several times with EtOAc. The combined organic extracts were washed with water, dried (Na_2SO_4), evaporated in vacuo, and recrystallizated with EtOH: yield 140 mg (80%); mp undefined (slow decomposition at 210–220 °C); ^1H NMR (DMSO) δ 4.44 (2 H, d, J = 2 Hz, 5-CH_2Cl), 6.65 (1 H, d, J = 2 Hz, 6-H), 8.6 (1 H, s, 2-H), 9.5 (1 H, br signal, NH, vanishes upon addition of D_2O). Anal. ($C_8H_5N_2O_2Cl$) C, H, N.

5-Bromo-6-aziridinylbenzimidazole-4,7-dione (18). To a stirred suspension of 800 mg of 5,6-dibromobenzimidazole-4,7-dione (2.61 mmol) in 200 mL of 1,2-dimethoxyethane was added 0.43 g of aziridine[40] (10 mmol). The mixture was stirred at room temperature for 3 h and during this period was decanted several times in order to remove impurities sticking to the glass. Evaporation of the solvent in vacuo at room temperature gave an oily residue, which was repeatedly washed with ether and MeOH, and finally recrystallized from MeOH–ethyl ether: yield 470 mg (67%); mp 300–302 °C dec; ^1H NMR (DMSO) δ 2.53 (4 H, s, aziridinyl-H), 8.14 (1 H, s, 2-H), 11.5–14.5 (1 H, very br signal, NH, vanishes upon addition of D_2O). Anal. ($C_9H_6BrN_3O_2$) C, H, N.

5-Bromo-6-(methylaziridinyl)benzimidazole-4,7-dione (19). To a stirred suspension of 200 mg of 5,6-dibromobenzimidazole-4,7-dione (0.66 mmol) in 50 mL of 1,2-dimethoxyethane was added 230 mg (2 mmol) of methylaziridine, and the resulting mixture was stirred at room temperature. Immediately after addition of methylaziridine, a complete solubilization was observed, and the solution became red-orange. The course of the reaction was followed by TLC (benzene–CH_3CN, 50:50). After several hours, complete disappearance of the starting quinone was observed. Evaporation of the solvent in vacuo gave an oily residue, which was washed several times with ether, redissolved in MeOH, and precipitated with ether. The obtained compound was pure on TLC (benzene–CH_3CN, 50:50): yield 70 mg (38%); mp undefined (decomposition); ^1H NMR (DMSO) δ 1.43 (3 H, d, J = 5 Hz, CH_3), 2.53 (3 H, m, H-2′ and H-3′), 3.30 (1 H, br s, NH, vanishes upon addition of D_2O), 8.12 (1 H, s, 2-H). Anal. ($C_{10}H_8BrN_3O_2$), C, H, N.

Tests against P388 Leukemia. The quinone derivatives reported in Table I were screened against P388 lymphocytic leukemia in mice. Tests were performed in accordance with the protocols of the National Cancer Institute.[41] $C_6D_2F_1$ mice were inoculated intraperitoneally on day 0 with 10^6 P388 lymphocytic leukemia cells. Treatment of groups of six of these mice was begun the following day (day 1).

For compounds **23**, **24**, and **25**, treatment was made on day 1 only; for compounds **7**, **11**, **15**, **17–20**, and **27** treatment was made on days 1–5. Compounds **6**, **16**, **21**, and **26** were tested in both regimens.

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Registry No. 4, 103151-22-0; **5**, 111210-09-4; **6**, 111210-04-9; **7**, 111210-05-0; **8**, 3363-56-2; **9**, 111210-10-7; **10**, 111210-11-8; **11**, 111210-06-1; **12**, 7711-50-4; **13**, 111210-12-9; **14**, 111210-13-0; **15**, 111210-07-2; **16**, 7711-39-9; **17**, 26573-35-3; **18**, 111237-56-0; **19**, 111210-08-3; **20**, 98436-82-9; **21**, 7711-63-9; **22**, 61587-95-9; **23**, 85311-42-8; **24**, 85311-43-9; **25**, 26558-10-1; **26**, 26557-29-9; **27**, 26557-83-5; **28**, 111210-14-1; **29**, 99922-32-4; aziridine, 151-56-4; 2-methylaziridine, 75-55-8.

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Cholecystokinin Antagonists. Synthesis and Biological Evaluation of 3-Substituted 1,4-Benzodiazepin-2-amines

Mark G. Bock,* Robert M. DiPardo, Ben E. Evans, Kenneth E. Rittle, 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 August 17, 1987*

Cholecystokinin (CCK) is a neuropeptide[1] that is found in different molecular sizes, a major one being the octapeptide, CCK-8. CCK occurs both in mammalian peripheral tissues and in the central nervous system.[2,3] The relationship between the activation of CCK receptors in the periphery and stimulation of pancreatic and biliary

†Department of Microbial Pharmacometrics.

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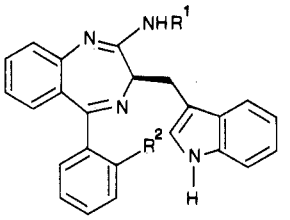
Table I. Physicochemical Data of 3-Substituted 1,4-Benzodiazepin-2-amines



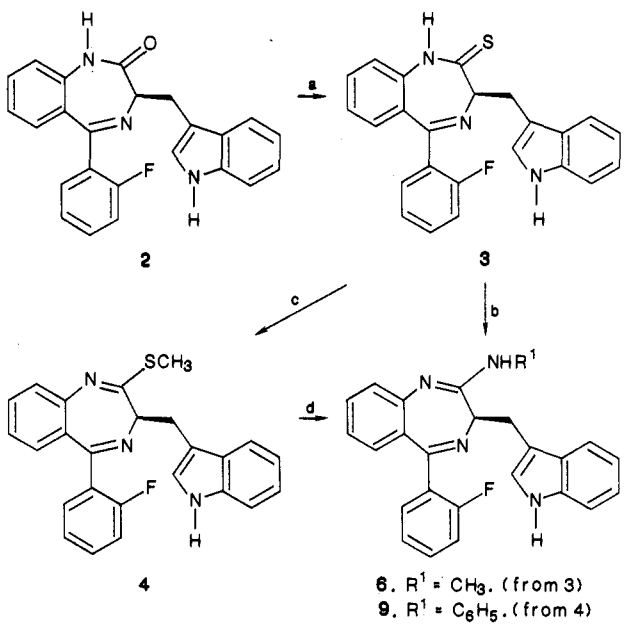
compd	R^1	R^2	proc	yield,[a] %	mp, °C	formula[b]	anal.[c]
5	H	F	A	73	133–136	$C_{24}H_{19}FN_4 \cdot 0.25H_2O$	C, H, N
6	CH_3	F	A	86	130–132	$C_{25}H_{21}FN_4 \cdot 0.3CHCl_3$	C, H, N
7	CH_3	$NHCH_3$	B	38	139–140	$C_{26}H_{25}N_5 \cdot 0.22CHCl_3$	C, H, N
8	$CH_2CH_2CH_3$	F	A	54	104–106	$C_{27}H_{25}FN_4 \cdot 0.25CH_3OH$	C, H, N
9	C_6H_5	F	C	47	127	$C_{30}H_{23}FN_4 \cdot 0.2H_2O$	C, H, N
10	CN	F	A	23	148–150	$C_{25}H_{18}FN_5 \cdot 1.05CHCl_3$	C, H, N
11	CH_2CO_2Et	F	A	74	107–110	$C_{28}H_{25}FN_4O_2 \cdot 0.1CHCl_3$	C, H, N
12	CH_2CO_2H	F	D	67	197	$C_{26}H_{21}FN_4O_2 \cdot 0.8CHCl_3 \cdot 0.8HOAc$	C, H, N

[a] Yield refers to analytically pure product. [b] All compounds were fully characterized spectroscopically; ^1H NMR confirmed the presence of solvate where indicated. [c] Combustion analyses were within ±0.4% of the theoretical values. Compound purity was further verified by HPLC and in all cases was >97%.

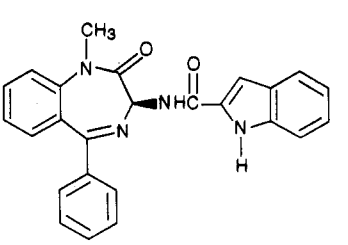
exocrine secretion, gall bladder contraction, and gut motility is now well-recognized.[4-7] The role of brain CCK is less well-defined, although the proposal has been advanced that it may function as a neurotransmitter or neuromodulator.[8-10] In addition to these, a variety of other functions have been attributed to CCK, including satiety,[11] sedation,[12] and antagonism of the analgesic effects of endogenous opiates.[13] Within this context, the development of effective agents, which interact competitively and selectively with the CCK receptor, is desirable to aid in further delineating the role of CCK in normal and pathological physiology.

Significant progress has recently been made in the design and synthesis of potent, nonpeptidal antagonists of CCK.[14-16] By many measures, the most promising agent to emerge from these studies is compound 1, (3*S*)(−)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-1*H*-indole-2-carboxamide (L-364,718), which has been demonstrated to have high affinity and selectivity for the peripheral CCK receptors.[17] Moreover, 1 has long-lasting in vivo efficacy, as well as good oral bioavailability.[17]

Scheme I[a]



[a] Key: (a) $(CH_3OC_6H_4)_2P_2S_4$, $PhCH_3$, 110 °C, 1.5 h; (b) R^1NH_2, $HgCl_2$, THF, 55 °C, 2 h; (c) CH_3I, $(n\text{-}Bu)_4NHSO_4$, $PhCH_3$-THF, NaOH (40%), 23 °C, 15 min; (d) R^1NH_2, >80 °C, 48 h.



1. L-364,718

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In addition to compound 1, modest CCK antagonist activity has been documented for other benzodiazepine structures as well.[18-22] However, the mechanism of action remains to be defined. Accordingly, we have continued to probe the structural requirements of the benzodiazepine ring system in an effort to determine which features are necessary for interaction with the peripheral CCK receptor. In the process, we have prepared CCK antagonists with various structure types[16] (vide infra) having different physical properties (e.g., aqueous solubility) and potentially different pharmacokinetic properties. This search is especially warranted in light of recent suggestions that propose that subtypes of peripheral CCK receptors may be distinguishable by choice of appropriate antagonists.[23]

The present paper describes the synthesis and pharmacological evaluation of the eight chemically novel 3-substituted 1,4-benzodiazepin-2-amines shown in Table I.

Chemistry. The 3-substituted 1,4-benzodiazepin-2-amines that form the basis of this study were synthesized by using the routes outlined in Scheme I. The starting material employed was the 3-(indolylmethyl)-1,4-benzodiazepin-2-one **2**, which was prepared from 2-amino-2′-fluorobenzophenone and D-tryptophan acid chloride as previously described by Evans et al.[15] Compound **2** was then converted to the thioamide **3** with 2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane (Lawesson's reagent) in refluxing toluene.[24] The thioamide **3** served as common intermediate for further elaboration to the target structures **5–12**.

In the first approach, compound **3** was converted to the corresponding iminomethylthioether **4** with iodomethane under phase-transfer conditions.[25] Thus, a suspension of **3** in a sodium hydroxide (40%) and toluene solvent mixture was treated with tetra-*n*-butylammonium hydrogen sulfate 23 °C, followed by iodomethane. Depending on the size of the reaction scale, tetrahydrofuran was added in varying amounts to prevent the thioamide **3** from clumping. In this way, **4** was obtained routinely in excess of 60% yield without competing alkylation at the indole nitrogen. The imino thioether **4** is sufficiently activated to permit transformation to amidines, like **9**, by heating with the appropriate amine.[26] In our experience with **4**, one prerequisite for its successful transformation to the corresponding amidine is that the amine with which **4** is reacted be sufficiently high boiling, to permit heating above 60–70 °C, under normal pressure. For example, aniline reacted with **4** at 82 °C to give **9** without incident. In contrast, the more nucleophilic methylamine could only be induced to react with **4** under forcing conditions (sealed pressure bomb, 120 °C), with the result that both the methylthio and the fluoro groups in **4** were displaced. An alternative method for preparing the amidines shown in Table I relies on a classical procedure requiring no conversion of the thioamide **3** to a more reactive form.[27,28] Accordingly, **3** was treated in dry tetrahydrofuran with the requisite amine, in the presence of mercuric chloride, to give the desired 1,4-benzodiazepin-2-amine (Table I). The resulting mercuric sulfide salt byproduct is readily removed from the reaction mixture (precipitation or chromatography), making workup and disposal convenient. This procedure is reliable and represents a variant of the method employed in our recently disclosed synthesis of 3-amino-1,4-benzodiazepin-2-ones.[29,30]

Table II. Receptor Binding Affinities for the 3-Substituted 1,4-Benzodiazepin-2-amines[a]

compd	[^{125}I]CCK		[^{125}I]gastrin: gastric glands
	pancreas	brain	
5	1.7	100	100
6	4.4	100	67
7	100	100	100
8	2.7	100	100
9	34	100	100
10	2.0	100	100
11	23	49	100
12	3.6	7.0	2.5
13[b]	100	100	100

[a] Receptor binding affinity is expressed as IC_{50}, the concentration (μM) of compound required for half-maximal inhibition of binding of [^{125}I]CCK-33 to CCK receptors in rat pancreatic or guinea pig brain tissues, or for half-maximal inhibition of binding of [^{125}I]gastrin to gastric glands.[14,31] [b] Chlordiazepoxide.

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.[14,17,31] Values shown are the means of triplicate determinations. The ability of the test compounds to inhibit specific [^{3}H]-(±)-L-364,718 rat pancreatic binding in the presence and absence of guanosine 5′-β,γ-imidotriphosphate (GppNHP, 100 μM) was examined to determine agonist or antagonist activity at peripheral CCK receptors.[32]

Discussion

The design principles that guided the development of the CCK antagonist L-364,718, 1, were enumerated in our earlier disclosures.[14,15] The starting point for the series of compounds that were subsequently prepared en route to 1 was the 3-alkyl-1,4-benzodiazepin-2-one **14**. As compounds 1 and **14** are representative of the 3-substituted 1,4-benzodiazepin-2-one structural class, we initiated a parallel line of investigation in which the key structural alteration was confined to the 2-position of the benzodiazepine ring system. Our intent was to test further the hypothesis that the 1,4-benzodiazepine core structure represents a versatile template useful for the construction of high-affinity, nonpeptidal ligands for the CCK receptor. Given that this notion has been substantiated for 3-sub-

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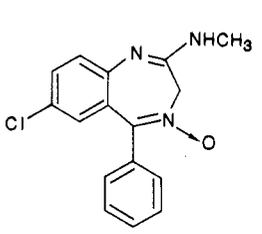

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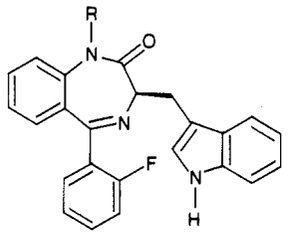

14, R = H
15, R = CH_3

stituted 5-phenyl-1,4-benzodiazepin-2-ones like 1 and 14, we focused on the 1,4-benzodiazepin-2-amines exemplified by **5**, to determine if our previous results could be extended to this class of compounds with similar effect. In the process, it was our minimum expectation that these modifications would lead also to compounds possessing different physical properties and, by inference, potentially different pharmacokinetic profiles.

The benzodiazepin-2-amines produced for this study (cf. Table I) were tested for displacement of the binding of [^{125}I]CCK to rat pancreas and guinea pig brain receptors; gastrin receptor interaction was measured as a function of the displacement of [^{125}I]gastrin from guinea pig gastric glands. These data are collected in Table II, where they are compared with the anxiolytic agent chlordiazepoxide, **13**. GppNHP did not affect the ability of compounds **5**, **6**, **8**, **10**, and **12** to displace [^{3}H]-(±)-L-364,718 binding, which is consistent with an antagonist (vs agonist) interaction of the compounds with the peripheral CCK receptor.[32]

The antianxiety agent chlordiazepoxide, **13**, having no substituent in the 3-position on the benzodiazepine nucleus, served as the reference benzodiazepin-2-amine for our studies. As anticipated, and in line with our previous results, this compound was inactive by our standards. However, introduction of a 3-indolylmethyl substituent at the 3-position imparted significant CCK antagonistic activity. The configuration at the sole chiral center in all cases is *R* (from D-tryptophan) and was based on previous design considerations.[14]

An examination of the results obtained in the pancreas CCK, the brain CCK, and gastrin binding assays reveals that, with one exception, all compounds exhibit selectivity for the peripheral CCK receptor. The anomaly is compound **7**. This compound was found to be inactive in all three assays and serves to illustrate the importance of the 2′-substituent on the 5-phenyl ring for activity.

The most potent and selective compounds among the benzodiazepin-2-amines in Table II proved to be the unsubstituted 2-amino derivative, **5**, and those bearing small substituents, e.g., **6**, **8**, and **10**. Interestingly, saponification of the ester function in **11** led to an apparent enhancement in potency and to a reduction in selectivity among the receptor types (cf. **12**).

An important result that derived from this study was that several of the more potent benzodiazepin-2-amines displayed a marked improvement in aqueous solubility vs their benzodiazepin-2-one counterparts. For example, compound **5** was found to be 1000-fold more soluble in 0.1 N HCl solution than **14**, whereas the two compounds were almost equipotent (1.7 μM vs 0.5 μM, respectively). A similar aqueous solubility enhancement was observed for compound **6** when compared with **15**. In this instance, however, the potency difference vs the pancreas CCK binding assay was more significant (4.4 μM vs 0.27 μM, respectively).

In sum, the CCK antagonist potencies and peripheral receptor selectivities of the 1,4-benzodiazepin-2-amines disclosed in this paper are similar to those of the 3-(indolylmethyl)-1,4-benzodiazepin-2-ones that were previously disclosed from these laboratories. In addition, 3-substituted 1,4-benzodiazepin-2-amines display a marked enhancement in aqueous solubility over their 3-substituted 1,4-benzodiazepin-2-one counterparts. The information garnered from this investigation may be applicable in the optimization of CCK antagonists as potential therapeutic agents, particularly when better aqueous solubility is deemed to be an important feature.

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 spectrometer with tetramethylsilane as an internal standard or Nicolet NT-360 (FT mode) spectrometer with an internal lock on the deuterium of the solvent. Fast atom bombardment (FAB) mass spectra were run on a Finnigan-Mat 731 instrument. HPLC was performed on a Hewlett-Packard 1084B instrument employing a Waters C-18 column. Flash chromatography was performed on silica gel (E. Merck, 0.04–0.063 μm), and 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). All reactions, except those performed in aqueous solvents, were carried out with use of standard techniques for the exclusion of moisture. Commercial chemicals were used as obtained without further purification, except for solvents, which were purified and dried by standard methods before use.

General Procedure for the Synthesis of 3-Substituted 1,4-Benzodiazepin-2-amines. Preparation of 5-(2-Fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-*N*-methyl-3*H*-1,4-benzodiazepin-2-amine (6). Procedure A. (*R*)-5-(2-Fluorophenyl)-1,3-dihydro-3-(1*H*-indol-3-ylmethyl)-2*H*-1,4-benzodiazepine-2-thione, **3** (2 g, 5 mmol), was dissolved in 30 mL of dry tetrahydrofuran and warmed to 55 °C. To this solution was added excess methylamine (20 equiv) in tetrahydrofuran and then mercuric chloride (2 g, 7.4 mmol). The reaction mixture became black immediately and was stirred for 2 h more. The reaction mixture was filtered and rotoevaporated to dryness, and the residue was partitioned between ethyl acetate and sodium thiosulfate solution. The organic phase was washed with sodium thiosulfate solution and brine, then dried (sodium sulfate), and concentrated to give 2 g of an off-white solid. Flash chromatography on silica gel (chloroform–methanol–ammonia, 97:3:0.3 v/v) afforded the analytical sample.

Preparation of (*R*)-5-(2-Fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-2-(methylthio)-3*H*-1,4-benzodiazepine (4). Benzodiazepine-2-thione **3** (2.66 g, 6.66 mmol) was suspended in a vigorously stirred solution of toluene–tetrahydrofuran (75 mL, 2:1 v/v) containing 30 mL of 40% sodium hydroxide solution. Tetra-*n*-butylammonium hydrogen sulfate (1.49 g, 4.38 mmol) was added to the suspension, followed by methyl iodide (456 μL, 7.14 mmol). The reaction mixture was stirred rapidly for 15 min, and the phases were separated. The aqueous phase was extracted with ethyl acetate, and the combined organic phases were washed with water and brine. The dried (magnesium sulfate) extracts were concentrated under reduced pressure to yield 2.8 g of crude product. Flash chromatography on silica gel (hexane–ethyl acetate, 2:1 v/v) afforded the analytical sample (1.8 g): mp 102–104 °C; TLC R_f 0.33 (2:1 hexane–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 3 H, SCH_3), 3.65 (dd, 1 H, J = 9, 6 Hz), 3.79 (dd, 1 H, J = 15, 6 Hz), 3.87 (dd, 1 H, J = 15, 9 Hz), 7.15 (m, 8 H), 7.4 (m, 4 H), 7.65 (d, 1 H, J = 8 Hz), 8.03 (br s, 1 H); MS, m/e 413 (M^+), 284, 130 (100). Anal. ($C_{25}H_{20}FN_3S$) C, H, N.

Preparation of (*R*)-3-(1*H*-Indol-3-ylmethyl)-*N*-methyl-5-[2-(methylamino)phenyl]-3*H*-1,4-benzodiazepin-2-amine (7). Procedure B. A stainless steel pressure bomb was charged with 130 mg (0.32 mmol) of 4 and 2 mL of methylamine. The vessel was closed and heated at 120 °C for 18 h. The bomb was cooled, and its contents were chromatographed on silica gel (chloroform–methanol–ammonia, 97:3:0.3 v/v) to afford the analytical product (R_f 0.34).

Preparation of (*R*)-5-(2-Fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-*N*-phenyl-3*H*-1,4-benzodiazepin-2-amine (9). Pro-

cedure C. A mixture of aniline (138 μL, 1.5 mmol) and compound 4 (170 mg, 0.42 mmol) was heated at 83 °C for 48 h. The reaction mixture was cooled and chromatographed directly on silica gel (hexane–ethyl acetate, 2:1 v/v) to give the analytical products (R_f 0.22).

Preparation of *N*-[5-(2-Fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-3*H*-1,4-benzodiazepin-2-yl]glycine (12). Procedure D. A solution of 220 mg (0.44 mmol) of 1,1-dimethylethyl [5-(2-fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-3*H*-1,4-benzodiazepin-2-yl]acetate (prepared according to procedure A) in 50 mL of dry ethyl acetate was cooled to 0 °C and saturated with hydrogen chloride gas. The reaction flask was capped, and the reaction mixture was warmed to room temperature over 5 h. Solvent and excess reagent were removed under reduced pressure to give 200 mg of an orange-brown powder. PTLC (chloroform–methanol–acetic acid, 84:15:1.5 v/v) on silica gel afforded the analytical sample.

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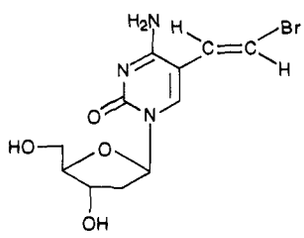
Synthesis and Antiviral Properties of (*E*)-5-(2-Bromovinyl)-2′-deoxycytidine-Related Compounds

A. Stanley Jones,*† Jon R. Sayers,† Richard T. Walker,† and Erik De Clercq‡

Chemistry Department, The University of Birmingham, Birmingham B15 2TT, Great Britain, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium. *Received June 15, 1987*

Treatment of 3′,5′-di-*O*-acetyl-(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (**2**) with *p*-chlorophenyl phosphorodichloridate and 1,2,4-triazole gave 1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-(*E*)-5-(2-bromovinyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**3**). Reaction of **3** with ammonia gave (*E*)-5-(2-bromovinyl)-2′-deoxycytidine (**1**), the overall yield from **2** being 60%. A similar 4-(1,2,4-triazol-1-yl) derivative (**4**) was obtained from 3′,5′-di-*O*-acetylthymidine by the use of phosphoryl chloride as the condensing agent. Treatment of thymidine with trimethylsilyl chloride and then with phosphoryl chloride and 1,2,4-triazole gave upon workup 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-4(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**5**). (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU) when similarly treated gave the corresponding (*E*)-5-(2-bromovinyl) compound **7**. A minor product formed in both cases was a 4-(1,2,4-triazol-1-yl) derivative in which the nucleoside 5′-hydroxyl group had been replaced by chlorine (**6** and **8**). Whereas compounds **4–6** and **8** did not exhibit a selective antiviral effect, compounds **1–3** and **7** proved almost as active as the reference compound BVDU. In particular, compound **7**, the 4-triazolyl derivative of BVDU, would seem worth pursuing for its potential as an inhibitor of herpes simplex virus type 1 and varicella-zoster virus.

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU) is a potent antiviral agent against herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV) in cell culture and animals, and the compound has been shown to be effective in the clinic.[1] (*E*)-5-(2-Bromovinyl)-2′-deoxycytidine (BVDC, **1**) has been shown to be almost as active as BVDU in cell culture. Its toxicity to cells is even less than that of BVDU, so its chemotherapeutic index is similar.[2] The clinical use of BVDC has not been reported. The synthesis of BVDC was first carried out by us by a route that was similar to that used for BVDU, namely by the formation of (*E*)-5-(2-carboxyvinyl)-2′-deoxycytidine (via the 5-chloromercuri and 5-chloropalladium derivatives of 2′-deoxycytidine) and reaction of this with *N*-bromosuccinimide.[3] Subsequent attempts to repeat these reactions have met with difficulties, and therefore an alternative synthesis has been developed.



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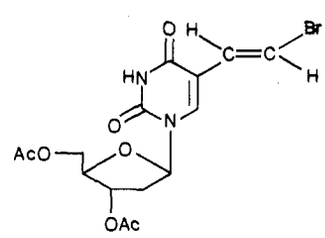

2

Chemistry

3′,5′-Di-*O*-acetyl-(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (**2**)[4] was treated with *p*-chlorophenyl phosphorodichloridate and 1,2,4-triazole according to the procedure described by Sung[5] to give 1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-(*E*)-5-(2-bromovinyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**3**) as a crystalline solid in 25% yield. This product was characterized by its UV and NMR spectra and elemental analysis. The NMR spectrum showed sharp singlets at δ 9.39 and 8.46, which were assigned to the H-5 and H-3 protons of the triazolyl ring. There was a noticeable downfield shift of the 2-vinylic and pyrimidine H-6 resonances compared to those of **2**. The λ_{max} of 340 nm was also consistent with the presence of the triazolyl ring. Treatment of **3** with ammonia gave (*E*)-5-(2-bromovinyl)-2′-deoxycytidine (BVDC, **1**), which was characterized by its UV and NMR spectra and elemental analysis. The low yield of **3** was due to loss upon workup. It was found that direct conversion of **2** to **1** without isolation of **3** gave an overall yield of 60%.

† University of Birmingham.
‡ Katholieke Universiteit Leuven.

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