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Señor
SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. _____ S. _____ D. _____

CALLE 72 No. 5-83 5º. PISO
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Re: P1.-GLAXO GROUP LIMITED.-Solicitud de Patente de Invención para
"DERIVADOS DE ARIL CETONA COMO INHIBIDORES DE LA
TRANSCRIPTASA REVERSA".-Clase A61.-Expediente número 00-
065.946.-

1. Me refiero al auto dictado por ese Despacho notificado mediante fijación en lista de 8 de Marzo de 2007.
2. De acuerdo con el Concepto Técnico No. **359** se tiene que:

"A efectos del examen de patentabilidad y como requerimiento de esta oficina, el solicitante deberá proporcionar, en un plazo que no excederá de 3 meses, los siguientes documentos relativos a la solicitud extranjera correspondiente a la misma invención que se examina y tales documentos son lo que a continuación se enumeran:

- 1- **JOURNAL OF MEDICINAL CHEMISTRY, US, AMERICAN CHEMICAL SOCIETY. WASHINGTON, año 1995, vol. 38, no. 10, pages 1657-1665, P.G. WYATT ET AL.**
- 2- **JOURNAL. MED. CHEM. (año 1985), vol. 28, nº (6), pages 699-707, SUGASAWA, TSUTOMU ET AL: "1-Azacycloalkyl-1,4-benzodiazepin-2-ones with antianxiety-antidepressant actions".**
- 3- **JUSTUS LIEBIGS ANN. CHEM. Año 1968, pages 712-73-8; CAPUANO, LILLY ET AL: "Darstellung von alpha-Ä2-alkoxycarbonyl-anilinoÜ- und alpha-Ä2-AcylanilinoÜ-carbonsureamiden".**

- 4- **SCI. PHARM; BRADL, E.: "Penicillin reactions. 1. Behavior towards penicillinase"; año 1971, vol. 39, N° (4), pages 267-92.**

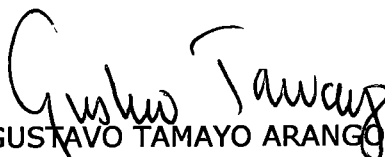
3. Para responder a los requerimientos del señor Examinador de conformidad con el Artículo 46 de la Decisión 486, anexo me permito presentar a ese Despacho copia de los siguientes documentos solicitados:

1. **JOURNAL OF MEDICINAL CHEMISTRY, US, AMERICAN CHEMICAL SOCIETY. WASHINGTON, año 1995, vol. 38, no. 10, pages 1657-1665, P.G. WYATT ET AL.**
2. **JOURNAL. MED. CHEM. (año 1985), vol. 28, n° (6), pages 699-707, SUGASAWA, TSUTOMU ET AL: "1-Azacycloalkyl-1,4-benzodiazepin-2-ones with antianxiety-antidepressant actions".**
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4. **SCI. PHARM; BRADL, E.: "Penicillin reactions. 1. Behavior towards penicillinase"; año 1971, vol. 39, N° (4), pages 267-92.**

4. El documento que acredita mi calidad de Representante Legal de la Sociedad peticionaria en el expediente de la referencia, fue presentado a ese Despacho mediante memorial de 8 de Septiembre de 2004.

5. En vista de todo lo anterior, ruego a usted proceder de conformidad y conceder el privilegio de patente solicitado.

Señor Superintendente,


GUSTAVO TAMAYO ARANGO
T.P. 37.172

Benzophenone Derivatives: A Novel Series of Potent and Selective Inhibitors of Human Immunodeficiency Virus Type 1 Reverse Transcriptase

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Received October 21, 1994*

A series of benzophenone derivatives has been synthesized and evaluated as inhibitors of HIV-1 reverse transcriptase (RT) and the growth of HIV-1 in MT-4 cells. Through the use of the structure-activity relationships within this series of compounds and computational chemistry techniques, a binding conformation is proposed. The SAR also indicated that the major interactions of 1h with the RT enzyme are through hydrogen bonding of the amide and benzophenone carbonyls and π -orbital interactions with the benzophenone nucleus and an aromatic function separated from the benzophenone by a suitable spacer group. The crystal structure of compound 1h has been determined. A number of compounds with potent inhibitory activity against HIV-1 RT and HIV in cellular assays at levels comparable with AZT and our efforts to identify a metabolically stable analogue are described.

Introduction

Since the identification of the human immunodeficiency virus (HIV) as the causative agent of AIDS,^{1,2} the search for safe and effective treatments for HIV infection has become a major focus for drug discovery groups worldwide. Investigations into the molecular biology of HIV³ have identified a number of viral targets for drug design, such as reverse transcriptase (RT), protease and integrase enzymes, and regulatory proteins (e.g., TAT and REV), as well as virus attachment and fusion. HIV RT, an essential enzyme in the life cycle of HIV, catalyzes the transcription of HIV-encoded single-stranded RNA into double-stranded DNA. The RNA-dependent DNA polymerase function of HIV RT does not have a parallel in mammalian metabolism and thus is a good target for a chemotherapeutic agent. Many nucleoside analogues after conversion to their 5'-triphosphates by cellular enzymes inhibit HIV RT and in consequence inhibit the growth of HIV in cell culture. However, adverse toxicity has limited the progression of many of these compounds into the clinic. Zidovudine⁴ (AZT, 3'-azido-2'-deoxythymidine) the first anti-HIV compound to be approved for use in the clinic is beneficial in the treatment of HIV infected patients. However, its clinical usage is compromised by serious side effects which are attributed to inhibition of cellular DNA polymerases, e.g., bone marrow suppression, and by the emergence of AZT resistant strains. Other nucleoside analogues didanosine (DDI, 2',3'-dideoxyinosine) and zalcitabine (DDC, 2',3'-dideoxycytosine) now approved for clinical use also suffer from serious side effects.⁴

The toxicity of the nucleoside analogues has stimulated considerable interest into alternative approaches

in the search for effective anti-HIV agents. Of particular prominence has been a structurally diverse group of compounds that inhibit the RT of HIV-1 but not HIV-2. Notable examples of such compounds include benzodiazepinones,⁵ dipyrroliodiazepinones,⁶ 2-pyridones,⁵ bis(heteroaryl)piperazines,⁷ 6-substituted pyrimidines,⁸ and imidazopyridazines.⁷ These compounds are non-competitive inhibitors of RT, and many bind close to the nucleotide binding site⁸ and are potentially devoid of the toxic side effects of the nucleoside analogues.

High-throughput screening at Glaxo against recombinant HIV-1 RT⁹ identified benzophenone 1a as a weak inhibitor of the enzyme with an IC₅₀ of 10 μ g/mL. In this paper we report the more significant results of the structural requirements of this series of compounds and our efforts to identify a potent, selective, and metabolically stable inhibitor of HIV-1.

Synthetic Chemistry

The benzophenone derivatives 1a-1 (Table 1) were obtained from the 2-hydroxybenzophenones 2a-j¹⁰ using one of two methods (Schemes 1 and 2). Anilines 4a-e were obtained from nitrophenols 5a-d (Scheme 3). Anilides 8a-d and 10 (Table 2) were obtained from the coupling of acids 7¹¹ and 9¹² with anilines 4b-e and 4c, respectively (Scheme 4). Compounds 1m,n (Table 3) were synthesized by acylation of 4-chlorophenol with the required acid chlorides, followed by a Fries rearrangement to give phenols 2k,l (Scheme 2). Alkylation of 2k and 2l with *N*-phenylbromacetamide gave anilides 1m,n (Table 3). Sodium borohydride reduction of 1g gave the benzhydrol 11 (Table 3).

Acid-catalyzed ketalization of the carbonyl of 2e (Scheme 5) with ethylene glycol, followed by alkylation using bromoacetonitrile, gave cyanomethyl ether 13. Reaction of 13 with benzylmagnesium bromide and subsequent treatment with acid gave the keto derivative 14 (Table 4). Carbodiimide-mediated coupling of acid 3e with cyclohexylamine, 2-methylbenzylamine, or 1,2,3,4-tetrahydroisoquinoline under standard conditions

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* Abstract published in *Advance ACS Abstracts*, April 15, 1995.

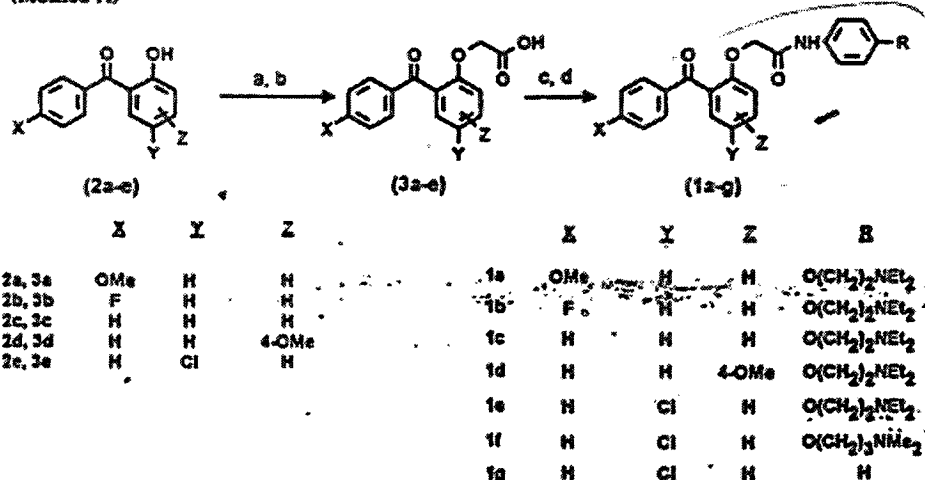
Table 1. Antiviral Activity

Wyatt et al.
related to I, IA, IC, III

no.	X	Y	Z	R	mp, °C	formula	method ^a	RT ^b	MTM ^c	TOX ^d	SYN ^e	p24 ^f
1a	OCH ₃	H	H	O(CH ₂) ₂ NEt ₂	136-137	C ₂₀ H ₂₀ N ₂ O ₃	A	6.8	0.44	5	0.34	0.18
1b	F	H	H	O(CH ₂) ₂ NEt ₂	141-142	C ₁₉ H ₁₈ FN ₂ O ₃	A	10	0.75	>0.5	0.016	<0.05
1c	H	H	H	O(CH ₂) ₂ NEt ₂	oil	C ₁₉ H ₂₀ N ₂ O ₃	A	2.3	<0.001	>0.5	0.005	0.0015
1d	H	H	4-OCH ₃	O(CH ₂) ₂ NEt ₂	89-90	C ₂₁ H ₂₂ N ₂ O ₄	A	6.3	<0.01	6	0.05	0.013
1e	H	Cl	H	O(CH ₂) ₂ NEt ₂	115-116	C ₁₉ H ₁₇ ClN ₂ O ₃	A	0.45	>10	10	NT	NT
1f	H	Cl	H	O(CH ₂) ₂ NMe ₂	119-120	C ₁₉ H ₁₉ ClN ₂ O ₃	A	0.097	<0.01	>0.5	0.0009	<0.0005
1g	H	Cl	H	H	118-119	C ₁₉ H ₁₇ ClNO ₃	A	0.01	0.0004	>0.005	0.0002	0.0012
1h	H	F	H	H	127-129	C ₁₉ H ₁₇ FN ₂ O ₃	B	0.003	0.09	10	0.014	0.006
1i	H	N-imidazolyl	H	H	amorph	C ₂₁ H ₁₈ N ₄ O ₃	B	>10	>10	10	NT	NT
1j	H	Cl	3-Cl	H	138-141	C ₂₁ H ₁₅ Cl ₂ N ₂ O ₃	B	>100	NT	NT	NT	NT
1k	H	Cl	4-Cl	H	123-129	C ₂₁ H ₁₅ Cl ₂ N ₂ O ₃	B	0.014	0.023	100	NT	NT
1l	H	Cl	6-Cl	H	123-124	C ₂₁ H ₁₅ Cl ₂ N ₂ O ₃	B	>100	NT	NT	NT	NT

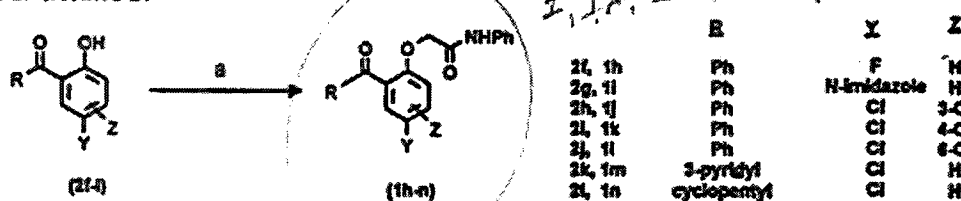
^a For methods, see the Experimental Section. ^b IC₅₀ versus RT of HIV-1 (μg/mL), AZT triphosphate control = 0.02 ± 0.005 μM. ^c IC₅₀ versus HIV-1 (strain RF)-infected MT-4 cells (μg/mL), AZT = 0.002-0.02 μM. ^d IC₅₀ versus uninfected MT-4 cells (mg/mL). ^e IC₅₀ versus syncytia formation in infected C8166-cells (μg/mL), AZT = 0.002-0.02 μM. ^f IC₅₀ versus p24 antigen formation in infected C8166-cells (μg/mL), AZT = 0.001-0.01 μM. ^g oxalate salt + 0.25H₂O. ^h Fumarate salt. ⁱ 0.75H₂O. ^j 0.5H₂O. ^k C: calcd, 63.62; found, 63.83. ^l 0.25H₂O.

Scheme 1. (Method A)^a



^a (a) NaH, BrCH₂CO₂Et, DMF; (b) NaOH, EtOH, H₂O; (c) SOCl₂; (d) 4a-e, NEt₃, PhH.

Scheme 2. (Method B)^a



^a (a) BrCH₂CONHPh, K₂CO₃, DMF.

gave the amides 16, 17, and 18, respectively (Table 4). The methods to compounds 12, 15, 21, and 23 (Table 4) are outlined in Scheme 5.

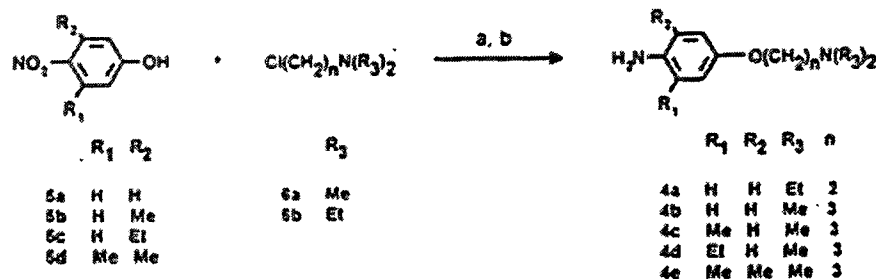
Biological Methods

The benzophenone derivatives described were initially tested for their ability to inhibit the poly(rA)-oligo-(dT)₁₂₋₁₆-directed RT activity of HIV-1. Selected com-

pounds were then progressed to a primary assay against HIV-1 (strain RF) in MT-4 cells and additional screens designed to measure the formation of syncytia (SYN) and p24 antigen (p24) in C8166 cells infected with HIV-1 (strain RF).⁷

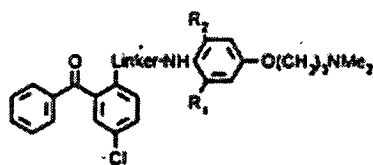
The resistance study on compound 8b was carried out using the following procedure. C8166 cells (1 × 10⁶ cells/mL) in RPMI 1640 growth medium were infected

Scheme 3*



* (a) K₂CO₃, DMF; (b) 10% Pd/C, EtOH

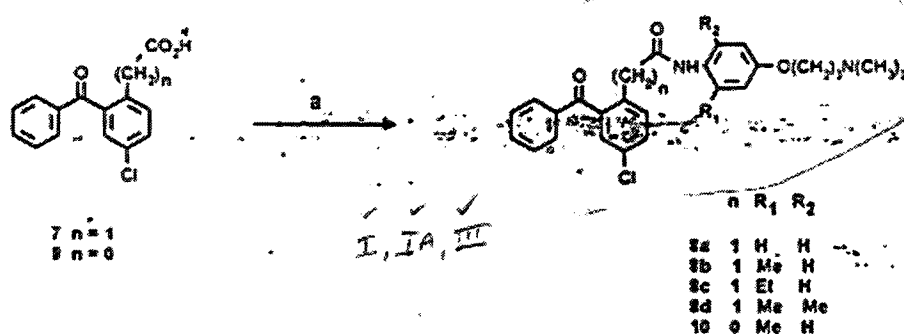
Table 2. Antiviral Activity



no.	linker	R ₁	R ₂	mp, °C	formula ^a	RT ^a	MTM ^a	TOX ^a	SYN ^a	p24 ^a
8a	-CH ₂ CO-	H	H	oil	C ₂₈ H ₂₇ ClN ₂ O ₃ ^b	0.2	0.21	10	NT	NT ^c
8b	-CH ₂ CO-	CH ₃	H	144-145	C ₂₇ H ₂₅ ClN ₂ O ₃ ^b	0.35	0.0044	>0.5	0.016	0.0029
8c	-CH ₂ CO-	Et	H	123-124	C ₂₈ H ₂₇ ClN ₂ O ₃ ^b	2.5	0.21	10	NT	NT
8d	-CH ₂ CO-	CH ₃	CH ₃	117-118	C ₂₈ H ₂₇ ClN ₂ O ₃ ^b	>50	NT	NT	NT	NT
10	-CO-	CH ₃	H	129-130	C ₂₈ H ₂₇ ClN ₂ O ₃ ^b	>100	NT	NT	NT	NT

^a For definitions, see Table 1. ^b 1.0H₂O: C, calcd, 69.73; found, 69.13. ^c 0.25H₂O: C, calcd, 6.03; found, 5.53.

Scheme 4*



* (a) 4b-e, HOBT, DCC, DMF.

with HIV-1 RF strain at a moi of 1 infectious dose/cell. The virus was adsorbed at room temperature for 3 h, after which cells were washed in RPMI 1640 growth medium to remove unabsorbed virus. 8b was initially added to the culture at 0.02 μ M. The culture was incubated until a cytopathic effect was detectable (3-4 days). Following a cycle of infection, supernatant fluids were titrated in fresh C8166 cells at a doubled concentration of 8b. The culture at the highest dilution of virus showing a cytopathic effect was harvested and passaged further in an increased 8b concentration as described above. Levels of HIV-1 infection were monitored regularly by analysis of p24 antigen in the culture supernatant using a commercial ELISA kit. Approximately after eight passages, 8b-resistant virus stock was prepared by passaging three times in 2 μ M 8b. These virus stocks were then titrated, and drug sensitivity of the 8b-resistant variant was determined in a MT-4 assay.⁷

The compound stability studies carried out in mouse serum and mouse S9 liver preparations were based on methods described by Gillette¹³ and Mazel.¹⁴

Discussion

Further screening of closely related structures to the initial lead (1a) available in the Glaxo compound library gave useful structure-activity information.

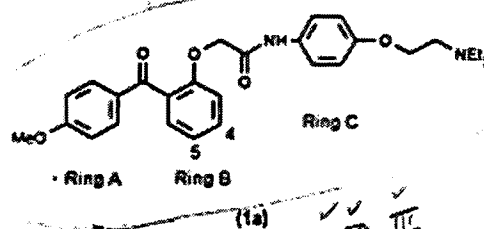
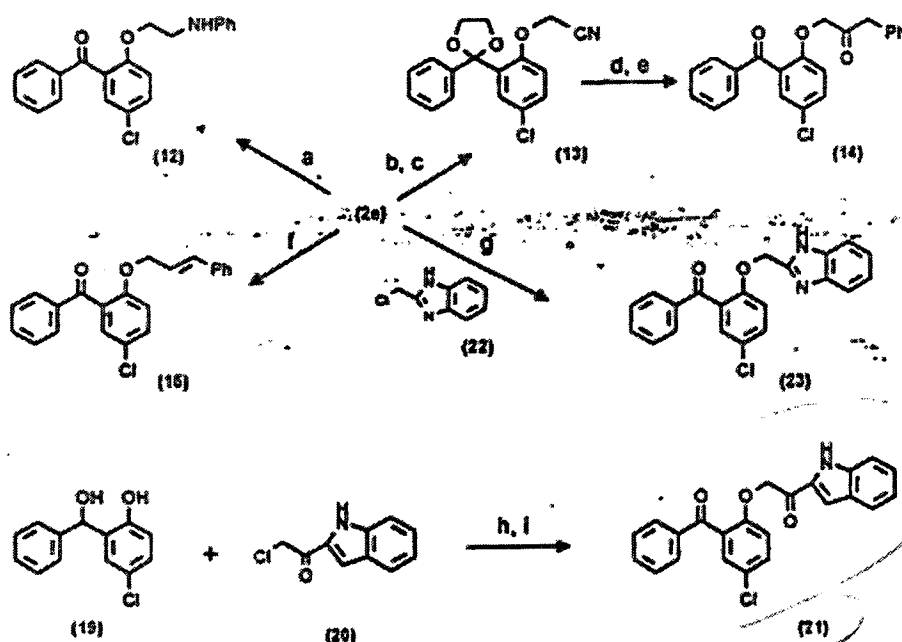


Table 3. Antiviral Activity

I, IA, II, III

no.	R	mp, °C	formula	RT ^a	MTM ^a	TOX ^a	SYN ^a	p24 ^a
1m		169-170	C ₂₀ H ₁₃ ClN ₂ O ₃	0.16	<0.01	10	NT	NT
1n		137-138	C ₂₀ H ₂₀ ClNO ₃	1.8	0.22	>100	NT	NT
1l		147-148	C ₂₁ H ₁₆ ClNO ₃	>100	NT	NT	NT	NT

^a For definitions, see Table 1.Scheme 5^a

^a (a) PhNHCH₂CH₂Cl, K₂CO₃, DMF; (b) HOCH₂CH₂OH, TsOH·H₂O, PhH; (c) BrCH₂CN, K₂CO₃, DMF; (d) PhCH₂MgBr, THF, -78 °C; (e) 1 N HCl, THF; (f) (E)-BrCH₂CH=CHPh, K₂CO₃, DMF; (g) NaH, DMF; (h) K₂CO₃, DMF; (i) PCC, CH₂Cl₂.

Replacement of the methoxy group of ring A of 1a (Table 1) with fluorine (1b) resulted in little change in activity, whereas removal of the substitution (1c) resulted in a modest increase in the inhibition of RT. Introduction of a methoxy group (1d) at the 4-position of ring B resulted in a slight loss of RT inhibition; however, introduction of a chlorine atom at the 6-position (1e) gave a 10-fold increase in activity in the RT assay but resulted in a toxic compound in the whole cell assay. A number of compounds with various (N,N-

dialkylamino)alkoxy substituents on ring C were tested (data not shown), and the most active was the (N,N-dimethylamino)propoxy derivative 1f. Compound 1f was found to be rapidly metabolized in both mouse serum and S9 mouse liver preparations (Table 6). Shortening of the amide linker to give 8a (Table 2) resulted in a loss of activity in both the enzyme and whole cell assays. The 2-(methylphenyl) amide 8b (Table 2) was synthesized to increase the steric crowding around the amide linker of 8a to add stability toward

Table 4. Antiviral Activity

no.	R	mp, °C	formula	RT ^a	MTM ^a	TOX ^a	SYN ^a	p24 ^a
12		131-132	C ₂₁ H ₁₉ ClNO ₃ ^b	>100	NT	NT	NT	NT
14		oil	C ₂₇ H ₁₇ ClO ₃	12	>100	>100	NT	NT
15		85-90	C ₂₇ H ₁₇ ClO ₃	>100	NT	NT	NT	NT
16		119-120	C ₃₁ H ₂₇ ClNO ₃	14.5	NT	NT	NT	NT
17		84-85	C ₂₃ H ₂₀ ClNO ₃	2.5	0.028	10	0.05	0.032
18		101-102	C ₂₇ H ₂₉ ClNO ₃	0.006	0.014	>0.5	0.011	0.016
21		125-126	C ₂₃ H ₁₉ ClNO ₃	0.063	0.7	10	>50	>5
23		144-145	C ₂₁ H ₁₅ ClN ₂ O ₃ ^c	0.51	0.31	>0.5	0.052	0.032

^a For definitions, see Table 1. ^b HCl salt. ^c 0.25H₂O.

Table 5. Metabolic Stability of Benzophenone Derivatives

no.	half-life, h	
	mouse serum ^a	mouse S9 liver ^b
1f	<0.25	>2
1g	<0.25	<0.25
8b	4.75	1.8
17	2.8	>6
18	1.2	2.8
21	<0.25	3.5
23	>6	>6

^a Stability of compound in mouse serum preparation; for method, see ref 13. ^b Stability of compounds in mouse S9 liver preparation; for method, see ref 14.

amidases. Compound 8b proved to be more active than 8a and was moderately stable in both metabolism tests (Table 5). Replacement of the methyl group with ethyl (8c) resulted in a reduction in activity. Inclusion of two *o*-methyl substituents (8d) or further reduction in the amide linker chain length (10) resulted in a total loss of activity (Table 2). The removal of the ring C aminoalkoxy group of 1f, to provide 1g, showed that this substituent was not essential for potent activity. Therefore, further analogues related to 1g were prepared. Replacement of the ring A with the more polar pyridyl ring (1m) or the saturated cyclopentyl ring (1n) (Table 3) resulted in a significant loss of activity. Reduction of the benzophenone carbonyl to afford the benzhydrol 11 (Table 3) resulted in a total loss of activity. The hydrogen-bonding acceptor capabilities of carbonyl and hydroxy groups are reported to be similar,¹⁵ and there

fore, the change in conformation of ring A relative to ring B, rather than a reduction of hydrogen-bonding capability, is probably the reason for the loss of activity of 11.

We next investigated the effect of substitution of ring B (Table 1, compounds 1g-1). Replacement of the 5-chloro substituent of ring B (1g) with fluorine (1h) resulted in little change in activity. Replacement of chlorine with a polar imidazole group (1i) resulted in a significant loss of activity. The dichloro-substituted compounds 1j-1 gave significant SAR information. The 4,5-dichloro-substituted 1k retained the activity of the monosubstituted 1g, whereas the 3,5- and 5,6-dichloro derivatives 1j, l were devoid of activity. The activity of 1k seems to suggest there is room to accommodate the two chlorines in the binding site and that the loss of activity of 1j, l was due to effects on the allowable conformations of the compounds.

The structure-activity data identified compound 1g (Table 1) as a lead compound. However, compound 1g was rapidly metabolized in mouse serum and S9 mouse liver preparations (Table 5). For a compound to be a suitable candidate for progression we required it to be metabolically stable (half-life > 6 h) in these tests. Thus we sought a derivative with comparable activity to 1g but with greater metabolic stability.

To investigate whether the amide of 1g, a likely site of metabolism, was required for activity, we synthesized the corresponding amine 12, ketone 14, and *trans*-vinyl 15 derivatives (Table 4). The loss of activity associated

with these modifications against HIV-1 RT suggests that the hydrogen-bonding capabilities of the amide is a major determinant of the activity of 1g.

Although the amide of 1g appeared to be required for potent activity, amide cleavage to release potentially toxic anilines was considered a possibility, and therefore, alternative amides were investigated. The significant loss of activity of the *N*-cyclohexylamide 16 (Table 4) indicated the anilino aromatic ring of 1g was essential for potent activity. The 2-methylbenzyl amide derivative 17 (Table 4) was synthesized to incorporate the amide linker of 1g and a phenyl ring, both required for potent activity, together with the *o*-methyl substituent of 8b found to increase metabolic stability. Compound 17 was less active than 1g in enzyme and cellular assays but was significantly more metabolically stable (Table 5). The bicyclic amide 18 (Table 4) synthesized as a constrained version of 17 proved to be a potent inhibitor of the enzyme and exhibited good activity in antiviral assays but was less metabolically stable than 17 (Table 5).

The potent activity of 18 suggests the amide hydrogen is not involved in hydrogen bonding to the enzyme and the major interaction is through the amide carbonyl. Therefore, compounds were synthesized that satisfied the requirements for both a hydrogen bond acceptor and aromatic residue but did not contain the potentially metabolically unstable amide linkage. The acylindole group of 21 (Table 4) was shown by molecular modelling to closely resemble the *trans*-*N*-phenylamide moiety of 1g. Indeed, 21 exhibited potent inhibition of HIV-1 RT; however, this level of activity was not translated into activity in antiviral assays. In the metabolism tests 21 (Table 5) proved to be surprisingly unstable; however, the major site of metabolism was not determined. The benzimidazole derivative 23 (Table 4) capable of acting as a hydrogen bond acceptor through an imidazole ring nitrogen exhibited moderate activity in both the enzyme and whole cell assays. This compound proved to be stable in both metabolism test systems (Table 5).

The generated SAR for this series identified the major interactions with the enzyme to be hydrogen bonding of the amide and benzophenone carbonyls and π -orbital interactions of the benzophenone nucleus and an aromatic function separated from the benzophenone by a suitable spacer group.

X-ray Crystallography and Discussion of the Binding Conformation

Crystals suitable for X-ray crystallography were obtained from the fluoro derivative 1h (Figure 1). The full crystal coordinate data for 1h are available as supplementary material. The conformation shown includes an internal hydrogen bond between the amide and the benzophenone carbonyl which is unlikely to occur in solution or during binding to the enzyme. In order to identify possible enzyme binding conformations of 1g, systematic searches were performed on 1g (IC_{50} vs HIV-1 RT = 0.01 μ g/mL) and the inactive dichloro derivatives 1j and 1l. A 15-deg step was used in SYBYL,¹⁶ with a 4-dimensional distance map calculated using the following distances: amide carbonyl-benzophenone carbonyl; amide carbonyl-centroid of ring B; amide carbonyl-centroid of ring A; amide linker oxygen-benzophenone carbonyl. A proposed "active"

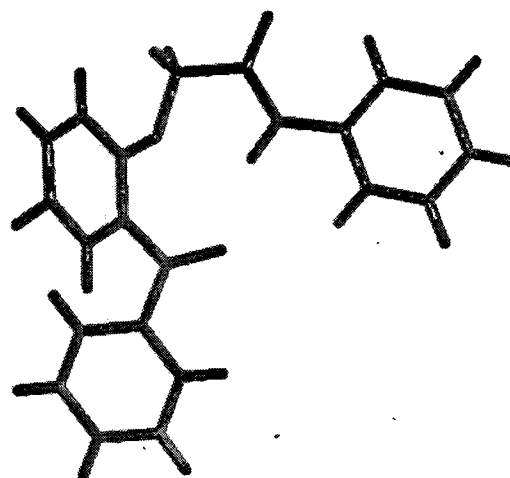


Figure 1. Crystal structure of 1h.

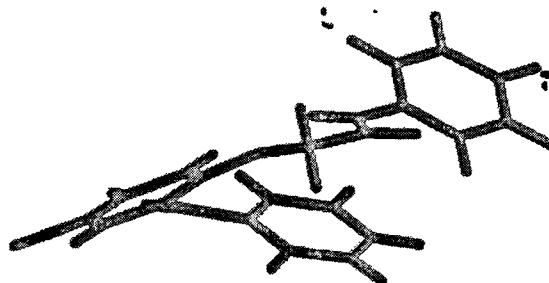


Figure 2. Proposed binding conformation of 1g.

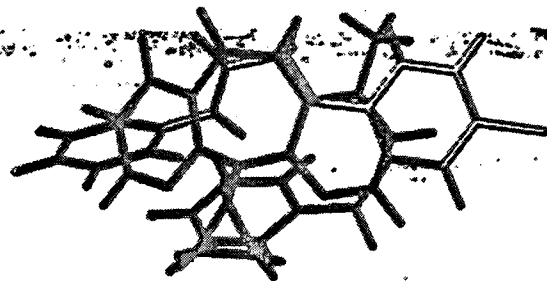


Figure 3. Proposed overlay of 1g with nevirapine.

distance map was produced by removing those conformations present in the map for 1j or 1l and present in 1g. This proposed 608 conformations within 5 kcal/mol of the lowest van der Waals energy for 1g. The conformations were clustered using the atomic RMS similarity tool within MACROMODEL.¹⁷ A representative of each set was minimised using MM2* forcefield and GB/SA solvent term. Of the low-energy conformations, the one shown in Figure 2 was felt to best match the SAR. This model was used for the design of further compounds such as indole 21. A proposed overlay of this conformation with nevirapine is shown (Figure 3). The comparison indicates that the benzophenones can make similar interactions with RT as those made by nevirapine, suggesting the two compounds bind at the same site. Consideration of this information together with comparisons with other non-nucleoside RT inhibi-

Table 6. Antiviral Activity

compound	HIV-1 RF (wild-type)		HIV-1 (8b-resistant)	
	IC ₅₀ ^a	TOX ^b	IC ₅₀ ^a	TOX ^b
8b	0.028	10	0.48	>10
AZT	0.0024	>1	0.0055	>1
3TC	0.043	>100	0.048	>100
Ro 31-8959	0.028	>1	0.0038	>1
TIBO R-32150	0.005	10	0.32	10
nevirapine	0.010	>10	0.22	>10
imidazopyridazine	0.004	>1	0.5	>1
L-697,661	0.0017	100	0.0023	100

^a IC₅₀ versus HIV-1 (strain RF) infected MT-4 cells (μg/mL). AZT = 0.002–0.02 μM. ^b IC₅₀ versus uninfected MT-4 cells (μg/mL).

tors could aid the design of compounds with potent activity against resistant HIV strains.

Selection of Resistant Virus

The rapid emergence of resistant strains has proved to be a problem with non-nucleoside anti-HIV agents progressed to clinical trials,¹⁸ and therefore it was important to ascertain whether this series of compounds selected for resistant virus. The selection of resistant virus was investigated using the benzophenone derivatives 8b (Table 2) (see Biological Methods). After eight passages of virus in the presence of increasing concentrations of 8b, a resistant strain was isolated that was 10-fold less sensitive to 8b. A number of known inhibitors of HIV-1 were tested against this virus strain in parallel with the wild-type strain to determine whether cross resistance occurred (Table 6). The nucleoside analogues AZT¹⁹ and 3TC¹⁹ and the protease inhibitor Ro 31-8959²⁰ were not cross resistant with the 8b-resistant strain. The non-nucleoside inhibitors, TIBO,⁵ nevirapine,⁵ and the imidazopyridazine⁷ proved to be cross resistant to the virus strain, also exhibiting a 10-fold loss of potency. In contrast, the non-nucleoside inhibitor L-697,661⁹ was not cross resistant and was equally potent against both the wild-type virus and 8b-resistant strain. However, 8b was inactive in a similar test using the HIV-1 strain A17 (supplied by Merck Sharp and Dohme Research Laboratories, West Point, PA) that was resistant to L-697,661. The A17 strain includes the mutation of the tyrosine-181 residue to cysteine, frequently associated with resistance to non-nucleoside RT inhibitors. This data shows the mutation in the 8b-resistant strain is not the commonly found tyrosine-181 to cysteine change but occurs in a region of the RT important for binding of TIBO, nevirapine, and the imidazopyridazine but not L-697,661.

Conclusions

A novel series of benzophenone derivatives have been synthesized, and a number are potent inhibitors of HIV-1 RT and HIV-1 in cellular assays. The benzophenone nucleus appears to be important for potent activity together with an aromatic function positioned by a suitable spacer group that contains a hydrogen bond acceptor function. In common with other non-nucleoside HIV-1 RT inhibitors, a member of this series of compounds (8b) rapidly induced resistant virus during passage of HIV-1 in the presence of the compound. Interestingly, the observed mutation was not the commonly observed tyrosine-181 to cysteine mutation, even though 8b was inactive against the A17 strain that contains this mutation. This result together with the

rapid onset of resistance during clinical use of other non-nucleoside HIV-1 RT inhibitors led to the cessation of work on this series of benzophenone derivatives.

Experimental Section

Methods and Materials. The ¹H NMR spectra for all compounds were recorded on a Bruker AM250 instrument, and the chemical shifts are reported as part per million (ppm) relative to internal tetramethylsilane (TMS). During workup, organic solutions were dried over MgSO₄ and evaporated on a Büchi rotatory evaporator with a bath temperature of 40 °C or below. Thin-layer chromatography was performed on silica plates (Merck Art. No. 5719), and flash column chromatography was carried out on silica (Merck Art. No. 9385). All dried solvents were purchased from the Aldrich Chemical Co (Suresse).

Method A: (2-Benzoylphenoxy)acetic Acid (3c). A solution of 2c²¹ (25.0 g, 126 mmol) in DMF (100 mL) was added dropwise to a stirred suspension of sodium hydride (80% dispersion in mineral oil; 3.8 g, 126 mmol) in DMF (100 mL). Stirring was continued for 1 h, and ethyl bromoacetate (14.0 mL, 126 mmol) was added. The mixture was heated at 70 °C for 16 h and then evaporated *in vacuo*. The residue was partitioned between water and EtOAc. The organic portion was washed with dilute aqueous NaOH and water, dried, and evaporated to give an oil (32.5 g). This was dissolved in EtOH (200 mL) and the solution treated with a solution of NaOH (7.0 g, 175 mmol) in water (50 mL). The mixture was heated under reflux for 1 h, cooled, and acidified with 1 N HCl. The oily precipitate was extracted with CH₂Cl₂, and the solution washed with water, dried, and evaporated to give an oil which crystallized from hexane to afford 3c (24.0 g, 74.3%) as white prisms: mp 169 °C; ¹H NMR (d₆-DMSO) δ 4.63 (s, 2H), 7.0–7.8 (m, 9H). Anal. (C₁₅H₁₁O₄) C, H.

2-(2-Benzoylphenoxy)-N-[4-[2-(diethylamino)ethoxy]phenyl]acetamide (1c). A mixture of 3c (2.56 g, 10.0 mmol) and thionyl chloride (20 mL) was heated at reflux for 30 min and then evaporated. The residue was dissolved in benzene and re-evaporated to give an oil. This was dissolved in benzene (20 mL) and added dropwise to a stirred solution of 4c²² (2.08 g, 10.0 mmol) and triethylamine (2.0 mL, 15.0 mmol) in benzene (80 mL). The mixture was heated at 70 °C for 16 h. The cooled solution was washed with dilute aqueous NaOH and water, dried, and evaporated to give an oil. This was purified by chromatography on silica gel using CH₂Cl₂–MeOH (9:1) to give 1c (1.70 g, 47%) as a colorless oil. Anal. (C₂₇H₃₀N₂O₄) C, H, N. A portion of this material (400 mg, 0.89 mmol) was heated in 2-propanol (10 mL) with fumaric acid (110 mg, 0.95 mmol). Upon cooling, 1c fumarate salt (450 mg, 90%) was deposited as white prisms: mp 144 °C; ¹H NMR (d₆-DMSO) δ 1.02 (t, J = 7.5 Hz, 6H), 2.70 (q, J = 7.5 Hz, 4H), 2.92 (t, J = 7.5 Hz, 2H), 4.04 (t, J = 7.5 Hz, 2H), 4.69 (s, 2H), 6.57 (s, 2H), 6.90 (d, J = 9 Hz, 2H), 7.07 (m, 2H), 7.35–7.70 (m, 7H), 7.80 (d, J = 9 Hz, 2H), 9.60 (s, 1H).

Method B: 2-(2-Benzoyl-4-fluorophenoxy)-N-phenylacetamide (1b). A mixture of 2f²³ (1.08 g, 5.0 mmol), K₂CO₃ (0.69 g, 5.0 mmol), and 2-bromo-N-phenylacetamide²⁴ (1.07 g, 5.0 mmol) in DMF (10 mL) was stirred under a nitrogen atmosphere for 4 h and then evaporated. The residue was partitioned between water and EtOAc. The organic portion was successively washed with water, 1 N HCl, and brine, dried, and evaporated to give an off-white solid (1.52 g) which crystallized from diisopropyl ether to give 1b (1.17 g, 67%) as white prisms: mp 127–129 °C; ¹H NMR (d₆-DMSO) δ 4.69 (s, 2H), 7.08 (t, J = 7.5 Hz, 1H), 7.22 (dd, J = 4, 9 Hz, 1H), 7.26–7.36 (m, 3H), 7.42 (dt, J = 2.5, 9 Hz, 1H), 7.52 (d, J = 7.5 Hz, 4H), 7.65 (d, J = 7.5 Hz, 1H), 7.82 (d, J = 7.5 Hz, 2H), 9.72 (br s, 1H). Anal. (C₂₇H₁₈FN₂O₂) C, H, F, N.

N-[3-(4-Amino-3-methylphenoxy)propyl]dimethylamine (4c). A mixture of 5b²¹ (20.0 g, 146 mmol) and K₂CO₃ (19.8 g, 143 mmol) in DMF (250 mL) was stirred for 15 min. A solution of N-(4-chloropropyl)dimethylamine²¹ (31.7 g, 261 mmol) in DMF (50 mL) was added dropwise and the mixture heated at 80 °C for 3 h. The mixture was evaporated and the residue treated with water and extracted with CH₂Cl₂.

Cl₂. The organic extract was washed with 1 N NaOH, dried, and evaporated to give an oil (27 g). This was dissolved in EtOH (300 mL) and hydrogenated in the presence of 10% palladium on charcoal (2.7 g). The reaction mixture was filtered and the filtrate evaporated to give 4c as an oil. (23.0 g, 76%); ¹H NMR (CDCl₃) signals at δ 2.0 (m, 2H), 2.2 (s, 6H), 2.3 (t, 2H), 2.55 (s, 3H), 4.0 (t, 2H). This material was used in the next step, the preparation of 8b, without further purification.

2-(2-Benzoyl-4-chlorophenyl)-N-[4-[3-(dimethylamino)propoxy]-2-methylphenyl]acetamide (8b). A mixture of 7¹ (2.0 g, 7.2 mmol) and 1-hydroxybenzotriazole hydrate (0.98 g, 7.2 mmol) in DMF (50 mL) was stirred for 10 min. A solution of 4c (1.52 g, 7.3 mmol) in DMF (50 mL) followed by *N,N'*-dicyclohexylcarbodiimide (1.5 g, 7.3 mmol) was added, and the mixture was stirred at 21 °C for 16 h and then filtered. The filtrate was evaporated and the residue treated with dilute aqueous NaOH and extracted several times with CH₂Cl₂. The combined extracts were evaporated to give an oil which was chromatographed on silica using CH₂Cl₂-MeOH (9:1) to give a solid which was crystallized from 2-propanol to afford 8b (1.30 g, 39%) as white prisms: mp 144–145 °C; ¹H NMR (d₆-DMSO) δ 1.79 (m, 2H), 1.99 (s, 3H), 2.12 (s, 6H), 2.30 (m, 2H), 3.82 (s, 2H), 3.90 (m, 2H), 6.61 (dd, *J* = 3, 9 Hz, 1H), 6.69 (d, *J* = 3 Hz, 1H), 6.98 (d, *J* = 9 Hz, 1H), 7.46 (d, *J* = 3 Hz, 1H), 7.5–7.8 (m, 7H), 9.27 (s, 1H). Anal. (C₂₇H₃₂ClN₂O₃) H, N, C: calcd, 69.7; found, 69.1.

2-[4-Chloro-2-(pyrid-3-ylcarbonyl)phenoxy]-N-phenylacetamide (1m). A mixture of 2k (280 mg, 1.2 mmol), 2-bromo-N-phenylacetamide (250 mg, 1.2 mmol), and K₂CO₃ (180 mg, 1.3 mmol) in DMF (5 mL) was stirred for 21 h and then diluted with EtOAc (50 mL). The solution was washed with water and saturated brine, dried, and evaporated to give a brown glass (428 mg). Crystallization from 2-propanol afforded 1m (245 mg, 56%) as yellow prisms: mp 169–170 °C; ¹H NMR (d₆-DMSO) δ 4.74 (s, 2H), 7.06 (t, *J* = 9 Hz, 1H), 7.21 (d, *J* = 9 Hz, 1H), 7.29 (t, *J* = 9 Hz, 2H), 7.36–7.57 (m, 4H), 7.66 (dd, *J* = 3, 9 Hz, 1H), 8.18 (dt, *J* = 2, 9 Hz, 1H), 8.77 (dd, *J* = 2, 6 Hz, 1H), 8.95 (d, *J* = 2 Hz, 1H), 9.88 (s, 1H). Anal. (C₂₂H₁₂ClN₂O₂) C, H, Cl, N.

2-[4-Chloro-2-(hydroxyphenylmethyl)phenoxy]-N-phenylacetamide (11). Sodium borohydride (200 mg, 5.3 mmol) was added to a solution of 1g (1.83 g, 5.0 mmol) in THF (50 mL) at 0 °C. The mixture was stirred for 4.5 h, during which time it was allowed to warm to 21 °C. Cold 2 N HCl was added, and the solution was concentrated. The residual oil was dissolved in EtOAc and the solution washed with 2 N HCl and brine. The dried solution was evaporated to an oil which was crystallized from cyclohexane-EtOAc (5:1) to give 11 (1.31 g, 71%) as white prisms: mp 147–148 °C; ¹H NMR (d₆-DMSO) δ 4.70 (AB q, *J* = 14, 20 Hz, 2H), 6.06 (d, *J* = 5 Hz, 1H), 6.10 (d, *J* = 5 Hz, 1H), 6.96 (d, *J* = 9 Hz, 1H), 7.10 (t, *J* = 9 Hz, 1H), 7.15–7.5 (m, 9H), 7.61 (d, *J* = 9 Hz, 2H), 10.07 (s, 1H). Anal. (C₂₇H₂₄ClNO₂) C, H, Cl, N.

2-[2-(Phenylamino)ethoxy]-5-chlorophenylphenylmethanone (12). A mixture of 2e (0.80 g, 3.4 mmol), K₂CO₃ (0.50 g, 3.8 mmol), and *N*-(2-chloroethyl)aniline²³ (0.53 g, 3.4 mmol) in DMF (20 mL) was heated at 100 °C under a nitrogen atmosphere for 2 h, cooled, and evaporated. The residue was dissolved in CH₂Cl₂, washed with 2 N K₂CO₃ and water, dried, and evaporated. The residue was purified by flash chromatography on silica gel using cyclohexane-EtOAc (9:1) to give an oil (0.6 g). A portion of the oil was dissolved in Et₂O and treated with HCl in Et₂O to afford 12-HCl salt: mp 131–132 °C; ¹H NMR (CD₃OD) δ 3.55 (t, *J* = 5 Hz, 2H), 4.30 (t, *J* = 5 Hz, 2H), 7.25 (m, 3H), 7.45–7.65 (m, 7H), 7.75 (m, 1H), 7.85 (m, 2H). Anal. (C₂₁H₁₉Cl₂NO₂) C, H, Cl, N.

[4-Chloro-2-(2-phenyl[1,3]dioxolan-2-yl)phenoxy]acetoneitrile (13). A mixture of 2e (5.0 g, 21.5 mmol), ethylene glycol (25 mL), toluene-4-sulfonic acid monohydrate (230 mg), and toluene (120 mL) was heated at reflux in a Dean-Stark apparatus for 24 h. The mixture was cooled, successively washed with saturated aqueous NaHCO₃, water, and brine, dried, and evaporated to give a yellow oil (5.65 g). A portion of this material (1.10 g), K₂CO₃ (0.59 g, 4.3 mmol), and bromoacetonitrile (0.30 mL, 4.3 mmol) in DMF (10 mL) was

stirred for 4.5 h. The mixture was diluted with EtOAc (100 mL) and successively washed with 2 N HCl, water, and brine. The dried solution was evaporated to give a dark oil (1.3 g) which was purified by flash chromatography on silica gel using cyclohexane-EtOAc (2:1, containing 0.1% NEt₃) to give 13 (1.03 g, 76%) as a white crystalline solid: mp 86–88 °C; ¹H NMR (d₆-DMSO) δ 4.00 (m, 4H), 4.95 (s, 2H), 7.13 (d, *J* = 8 Hz, 1H), 7.25–7.75 (m, 7H). Anal. (C₁₇H₁₃ClNO₂) C, H, Cl, N.

1-(2-Benzoyl-4-chlorophenoxy)-3-phenylpropan-2-one (14). A solution of 13 (936 mg, 3.0 mmol) in anhydrous THF (15 mL) was stirred at –78 °C under an atmosphere of N₂. Benzylmagnesium bromide (2 M) in THF (1.5 mL, 3.0 mmol) was added, and the solution was stirred for 5 h. Additional 2 M benzylmagnesium bromide in THF (0.5 mL, 1.0 mmol) was added, and stirring was continued for 2.5 h. The solution was diluted with EtOAc, washed with aqueous NH₄Cl and brine, dried, and evaporated to give a foam (1.12 g). This was purified by flash chromatography on silica gel using cyclohexane-EtOAc (5:1, containing 0.2% NEt₃) to give an oil (152 mg). The bulk of this material (128 mg) was dissolved in THF (6 mL), and 1 N HCl (6 mL) was added. The mixture was heated at 70 °C for 3 h and then stirred at room temperature for 16 h. The mixture was extracted with ether and the organic extract washed with brine and dried. Evaporation gave a gummy solid (178 mg) which was purified by flash chromatography on silica gel using cyclohexane-EtOAc (5:1) to give 14 (65 mg, 7.0%) as an oil: ¹H NMR (CDCl₃) δ 3.45 (s, 2H), 4.47 (s, 2H), 6.70 (d, *J* = 8 Hz, 1H), 7.00–7.65 (m, 8H), 7.86 (d, *J* = 4 Hz, 1H); MS (TOF) *m/z* 365.3 (M⁺) (calcd for C₂₂H₁₇ClO₂ 364.83).

2-[3-Phenyl-2(E)-propenoxy]-5-chlorophenylphenylmethanone (15). A mixture of 2e (1.00 g, 4.30 mmol), K₂CO₃ (0.71 g, 5.16 mmol), and *trans*-cinnamyl bromide (0.85 g, 4.3 mmol) in DMF (50 mL) was heated at 60 °C for 2 h, cooled, and evaporated. The residue was dissolved in CH₂Cl₂, washed with dilute NaOH and water, dried, and evaporated. The residue was purified by flash chromatography on silica gel using cyclohexane-EtOAc (85:15) to give an oil which was crystallized from diisopropyl ether to afford 15 (0.73 g, 49%) as white prisms: mp 89–90 °C; ¹H NMR (CDCl₃) δ 4.60 (d, *J* = 5 Hz, 2H), 6.00 (td, *J* = 5, 16 Hz, 1H), 6.26 (d, *J* = 16 Hz, 2H), 6.96 (m, 1H), 7.25 (m, 5H), 7.44 (m, 4H), 7.55 (m, 4H), 7.82 (m, 2H). Anal. (C₂₂H₁₇ClO₂) C, H, Cl.

2-(2-Benzoyl-4-chlorophenoxy)-1-(3,4-dihydro-1H-isoquinolin-2-yl)ethanone (18). Triethylamine (0.35 mL, 2.5 mmol) and 1,2,3,4-tetrahydroisoquinoline²¹ (0.32 mL, 2.5 mmol) were added to stirred solution of 2e (531 mg, 2.0 mmol), 1-hydroxybenzotriazole hydrate (270 mg, 2.0 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (480 mg, 2.5 mmol) in THF (25 mL). The mixture was stirred for 19 h, diluted with EtOAc, and washed sequentially with water, saturated aqueous NaHCO₃, water, 2 N HCl, water, and brine. The dried solution was evaporated to give 18 (772 mg, 95.1%) as a white foam. A portion of this material was crystallized from ether to give white prisms: mp 101–102 °C; ¹H NMR (d₆-DMSO) δ 2.76 (m, 2H), 3.51 (t, *J* = 6 Hz, 2H), 3.59 (t, *J* = 6 Hz, 2H), 4.52 (d, *J* = 7 Hz, 2H), 4.95 (s, 2H), 7.10 (d, *J* = 9 Hz, 1H), 7.17 (s, 2H), 7.35–7.70 (m, 5H), 7.81 (d, *J* = 7 Hz, 2H). Anal. (C₂₆H₂₂ClNO₂) C, H, Cl, N.

2-(2-Benzoyl-4-chlorophenoxy)-1-(1H-indol-2-yl)ethanone (21). A solution of phenol 19²⁴ (1.10 g, 4.67 mmol) and K₂CO₃ (645 mg, 4.67 mmol) in DMF (50 mL) was stirred at room temperature for 30 min. Indole 20²⁷ (904 mg, 4.67 mmol) was then added, and the solution stirred for 5 h. The reaction was evaporated to afford an oil which was partitioned between EtOAc (50 mL) and brine (50 mL). The organic phase was washed with brine, dried, and evaporated to a brown oil. Flash chromatography on silica gel using CH₂Cl₂-MeOH (100:1) afforded a brown oil which was crystallized from cyclohexane-Me₂CO to afford a solid (726 mg). A portion of this material (600 mg) in CH₂Cl₂ (5 mL) was treated with pyridinium chlorochromate (363 mg, 1.68 mmol), and the mixture was stirred for 1 h. Diethyl ether (100 mL) was then added, and the resulting suspension was filtered through Celite. The filter bed was washed with EtOAc, and the combined filtrates were

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evaporated to afford a brown oil. Purification by flash chromatography on silica gel using cyclohexane-EtOAc (5:1) gave 21 (284 mg, 48%) as a white crystalline solid: mp 125–126 °C; ¹H NMR (*d*₆-DMSO) δ 5.50 (s, 2H), 7.04–7.90 (m, 13H), 11.85 (br s, 1H). Anal. (C₂₂H₁₅ClNO₂) C, H, Cl, N.

[2-(1*H*-Benzimidazol-2-ylmethoxy)-5-chlorophenyl]phenylmethanone (23). A solution of 2e (0.5 g, 2.15 mmol) in DMF (5 mL) was added dropwise to a stirred suspension of sodium hydride (60% suspension in mineral oil; 95 mg, 2.37 mmol) in DMF (10 mL). The mixture was stirred for 10 min, and a solution of 22¹ (0.36 g, 2.16 mmol) in DMF (10 mL) was added dropwise. The mixture was heated at 80 °C for 16 h and then evaporated. The residue was partitioned between water and CH₂Cl₂. The organic phase was dried and evaporated to give an oil. Purification by chromatography using CH₂Cl₂-MeOH (19:1) gave an oil which crystallized from 2-propanol to afford 23 (0.18 g, 23%) as pale yellow prisms: mp 144–145 °C; ¹H NMR (*d*₆-DMSO) δ 5.31 (s, 2H), 7.18 (m, 2H), 7.41–7.77 (m, 10H), 12.40 (br s, 1H). Anal. (C₂₁H₁₅ClN₂O₂·0.5H₂O) H, Cl, N, C: calcd, 67.8; found, 68.5.

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Supplementary Material Available: Atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and hydrogen coordinates for compound 1h (5 pages); structure factor listings for compound 1h (9 pages). Ordering information is given on any current masthead page.

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1-Azaacycloalkyl-1,4-benzodiazepin-2-ones with Antianxiety-Antidepressant Actions

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A series of 1-azaacycloalkyl-1,4-benzodiazepin-2-ones were synthesized from 1-azaacycloalkyl-2-benzoylanilines 2-4 and corresponding imines 5 and 6 and then evaluated for their central nervous system activities. Pharmacological data showed that some of these compounds have potent antidepressant properties, as assessed by their antagonism of tetrahydrozoline (THZ) induced ptosis and their inhibition of [³H]norepinephrine uptake into rat brain synaptosomes, as well as their moderate anxiolytic properties of preventing of pentylenetetrazol (PTZ) convulsion, suppressing conflict behavior, and displacing potential for [³H]diazepam binding. Introduction of a halogen substituent at position 7 of the 1,4-benzodiazepine ring lengthened the anti-PTZ effect, although the peak effect was slightly reduced and clearly enhanced the anti-PTZ and anxiolytic properties. Introduction of Cl to the ortho position of the phenyl ring at position 5 greatly reduced the antidepressant properties. The secondary amine function of the azaacycloalkyl ring at position 1 was essential for the production of the antidepressant properties. Of these new series, 7-fluoro-5-(2-fluorophenyl)-1,3-dihydro-1,4-pyridazinyl-2H-1,4-benzodiazepin-2-one (10d) has the potential to become a useful antidepressant drug with a moderate anxiolytic property.

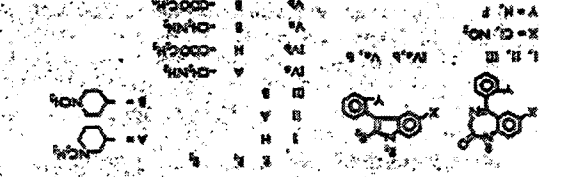
As the chemistry, psychopharmacology, and structure-activity relationships of 1,4-benzodiazepines (1) have been clearly established, the possibility for finding a new type of 1, which is readily accessible by simple modification and yet retains favorable psychopharmacological properties, is small. However, our exclusive ortho acylation reaction of anilines using boron trichloride and nitriles¹ made possible the one-step synthesis of 2-acyl-N-azaacycloalkyl-1,4-benzodiazepines (7-12) having a piperidine or pyrrolidine benzodiazepines (7-12) having a piperidine or pyrrolidine moiety directly attached at position 1 (Scheme 1). Although detailed investigation of the structure-activity relationships of 1 revealed that substitution of basic aminoconjugate side chains at position 1 generally weakens anxiolytic activity,² we found that some of our new 1,4-benzodiazepines possess the pharmacological features of potent antidepressants together with those of anxiolytics. The combined anxiolytic-antidepressant actions of benzodiazepine derivatives have been reported in recent years.³

Chemistry. With use of 2 and 3 (R₂ = allyl) as starting material, 7 and 8 were synthesized by the conventional reagent (method A, Scheme II). However, 13 (R₂ = CH₃, X = 4-NO₂, Y = 2-Cl) gave 15 (X = 4-NO₂, Y = 2-Cl) as the main product (25%), so the desired 7a (Table I) was synthesized by nitration of 7b. Alternatively, 2 and 4 (R₂ = CH₃) were condensed with phthaloylacetyl chloride to give 16 and 17, which were subjected to hydrazinolysis (method B). This method generally gave 15 as a byproduct; 16 (X = 9-Cl, Y = 2-F) gave 15 (X = 9-Cl, Y = 2-F) in 50% yield besides the target compound 7a (14%). Compounds 7 and 9 were obtained more conveniently, when the 2-acylanilines 5 and 6 were condensed with glycine ethyl ester hydrochloride to give imino esters 18 and 19, which were cyclized under acidic conditions in which polyphosphoric acid (PPA) was found to be most favorable. Compound 10 could also be obtained directly from 5 (R₂ = H) (method C). To synthesize 1-piperidinyl-1,4-benzodiazepin-2-ones 10 and 11, 2 and 3 (R₂ = CH₃) were converted into (benzyl-

oxycarbonyl)piperidinyl derivatives 24 and 25 by successive treatment with ethyl chloroformate, 6 N hydrochloric acid, and benzyl chloroformate via compounds 20 and 21 and compounds 22 and 23. Method A and B were used to cyclize 24 and 25 to the corresponding 1,4-benzodiazepin-2-ones 26 and 27. Decarboxylation was conducted by treatment with a solution of aluminum trichloride and anisole in dichloromethane and nitromethane⁴ or by hydrolysis with trifluoroacetic acid (me-

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(3) After the completion of our work, we were informed about a patent application from Sumitomo Chemical Co., Ltd., Osaka, Japan, in 1983 (Chem. Abstr. 1983, 107:130, 1970; Chem. Abstr. 1970, 72, 100773), which partly anticipates our work. It was stated that compound II could be obtained by treatment of I with 1-ethyl-3-chloropiperidine in the presence of NaH in toluene and DMF in an unspecified yield. Alternatively, II could be obtained by oxidative ring closure of IVa in an unspecified yield. But no experimental data was given on the synthesis of IVa, the starting material, or compounds III and Va. (Con-

found III was supposed to have been obtained from I or Va in a reaction similar to that for obtaining II.) During our work, we confirmed that treatment of I (X = Y = Cl) with 4-chloro-N-methylpiperidine (VI) in the presence of NaH in toluene and DMF (1:1) under reflux for 4 h gave no trace of III (X = Y = Cl), with I being recovered in quantitative yield. Also, treatment of IVb (X = Cl, Y = H) with VI under similar conditions or by transfer catalysis (benzyltrimethylammonium chloride) did not give the desired piperidinyl imino ester Vb (X = Cl, Y = H), with the starting material being recovered in almost quantitative yield.

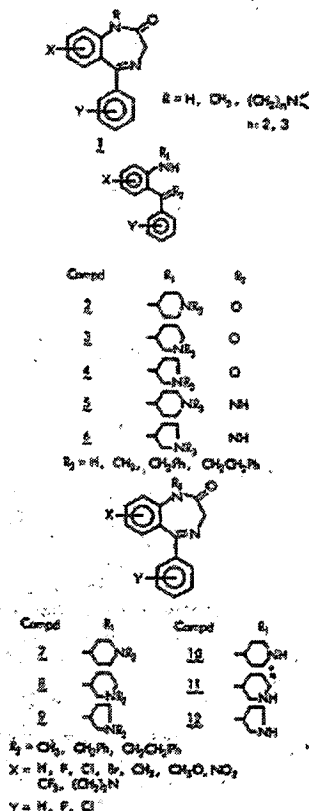


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Sugasa.

Scheme I



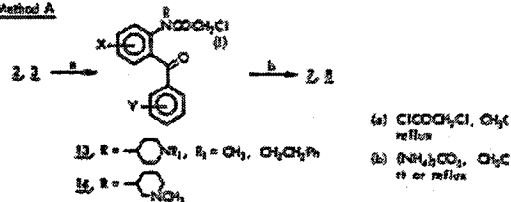
thod D, Scheme III). In a more facile reaction, 10 and 12 could be obtained via [N-(ethoxycarbonyl)piperidinyl]- and [N-(ethoxycarbonyl)pyrrolidinyl]-1,4-benzodiazepin-2-ones 28 and 29 produced by dealkylative ethoxycarbonylation of 7 ($R_2 = \text{CH}_3$) and 9 ($R_2 = \text{CH}_2\text{Ph}$) followed by mild hydrolysis with use of dialkyl sulfide and methanesulfonic acid.⁷ However, in the present study, we adopted an even more convenient method in which demethylation was conducted with α -chloroethyl chloroformate in a one-pot operation⁸ (method E). Compound 28 could also be obtained from 20 by method A or B. Similarly, N-acetyl derivative 30 was synthesized from 31, which had been prepared by acetylation of 22.

3-Hydroxy derivatives 34 were produced by successive treatment of the N-benzoyloxycarbonyl derivative 26 with 30% hydrogen peroxide and acetic anhydride followed by simultaneous deprotection with aluminum trichloride and anisole as described in method D via 32 and 33 (method F, Scheme IV).

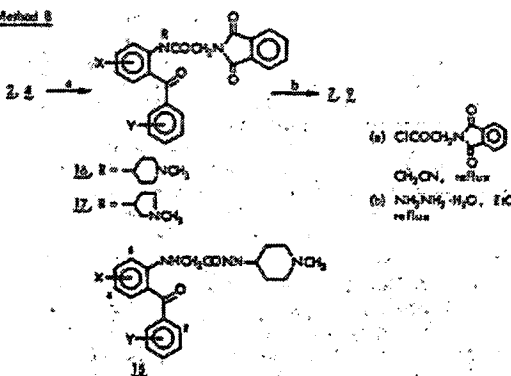
Pharmacology. The antidepressant and anxiolytic activities of 1-azacycloalkyl-1,4-benzodiazepin-2-ones were primarily assessed by their prevention of tetrabenazine (TBZ) induced ptosis and pentylentetrazol (PTZ) intoxication in mice, respectively. The antianxiety activities of selected compounds were followed up by observing anticonflict effect in rats. Also examined with selected compounds were the uptake inhibition of [³H]nor-

Scheme II

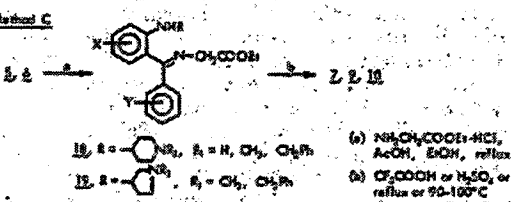
Method A



Method B



Method C



epinephrine (NE) into rat brain synaptosomes⁹ and the displacing potential for [³H]diazepam (DZ) binding in rat brain.¹⁰ Pharmacological data are summarized in Tables III-V.

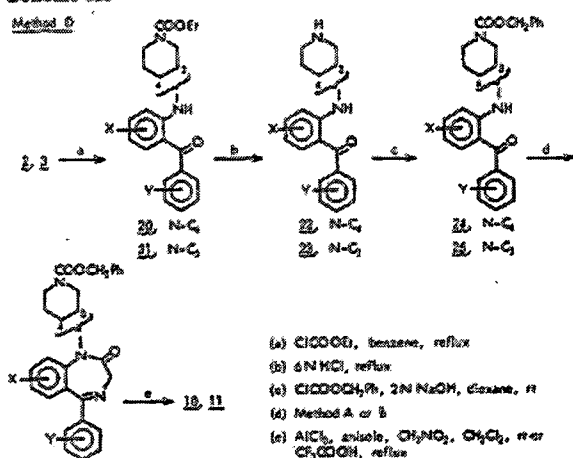
Most of the test compounds antagonized TBZ-induced ptosis in mice, when administered 1 h prior to TBZ. The potency was generally higher in compounds with the secondary amine function on the piperidine or pyrrolidine ring in position 1 of 1,4-benzodiazepine (NH) than their corresponding N-methyl congeners (NCH₃). This rule applied to six pairs of compounds: 7d-10d, 7e-10e, 7g-10g, 7h-10h, 7i-10i, and 9a-12a. However, no large difference was seen in their potencies when the test was carried out 4 h after administration. For example, the respective ED₅₀ values of 7i and 10i with the pretreatment schedule of 1 h were 2.88 and 0.76 mg/kg, while the values with the 4-h pretreatment schedule were nearly equal, 0.69 and 0.64 mg/kg, po. Upon oral administration, the most active compound was 10a, with equivalent activity shown by its NCH₃ compound (7a). However, 10a was about 4 times as potent as 7a when administered subcutaneously. This result indicates that the antidepressant profile judged from the anti-TBZ effect may be intrinsic for the NH compounds and that biotransformation, i.e., demethylation, is required for the NCH₃ compounds to elicit this action. Biochemical data on the uptake inhibition of [³H]NE support this assumption. The NH compounds 10d and 10h prevented the uptake of NE with the respective IC₅₀ values of 0.08 and 0.56 μM , being 70-700 times as potent

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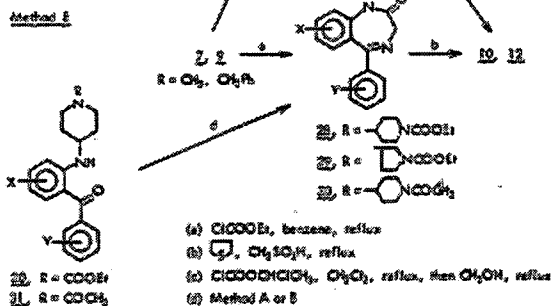
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 (10) Möhler, H.; Okada, H. *Life Sci.* 1977, 20, 2101.

Scheme III

Method D

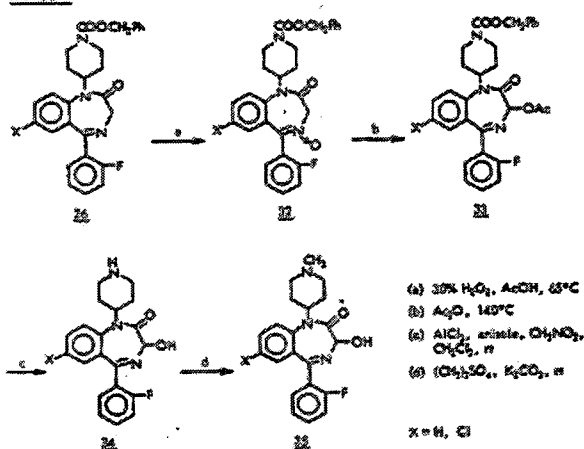


Method E



Scheme IV

Method F



as their corresponding NCH_3 compounds 7d ($53.7 \mu\text{M}$) and 7h ($39.9 \mu\text{M}$). Imipramine antagonized TBZ-induced ptosis with an ED_{50} value of 2.14 mg/kg , po and its IC_{50} value for NE-uptake inhibition was $0.09 \mu\text{M}$. Thus, 7a and 10a were more active in the present tests than imipramine.

The introduction of substituents to the benzodiazepine moiety and/or the phenyl ring at position 5 gave rise to interesting changes in both antidepressive and anxiolytic properties. In general, halogen substitution at position 7 of the benzodiazepine ring (X) caused a decrease in the peak effect and an increase in the duration of action of the anti-TBZ activity as substituents increased in molecular size. Compound 10a (X = H, $\text{ED}_{50} = 0.26 \text{ mg/kg}$) was the

most active one when 1-h pretreatment was used, followed by 10c (X = F, 0.39 mg/kg), 10f (X = Cl, 0.48 mg/kg), and 10i (X = Br, 0.76 mg/kg). However, 10f and 10i preserved obvious activities even at 24 h after administration, their respective ED_{50} values being 1.47 and 1.26 mg/kg in contrast to no effects with 10a or 10c at a dose of 32 mg/kg . The NCH_3 compounds behaved in the same manner. The N-(ethoxycarbonyl)piperidine 28 and N-acetylpiperidine 30 series were also active but weaker in potency.

Benzodiazepine derivatives without halogen substituents have been shown to lack antianxiety properties.¹ This rule applies to our 7a and 10a. Introduction of F at the X position failed to increase the activity; 10c as well as 10a showed neither anti-PTZ nor anticonflict effects with K_i values in the micromolar range in the [^3H]diazepam binding test. However, introduction of Cl (10f) or Br (10i) resulted in activities 2–3 times as potent as 10a in the affinity to benzodiazepine receptors, accompanied by weak but significant in vivo potencies. 10f became positive in the anticonflict test with an ED_{50} value of 11.81 mg/kg , po. This compound also showed a very weak but dose-dependent antagonism in the PTZ test, if given orally 4 h prior to PTZ ($\text{ED}_{50} = 47.17 \text{ mg/kg}$, po). One exception was 7c (X = F, N = CH_3), which had an ED_{50} value of 25.79 mg/kg in the PTZ test in spite of the large K_i value of $2.42 \mu\text{M}$. However, this compound showed no significant activity in the anticonflict test.

According to the structure-activity relationship, the larger the halogen substituent at position 7 was, the longer was the duration of the antidepressive properties and the more active were the antianxiety effects.

The effects of substituents other than halogen at position 7 (CH_3 , 7k; OCH_3 , 7l; $\text{N}(\text{CH}_3)_2$, 7m; NO_2 , 7n) and of halogen substituents at positions other than 7 (8-F, 7o; 8-Cl, 7p; 8- CF_3 , 7g; 7,8-Cl, 7r; 9-Cl, 7s) were assessed for compounds with F on the ortho position of the phenyl ring (Y = F). The anti-TBZ activity was preserved in compounds receiving any substitution at the 7-position, except for 7n. 7p (8-Cl, Y = F) was completely devoid of anti-TBZ activity, but its comparable compound, 7g (7-Cl, Y = F), was very active. Thus, a substituent at position 8 seemed to be undesirable for the anti-TBZ activity, which reappeared when the substituent was moved to position 9 (7s; 9-Cl, Y = F). All these modifications led to marked decreases in anti-PTZ effects.

Antidepressive and anxiolytic properties were affected in opposite ways by the introduction of a halogen substituent at Y. Although the antidepressant activities were little affected by F substitution, the affinities to benzodiazepine receptors were remarkably enhanced as indicated by the great changes in K_i values (7a,b, 7c,d, 7f,g', 7i,j, 10a,b, 10c,d, 10f,g', 10i,j etc.). As mentioned above, 7a and 10a (X and Y = H) had very low affinities for benzodiazepine receptors (K_i in μM) and no apparent anti-PTZ and anticonflict effects. But 7b and 10b (X = H, Y = F) showed significant affinities for the receptor with the respective K_i values of 0.2 and $0.15 \mu\text{M}$; they were about 10 times as potent as those of 7a and 10a, even though the potentials were still insufficient for producing significant anti-PTZ activity. The manifestation of in vivo efficacies by F substitution on Y were achieved first in 7d and 10d (X and Y = F). Compounds 7f, 7i, 10f, and 10i, having Cl or Br at X and no halogen at Y, could antagonize PTZ-induced seizure with ED_{50} values between 20 and 40 mg/kg . They were more active in inhibiting conflict behaviors ($\text{ED}_{50} = 8\text{--}20 \text{ mg/kg}$, po). Additional F substitution at the Y position yielded potencies in the PTZ test as high as that of chlordiazepoxide, as seen in 7g', 7j, and

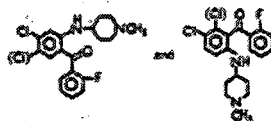
Table I. 1-Azacyclopentyl-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-ones

compd ^a	X	Y	R	method	yield, %	mp, °C	recryst ^b solvent	formula	anal. ^d C, H, N
7a	H	H	CH ₃	B	59	157-159	D-E	C ₂₁ H ₂₃ N ₃ O	C, H, N
7b	H	2F	CH ₃	C ^c	85	122-125	D-E	C ₂₁ H ₂₃ N ₃ O	C, H, N
7c	7-F	H	CH ₃	B	48	236-238 dec	F-G	C ₂₀ H ₂₁ N ₃ O-2HCl	C, H, Cl, F, N
7c'	7-F	H	CH ₃	C ^c	84	223-228 dec	D-I	C ₂₀ H ₂₁ N ₃ O-CF ₃ COOH	C, H, Cl, F, N
7d	7-F	2-F	CH ₃	B	61	146-148	D-E	C ₂₁ H ₂₃ N ₃ O	C, H, N, F
7e	7-F	2-F	CH ₃	C ^c	54				
7f	7-F	2-Cl	CH ₃	C ^c	85	139-140	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
7f'	7-Cl	H	CH ₃	C ^c	53	148-150	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, N
7g	7-Cl	2-F	CH ₃	A	71	250-251 dec	F-G	C ₂₀ H ₂₁ N ₃ O-2HCl	C, H, Cl, N
7g'	7-Cl	2-F	CH ₃	B	44	147-149	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
7h	7-Cl	2-Cl	CH ₃	C ^c	74	199-200 dec	H-5% H ₂ O	C ₂₀ H ₂₁ N ₃ O-2HCl	C, H, Cl, F, N
7i	7-Br	2-Cl	CH ₃	B	42	146-147	C-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, N
7j	7-Br	H	CH ₃	C ^c	51	247-252 dec	D-G	C ₂₀ H ₂₁ N ₃ O-2HBr	C, H, Br, F, N
7k	7-CH ₃	2-F	CH ₃	B	71	170-171	C-E	C ₂₀ H ₂₁ N ₃ O	C, H, Br, F, N
7l	7-CH ₃	2-F	CH ₃	B	59	139-141	C-E	C ₂₀ H ₂₁ N ₃ O	C, H, F, N
7m	7-NO ₂	2-F	CH ₃	B	68	76-77	E-G	C ₂₀ H ₂₁ N ₃ O-2HCl	C, H, F, N
7n	8-F	2-F	CH ₃	A	58	142-144	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, F, N
7o	8-F	2-F	CH ₃	B	23	199-201	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, F, N
7p	8-Cl	2-F	CH ₃	B	60	200-201	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
7q	8-Cl	2-F	CH ₃	B	22	203-205 dec	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
7r	8-Cl	2-F	CH ₃	B	41	121-122	B-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
7s	8-Cl	2-F	CH ₃	B	26	168-169	E-G	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
7t	9-Cl	2-F	CH ₃	B	14	181-182	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
7u	7-Cl	4-F	CH ₃	B	80	powder	C-E	C ₂₀ H ₂₁ N ₃ O	C, H, F, N
7v	7-Cl	H	CH ₂ CH ₂ Ph	B	71	172-173	F	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, N
7v'	7-Cl	H	H	A	67	217-219 dec	F	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, N
10a	H	H	H	D-B	60	281-283 dec	F	C ₂₀ H ₂₁ N ₃ O-2HBr	C, H, Br, N
10b	H	2-F	H	C-E	64				
10c	7-F	H	H	A-E	64				
10d	7-F	2-F	H	C ^c	69				
10e	7-F	2-F	H	D-B	71	166-167	F-I	C ₂₀ H ₂₁ N ₃ O	C, H, F, N
10f	7-Cl	2-F	H	C-E	64	121-122	A-E	C ₂₀ H ₂₁ N ₃ O	C, H, F, N
10f'	7-Cl	2-F	H	C-E	49	150-151	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, F, N
10g	7-Cl	2-F	H	C ^c	65				
10g'	7-Cl	2-F	H	C-E	61	275-276 dec	E-G	C ₂₀ H ₂₁ N ₃ O-2HCl	C, H, F, N
10h	7-Cl	2-F	H	D-B	39	276-278 dec	F-G	C ₂₀ H ₂₁ N ₃ O-2HCl	C, H, Cl, F, N
10i	7-Cl	2-F	H	C-E	75	191-192	E-G	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, N
10j	7-Cl	2-F	H	D-B	34	262-265 dec	D-F	C ₂₀ H ₂₁ N ₃ O-0.5H ₂ O	C, H, Br, Cl, N
10k	7-Cl	2-F	H	D-A	56	197-199 dec	F-I	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
10l	7-Cl	2-F	H	C-E	56	261-262 dec	F-H ₂ O	C ₂₀ H ₂₁ N ₃ O-2HBr	C, H, F, N
10m	7-Cl	2-Cl	H	C ^c	45	powder			
10n	7-Br	H	H	D-A	54				
10o	7-Br	2-F	H	B-E	48	194-198 dec	E-G	C ₂₀ H ₂₁ N ₃ O	C, H, Br, N
8	7-Cl	2-F	CH ₃	B-E	51	202-204 dec	E-G	C ₂₀ H ₂₁ N ₃ O	C, H, Br, F, N
9a	7-Cl	2-F	CH ₃	A	44	powder			
9b	7-Cl	2-F	CH ₃	P	42	113-114	C-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
9c	7-Cl	2-F	CH ₂ Ph	B	20	141-143	E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
11	7-Cl	2-F	H	D-A	77	156-157	D-E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
12a	7-Cl	2-F	H	C-E	34	133-136	E	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, N
12b	7-Cl	2-F	H	C-E	62	220-221 dec	A-H ₂ O	C ₂₀ H ₂₁ N ₃ O-0.5H ₂ O	C, H, Br, F, N
28a	H	H	COOEt	A	11	138-140	F	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, N
28b	7-Cl	2-F	COOEt	B	85	160-161	A-D	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, F, N
28c	7-Cl	2-F	COOEt	A	73	powder			
30	H	H	COCH ₃	A	58	208-209	D-G	C ₂₀ H ₂₁ N ₃ O	C, H, Cl, N

^a All compounds exhibited IR (max ca. 1680 cm⁻¹) and ¹H NMR (CDCl₃) δ 3.7-3.9 and 4.7-4.8 (2 H, AB, J = 10 Hz, COCH₂N) spectra consistent with the structures. ^b Isolated yield based on corresponding 2, 3, 4, 5, and 6. ^c A = acetone, B = benzene, C = hexane, D = dichloromethane, E = ether, F = methanol, G = 2-propanol, H = tetrahydrofuran, I = ethyl acetate. ^d All compounds were analyzed (C, H, halogens, and N), and the values obtained were within ±0.5% of the theoretical values besides the following data. Calcd (found): 7i C, 71.77; (71.14%); 9 Cl, 17.63 (16.52); 10h C, 61.86 (59.65); 10i C, 60.31 (59.71), Br 20.08 (20.57); 28a C, 69.88 (70.57); 28b C, 61.60 (62.23).

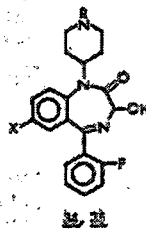
Footnotes to Table I Continued

^aCF₃COOH was used. ^bPPA was used. ^cH₂SO₄ was used. ^dSee Experimental Section. ^eStarting materials were a 3:1 mixture of ^fCrude



2 (X = 6-Cl, R₂ = CH₃, Y = 2-F, ca. 70% purity) was used. ^a28 was prepared from 20. ^b5 (X = Y = R₂ = H) was used as starting material. ^c5 (X = Y = F, R₂ = H) was used as starting material. ^dCICOCHClCH₃ was used; otherwise stated ClOOEt was used. ^e5 (X = 4-Cl, Y = 2-F, R₂ = H) was used as starting material. ^f12a (2.9 mmol) was methylated with 37% CH₃O (3.0 mmol) and Raney Ni (5 mL of EtOH) in a solution of MeOH (15 mL) with a H₂ stream at room temperature for 2 h. ^g9c was used as starting material. ^h4-Chloro-N-3-pyrrolidinylaniline dihydrochloride was treated as described in ref 2 and the resulting 4 (X = 4-Cl, Y = 2-Cl, R₂ = H) was subjected to ethoxycarbonylation, giving oily 4 (X = 4-Cl, Y = 2-Cl, R₂ = COOCH₂Ph, 38%); IR ^{max} 3290, 1690, 1620 cm⁻¹; ¹H NMR (CDCl₃) δ 5.12 (2 H, s, CH₂Ph), 9.1 (1 H, d, J = 6 Hz, NH). This was converted into 12b by using method A.

Table II. 1,3-Dihydro-5-(2-fluorophenyl)-3-hydroxy-1-(4-piperidinyl)-2H-1,4-benzodiazepin-2-ones



compd ^a	X	R	method	yield, ^b %	mp, °C	recrystn ^c solvent	formula	anal. ^d
34a	H	H	F	67	190-192	D-I	C ₂₂ H ₂₀ FN ₂ O ₂	C, H, F, N
34b	Cl	H	F	57	215-216 dec	F	C ₂₁ H ₁₉ ClFN ₂ O ₂	C, H, Cl, F, N
35	Cl	CH ₃	e	43	197-198	D-I	C ₂₁ H ₂₂ ClFN ₂ O ₂	C, H, Cl, F, N

^aAll compounds exhibited IR (^{max}CHCl₃ 3450-3460, 1670-1680 cm⁻¹) and ¹H NMR [(CDCl₃) δ 4.9-5.0 (1 H, s, NC(OH)HCO)] spectra consistent with the structures. ^bYield based on the corresponding 26. ^cSee footnote c in Table I. ^dAll compounds were analyzed (C, H, halogens, and N), and the values obtained were within ±0.3% of the theoretical values. ^eSee Experimental Section.

10j. In the conflict behavior test, 7g' and 7j were much more active than CDP. Further increases in anti-PTZ potencies were achieved by introducing Cl at Y, as indicated by comparisons of the anti-PTZ activities between 7e, 7h, 10e, and 10h and their corresponding F-substituted 7d, 7g', 10d, and 10g'. In contrast, obvious decreases in antidepressive effects were noted by this modification; i.e., anti-TBZ effects and inhibitory potentials for NE uptake of the Cl-substituted compounds were about 1/10 to 1/50 as active as those of the corresponding F-substituted compounds. From these data, it can be concluded that the anticonvulsant and antianxiety effects were readily augmented by the substitution at Y in the order of H << F < Cl, while the order for the anti-TBZ effect and the preventive effect on NE uptake was H = F >> Cl. These orders were also found for a limited number of compounds having a pyrrolidine ring at position 1 (9a,b and 12a,b).

1-(3-Piperidinyl)-1,4-benzodiazepin-2-one derivatives with Cl substituents at the X and Y positions (8, 11) were tested. In comparison with their corresponding 4-piperidinyl derivatives (7h, 10h), antidepressive potencies were found to be significantly reduced, while antianxiety effects were well preserved. This finding suggests that the three carbon atoms between N at position 1 and N of the piperidine ring may be suitable for producing the antidepressant activity.

The introduction of OH at position 3 led to an apparent reduction in the antidepressive properties, while the anxiolytic effects were not influenced (35, 34b).

The test compounds had weak acute toxicities. The most striking structural feature influencing toxicity was CH₃ substitution for R; the toxicity increased considerably when the NH was replaced by NCH₃. However, even the most toxic compound so far tested (7d) had an LD₅₀ value

very close to those of conventional tricyclic antidepressants.

With benzodiazepine compounds, antianxiety with antidepressant activities have been reported for triazolo-benzodiazepine derivatives, alprazolam,¹¹ adinazolam,¹² and U-43,485F.¹³ Since the antianxiety effect of these triazolo-benzodiazepines generally predominated over the antidepressive properties, our compounds, i.e., 7a, 10a, 7d, 10d, etc., should be more suitable for clinical use as potent antidepressants.

In conclusion, 1-(4-piperidinyl)- or 1-(3-pyrrolidinyl)-1,4-benzodiazepin-2-ones appear to have potent antidepressant properties together with antianxiety properties. Halogen substitution, the most conventional modification for increasing the antianxiety effect of benzodiazepines, yielded interesting changes in both antidepressive and antianxiety properties. Following introduction of a halogen at position 7 of the 1,4-benzodiazepine ring (X), the antidepressive properties, assessed by antagonism to TBZ-induced ptosis in mice, showed decreases in their peak effects but became long lasting. On the other hand, the antianxiety properties were obviously enhanced. Further increases in the anti-PTZ effect were attained with a halogen substituent in the ortho position of the phenyl ring at position 5 (Y). The antidepressant properties were greatly reduced when Cl was introduced to Y. The experiment on uptake inhibition of [³H]norepinephrine into rat brain synaptosomes revealed that the secondary amine

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Table III. Biological Activities (Antidepressive Effects)

no.	TBZ ^a ED ₅₀ mg/kg (95% CL)		³ H[NE] IC ₅₀ μ M
	po ^d	sc ^d	
7a	0.38 (0.14-0.84)	0.26 (0.15-0.47)	
7b		0.21 (0.11-0.33)	
7c			
7c'	1.41 (0.85-2.44)		
7d	1.62 (0.78-4.25)	0.27 (0.15-0.45)	53.7
7e	59.24 (36.74-148.6)		
7f	0.74 (0.41-1.22)	0.85 (0.52-1.14)	
7f'	0.59 (0.33-0.95)		
7g	2.13 (1.25-3.55)	1.30 (0.68-2.56)	
7g'	1.12 (0.45-1.94)		
7h	26.14 (14.24-49.03)	23% at 50 mg/kg ^e	39.9
7i	2.68 (1.36-5.31)		
7j	3.85 (2.49-5.93)	2.45 (1.46-4.64)	
7k		0.84 (0.41-1.52)	
7l		1.24 (0.68-2.39)	
7m		0.70 (0.31-1.20)	
7n		17.74 (10.83-30.23)	
7o		0.94 (0.57-1.43)	
7p		34% at 50 mg/kg	
7q		NE at 50 mg/kg	
7r		64% at 50 mg/kg	
7s		2.66 (1.57-4.41)	
7t		53% at 50 mg/kg	
7u		19.26 (8.32-172.7)	
7v		7.88 (4.38-14.96)	
10a	0.26 (0.14-0.45)	0.06 (0.02-0.11)	0.029
10b		0.07 (0.05-0.11)	
10c	0.39 (0.19-0.65)		0.059
10d	0.38 (0.16-0.70)		0.079
10d'			
10e	13.48 (5.73-27.70)		0.877
10f	0.48 (0.24-0.96)	0.12 (0.06-0.22)	
10f'	0.48 (0.27-0.79)		0.172
10g	0.67 (0.46-1.01)	0.05 (0.04-0.12)	
10g'	0.76 (0.51-1.12)		0.118
10h	9.59 (5.52-15.08)	9.94 (2.40-33.19)	0.562
10i	0.76 (0.40-1.47)		0.402
10j	5.37 (3.43-8.29)		0.258
8	21% at 100 mg/kg		
9a	3.69 (2.27-6.51)		
9b	61% at 100 mg/kg		
9c	58% at 50 mg/kg		
11	57% at 50 mg/kg		
12a	0.40 (0.22-0.68)	25.92 (9.43-305.1)	
12b	34% at 50 mg/kg	12.57 (6.58-21.78)	
28a		0.65 (0.39-1.11)	
28b		67% at 50 mg/kg	
28c		16% at 50 mg/kg	
30		3.25 (1.76-6.06)	
34a		0.23 (0.10-0.49)	
34b	6.51 ^f		
35	9.79 (4.58-19.22)		
IMP ^g	2.14 (1.32-3.37)	0.80 (0.40-1.86)	0.055
CDP ^h			

^aAntagonism of tetrabenazine-induced ptosis in mice. ED₅₀ values and their 95% confidence limits were calculated by regression analysis ($n = 8$). ^bUptake inhibition of [³H]norepinephrine into rat brain synaptosomes. Presumed IC₅₀ values were obtained by graphical interpolation. ^cOral administration. ^dSubcutaneous administration. ^ePercent antagonism at that dose. ^fNo effect at that dose. ^gEstimated ED₅₀ 95% confidence limits not given because of poor dose dependency. ^hImipramine. ⁱChlordiazepoxide.

function of the azacyclic ring at position 1 was essential for the production of the antidepressant properties.

Although animal data on antianxiety effects correlate well with clinical dosages, there is no conclusive antidepressant test for predicting effective clinical dosages. Irrespective of differences in preclinical potencies, daily clinical dosages of each known antidepressant are mostly in the same range (30-100 mg/man per day). This also applies to our drug candidate. Furthermore, obviously beneficial effects in depressive patients are usually produced following long-term treatments with an antide-

pressant drug. Thus, if the antianxiety effect of our antidepressant candidate is as potent as CDP, accompanying effects such as sedation or muscle relaxation will become so potent as to overwhelm the essential antidepressive effect following long-term treatments with large doses. Therefore, we think the antianxiety effect of our antidepressant candidate should not exceed that of CDP, the weakest minor tranquilizer. This rationale led us to select compound 10d as the most suitable candidate from the new series for development into an antidepressant drug.

Experimental Section

Melting points were determined on a Yanagimoto micromelting apparatus and are uncorrected. IR spectra were recorded in CHCl₃ solution by a Hitachi 260-10 IR spectrophotometer. Wave numbers are expressed in reciprocal centimeters. NMR spectra were taken in CDCl₃ solution on a Varian EM-390 or T-60 spectrophotometer. Chemical shifts are expressed as δ values (parts per million) from tetramethylsilane. Column chromatography was conducted with use of silica gel (E. Merck, 70-230-mesh ASTM) and aluminum oxide (E. Merck, Standardisirt). Silica gel GF and aluminum oxide F254 (E. Merck) were used for analytical thin-layer chromatography (TLC). In cases where products were isolated by solvent extraction, the procedure generally followed was to extract the aqueous layer with two to three portions of the indicated solvent, then wash the organic layer with saturated NaCl-H₂O or H₂O, and dry it over Na₂SO₄ or MgSO₄.

Method A. To a stirred solution of a 2-acyl-N-azacycloalkylaniline hydrochloride 2 or 3 ($R_2 = \text{CH}_3, \text{CH}_2\text{CH}_2\text{Ph}$, 6 mmol) in CH₃CN (20 mL), prepared by adding of 1 equiv of HCl gas-MeOH to a solution of 2 or 3 in CH₃OH and concentrating the mixture, was added chloroacetyl chloride (6×2 mmol) and the solution was refluxed for 1-20 h. TLC (Al₂O₃ GF, CHCl₃-AcOEt = 5:1) was used to check for completion of the reaction. After removal of the excess reagent and solvent under reduced pressure, the resulting chloroacetyl derivative hydrochlorides 13 or 14 was dissolved again in CH₃CN (50 mL) containing NaI (6×3 mmol) and the solution was warmed at 70 °C for 2 h. The exchange of the chloroacetyl for the iodoacetyl was checked by monitoring with TLC (Al₂O₃ GF, CH₂Cl₂) and ¹H NMR [CDCl₃] δ 4.1 and 3.9 (AB q, $J = 12$ Hz, COCH₂Cl), ca. 3.4 and 3.7 (AB q, $J = 10$ Hz, COCH₂I). After the solution cooled, (NH₄)₂CO₃ (6 g) was added and the mixture was stirred at room temperature for 10 days. In an alternate procedure, prior to adding (NH₄)₂CO₃, CH₃CN was evaporated, H₂O was added, and the mixture was extracted with CH₂Cl₂. Evaporation of the solvent and addition of acetone gave the iodoacetyl derivative hydrochlorides of 13 or 14. These were dissolved in CH₃CN (50 mL) containing (NH₄)₂CO₃ (6 g), and the mixture was warmed in a sealed vessel at 70 °C for 10 h. After the mixture cooled, the solvent was evaporated; H₂O was added, and the mixture was extracted with CH₂Cl₂. The residue was purified on Al₂O₃ (60 g, benzene and benzene containing 1-5% EtOAc), giving 7 or 8.

Method B. A solution of a 2-acyl-N-azacycloalkylaniline 2 or 4 ($R_2 = \text{CH}_3$) (10 mmol) and phthaloylchloride (10 $\times$ 2 mmol) in CH₃CN (30 mL) was refluxed for 20-140 h. The reaction was monitored by TLC (Al₂O₃ GF, CH₂Cl₂-AcOEt = 5:1). The solvent was evaporated and then ice-2 N NaOH was added and the mixture was stirred at room temperature for 30 min and extracted with CH₂Cl₂. The extract was purified on Al₂O₃ (100 g, CH₂Cl₂) to obtain analytically pure 16 or could be directly used for the next step. The phthaloylchloride derivative 16 or 17 was dissolved in 95% EtOH (60 mL) containing 80% NH₄NH₂-H₂O (10 $\times$ 3 mmol) and the solution was refluxed for 0.5-2 h. The solution was concentrated and the precipitate was filtered off. After concentration of the filtrate, the residue was purified on Al₂O₃ (50 g, benzene, benzene containing 2-5% AcOEt and benzene containing 5% MeOH), giving 7 or 9 and the corresponding 15 as the more polar fraction.

Method C. A solution of a 2-acyl-N-azacycloalkylanilineimine 5 or 6 ($R_2 = \text{CH}_3, \text{CH}_2\text{Ph}$) (0.2 mol) in EtOH (700 mL) containing glycine ethyl ester hydrochloride (0.2 $\times$ 1.2 mol) and AcOH (0.2 mol) was refluxed for 3 h. After removal of the solvent, the residue was made alkaline with dilute NH₄OH and extracted with CH₂Cl₂.

Table IV. Biological Activities (Minor Tranquilizing Effects)

no.	PTZ ^a ED ₅₀ , mg/kg (95% CL)		conflict ^b ED ₅₀ , mg/kg, po (95% CL)	[³ H]DZ ^c K _d , μM
	po	sc		
7a	NE at 50 mg/kg	NE at 10 mg/kg		2.3
7b	37.5% at 50 mg/kg			0.2
7c	25.79 (17.62-35.90)		20% at 16 mg/kg	2.42
7c'	29.96 (18.28-94.90)			
7d	26.92 (18.90-31.75)	5.97 (4.41-8.50)	11.36 (6.66-25.45)	0.094
7e	5.20 (3.09-8.15)			0.014
7f		50% at 20 mg/kg		
7f'	28.90 (21.77-41.13)		8.40 (3.78-14.34)	0.49
7g	2.70 (1.66-3.96)			
7g'	5.28 (3.95-6.96)	0.71 (0.37-1.11)	3.67 (1.71-7.11)	0.042
7h	3.24 (2.02-4.87)	1.69 (0.78-4.85)		0.021
7i	21.36 (13.74-37.28)		17.35 ^d	0.415
7j	1.81 (1.07-2.31)	0.79 (0.50-1.24)	1.23 (0.79-2.59)	0.049
7k		NE at 10 mg/kg		0.26
7l		NE at 10 mg/kg		1.26
7m		NE at 10 mg/kg		8.3
7n		NE at 10 mg/kg		8.3
7o	NE at 50 mg/kg	NE at 10 mg/kg		24
7p	NE at 50 mg/kg	NE at 10 mg/kg		0.44
7q		NE at 20 mg/kg		0.69
7r	NE at 50 mg/kg	NE at 10 mg/kg		17
7s		NE at 10 mg/kg		0.80
7t		NE at 10 mg/kg		4.7
7u		NE at 10 mg/kg		68
7v		NE at 10 mg/kg		23
10a	NE at 200 mg/kg	NE at 10 mg/kg	20% at 32 mg/kg	1.6
10b	NE at 50 mg/kg	NE at 10 mg/kg		1.3
10c	12.5% at 50 mg/kg		60% at 32 mg/kg	0.15
10d	32.11 (2.137-46.27)		50% at 16 mg/kg	2.0
10d'	27.55 (19.37-42.22)			0.15
10e	29.44 (20.35-43.04)			
10f	36.45 (26.12-49.63)	NE at 10 mg/kg		0.012
10f'	NE at 50 mg/kg		11.81 (6.15-33.94)	0.35
10g	11.39 (8.68-15.78)	8.52 (5.28-16.64)		
10g'	14.17 (9.91-20.25)		6.51 (4.22-10.04)	0.036
10h	12.89 (8.84-17.94)			0.017
10i	38.78 (27.95-53.20)			0.57
10j	2.70 (2.00-3.91)		4.54 ^d	0.033
8	12.03 (7.92-18.99)			0.016
9a	1.10 (0.72-1.72)			0.2
9b	5.78 (3.95-9.41)			0.05
9c	1.52 (0.86-3.18)			0.27
11	16.17 (11.71-22.28)			0.013
12a	20.12 (14.27-32.17)		60% at 8 mg/kg	0.73
12b	9.63 (6.42-15.50)			0.027
28a		NE at 10 mg/kg		2.3
28b	9.09 (5.81-13.95)	11.14 (5.96-26.04)		0.038
28c	1.95 (1.07-3.96)			0.02
30		NE at 10 mg/kg		1.5
34a		NE at 10 mg/kg		0.36
34b	12.50 (8.35-18.59)		8.80 (5.15-24.51)	0.14
35	2.41 (1.70-3.40)			0.074
IMP				
CDP	2.70 (1.66-3.96)		6.49 (3.10-10.11)	0.202

^aAntagonism of pentylentetrazol-induced convulsion in mice. ED₅₀ values and their 95% confidence limits were calculated by the probit method (n = 8). ^bAnticonflict effects in rats. ED₅₀ and 95% confidence limits were calculated by the probit method (n = 5-8). ^cDisplacing potential to [³H]diazepam binding in rat cerebral cortex. Presumed K_d values were calculated with use of IC₅₀ values obtained by graphical interpolation. ^dEstimated ED₅₀. 95% confidence limits not obtained because of poor dose dependency.

The extract was crystallized from Et₂O-hexane to obtain analytically pure iminoglycine ester 18 or 19 or used directly for the next step.

Cyclization Using CF₃COOH. An iminoglycine ester 18 or 19 (0.2 mol) was dissolved in CF₃COOH (600 mL) and the solution was refluxed for 20 h. The solvent was evaporated under reduced pressure, benzene was added to the residue, and the solvent was again evaporated to remove residual CF₃COOH. Addition of benzene to the resulting syrup followed by Et₂O generally gave crystalline 7 or 9-CF₃COOH. The crystalline or oily 7 or 9-CF₃COOH was alkalinized with ice-concentrated NH₄OH (20 mL) and extracted with CH₂Cl₂. Crystallization of the extract gave pure 7 or 9 or the corresponding salt. The filtrate of 7 or 9-CF₃COOH was concentrated and the residue was made alkaline with ice-concentrated NH₄OH and extracted with CH₂Cl₂. From

the extract, 2 or 4 was recovered in 5-10% yield. When the starting material was 5 (R₂ = H), CF₃COOH was evaporated and the residue was extracted with CH₂Cl₂. The CH₂Cl₂ layer was washed successively with 5% AcOH and H₂O. The combined acidic layer was made alkaline with concentrated NH₄OH and extracted with CH₂Cl₂. Purification of the extract gave 10. The above-mentioned CH₂Cl₂ layer was washed with dilute NH₄OH and concentrated to recover 2 (R₂ = H) in 10-20% yield.

Cyclization Using H₂SO₄. An iminoglycine ester 18 or 19 (1.5 mol) was dissolved in concentrated H₂SO₄ (30 mL) and the mixture was warmed 90 °C for 13 h with stirring. After cooling the mixture was poured onto ice (150 g) and made alkaline with NaOH (49 g) and ice-H₂O (200 mL). The mixture was extracted with benzene. Crystallization of the extract gave 7 or 9. After concentration of the mother liquor, CF₃COOH (0.3 mL) and Et₂O

Table V. Biological Activities (Acute Toxicities)

no.	mouse LD ₅₀ ^a mg/kg, po (95% CL)
7a	425 (385-472)
7d	267 (230-328)
7f	548 (510-588)
7g	489 (425-553)
10a	1239 (1113-1386)
10d	939 (805-1213)
10f	1393 (1293-1506)
10g	1480 (1306-1666)
IMP ^b	310 (293-330)
CDP ^c	877 ^d

^aLD50 values were calculated by the probit method ($n = 10$). 95% confidence limits were included parentheses. ^bImipramine. ^cChlordiazepoxide. ^dEstimated LD₅₀ obtained by the up and down method ($n = 5$).

(30 mL) were added to the residue. The crystallized 7 or 9- CF_3COOH were filtered off and converted to free 7 or 9 as described above. From the mother liquor of 7 or 9- CF_3COOH , 2 or 4 were recovered in a manner similar to the above.

Cyclization Using PPA. A mixture of an iminoglycine ester 18 (0.1 mol) and PPA (250 g) was warmed at 100 °C (bath temperature) with stirring for 4 h. After cooling, ice (500 g) and concentrated NH_4OH (320 mL) were added, and the mixture was extracted with CH_2Cl_2 . Ether was added to the extract and the resulting crude 7 was separated and recrystallized from CH_2Cl_2 -ether to obtain pure 7. Purification of the mother liquor in a similar manner after its conversion into 7- CF_3COOH gave additional pure 7.

Method D. To a refluxing solution of 2-acyl- N -azacycloalkylaniline 2 or 3 ($R_1 = \text{CH}_3$, 15 mmol) in dichloroethane (50 mL) was added dropwise ethyl chloroformate (15 $\times$ 3 mmol) over a period of 10 min and the solution was refluxed for 1 h. To complete the reaction, NaHCO_3 (15 mmol) was added to the solution and the mixture was refluxed a further 1 h. TLC (SiO_2GF , CHCl_3 - $\text{CH}_3\text{OH} = 5:1$) was used to check for the completion of the reaction. After cooling, ice- H_2O was added, the benzene layer was separated, and the H_2O layer was extracted with benzene. The combined benzene extract was crystallized to obtain a pure sample of 20 or 21 or could be directly used for the next step. An N -ethoxycarbonyl derivative 20 or 21 was dissolved in concentrated HCl - H_2O (1:1, 50 mL) and the solution was refluxed for 20 h. TLC ($\text{Al}_2\text{O}_3\text{GF}$, CH_2Cl_2) was used to check for completion of the reaction. The solution was concentrated in vacuo, giving crude 22 or 23, which was crystallized to obtain a pure sample or used directly for the next step. To a stirred mixture of 22 or 23 (15 mmol) in dioxane (50 mL) containing 2 N NaOH (19 mL) was added dropwise a solution of benzyl chloroformate (15 $\times$ 2.2 mmol) in dioxane (10 mL) with ice-cooling. The mixture was stirred at room temperature for 1.5 h. TLC ($\text{Al}_2\text{O}_3\text{GF}$, CH_2Cl_2 or SiO_2GF , CHCl_3 - $\text{CH}_3\text{OH} = 5:1$) was used to check for completion of the reaction. H_2O was added and the mixture was extracted with benzene. The extract was purified on Lobar column B (CH_2Cl_2 - $\text{AcOEt} = 10:1$), giving crude 24 or 25. These were converted to 26 or 27 by use of method A or B. Compound 26 or 27 (6 mmol) was dissolved in a solution of CH_2Cl_2 (50 mL) and CH_3NO_2 (50 mL) containing AlCl_3 (6 $\times$ 6 mmol) and anisole (6 $\times$ 6 mmol) and the mixture was stirred at room temperature for 20 h. Ice- H_2O was added and the mixture was washed with Et_2O . The Et_2O layer was washed with dilute HCl . The combined acidic layer was made alkaline with 2 N NaOH and the mixture was extracted with Et_2O . Crystallization of the extract gave 10 or 11. Alternatively, compound 26 or 27 (40 mmol) was dissolved in CF_3COOH (53 mL) and the solution was refluxed for 2 h. After removal of the solvent in vacuo, ice- H_2O was added and the mixture was extracted with Et_2O . The H_2O layer was made alkaline with dilute NH_4OH and extracted with CH_2Cl_2 . The extract was crystallized, giving 10 or 11.

Method E. Procedure Using Ethyl Chloroformate. To a refluxing solution of 1-(1-methyl-4-piperidinyl)- or 1-(1-benzyl-3-pyrrolidinyl)-1,4-benzodiazepin-2-one (7, $R_1 = \text{CH}_3$; 9, $R_1 = \text{CH}_2\text{Ph}$) (50 mmol) in dichloroethane (150 mL) was added dropwise ethyl chloroformate (50 $\times$ 3 mmol) over a period of 5 min and the solution was refluxed for 0.5 h. After cooling, the

solution was washed with dilute HCl and the organic layer was evaporated to obtain crude 28 or 29. The acidic layer was made alkaline with concentrated NH_4OH and extracted with CH_2Cl_2 . From the extract, 7 ($R_1 = \text{CH}_3$) was recovered in a yield of less than 5%. Compound 28 or 29 was dissolved in methanesulfonic acid (41 mL) containing tetrahydrothiophene (48 $\times$ 5 mmol) and the mixture was warmed at 120 °C with stirring for 0.5 h. After cooling, ice- H_2O was added and the mixture was washed with CH_2Cl_2 . The acidic layer was made alkaline with concentrated NH_4OH and extracted with CH_2Cl_2 . Crystallization of the extract gave 10 or 12.

Procedure Using α -Chloroethyl Chloroformate. To a stirred solution of 7 ($R_1 = \text{CH}_3$, 0.12 mol) in dichloromethane (230 mL) was added dropwise a solution of α -chloroethyl chloroformate (14.7 mL, 0.13 mol) in dichloromethane (70 mL) at -5 °C over 15 min and the solution was refluxed for 1.5 h. After cooling, CH_3OH (100 mL) was added with stirring at 20 °C and the solution was refluxed for 2 h with evolution of CO_2 . After removal of the solvent, the residue was dissolved in CH_3OH (150 mL), and the solution was decolorized with active charcoal and concentrated. Purification of the residue by crystallization gave 10- HCl .

1,3-Dihydro-5-(2-fluorophenyl)-1-(1-methyl-4-piperidinyl)-7-nitro-2H-1,4-benzodiazepin-2-one (7n). To a stirred solution of 1,3-dihydro-5-(2-fluorophenyl)-1-(1-methyl-4-piperidinyl)-2H-1,4-benzodiazepin-2-one (7b; 2.1 g, 5.9 mmol) in concentrated H_2SO_4 (10 mL) was added dropwise a solution of KNO_3 (571 mg, 5.9 mmol) in concentrated H_2SO_4 (5 mL) at -8 °C. The solution was allowed to stand at -5 °C for 6 h and then at room temperature for 15 h. The reaction mixture was poured onto ice (200 g), made alkaline with concentrated NH_4OH , and extracted with CH_2Cl_2 . The extract was chromatographed on Al_2O_3 (200 g, CH_2Cl_2 and EtOAc). The eluate of EtOAc was collected and recrystallized, giving 7n (544 mg).

1,3-Dihydro-1-[1-(ethoxycarbonyl)-4-piperidinyl]-5-phenyl-2H-1,4-benzodiazepin-2-one (28) from N -[1-(ethoxycarbonyl)-4-piperidinyl]-2-benzoylaniline (20). Compound 20 was treated according to method A or B. Crude 28 was chromatographed on a Lobar column (EtOAc) or crystallized from the appropriate solvent to obtain a pure sample of 28.

1-(1-Acetyl-4-piperidinyl)-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (30). 2-Benzoyl- N -(4-piperidinyl)aniline (22; $X = Y = \text{H}$, 2.8 g) was dissolved in Ac_2O (5 mL) and the solution was kept at room temperature for 0.5 h. Ice and K_2CO_3 were added, and the mixture was stirred for 0.5 h and extracted with ether. The extract was chromatographed on Lobar column B (EtOAc), giving oily 31 (3 g, $X = Y = \text{H}$). Compound 31 was treated by use of method A, the extract was chromatographed on Al_2O_3 (CH_2Cl_2), and the eluate was crystallized to obtain pure 30.

Method F. To a solution of 1-[1-(benzyloxycarbonyl)-4-piperidinyl]-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (26; 8.9 mmol) in AcOH (50 mL) was added 30% H_2O_2 (3.5 mL, 8.9 $\times$ 4.5 mmol) and the solution was warmed at 65 °C for 16 h. TLC (SiO_2GF , CH_2Cl_2 - $\text{EtOAc} = 1:1$) was used to check for completion of the reaction. After cooling, NaHSO_3 (3.5 g) was added and the solution was concentrated to about one-fourth volume. Ice and concentrated NH_4OH were added, and the mixture was extracted with CH_2Cl_2 . The extract was purified on SiO_2 (15 g, EtOAc -benzene = 1:1), giving oily N -oxide 32: IR $\nu_{\text{max}}^{\text{CHCl}_3}$ 1680 cm^{-1} ; ^1H NMR (CDCl_3) δ 4.6 (2 H, s, $\text{COCH}_2\text{N}-\text{O}$), 5.1 (2 H, s, OCH_2Ph). A solution of compound 32 (8.9 mmol) in Ac_2O (25 mL) was heated at 140 °C for 1 h and then excess Ac_2O was removed under reduced pressure. The residue was purified on SiO_2 (10 g, CH_2Cl_2 - $\text{EtOAc} = 5:1$), giving the oily 3-acetoxy derivative 33: IR $\nu_{\text{max}}^{\text{CHCl}_3}$ 1610, 1690 cm^{-1} ; ^1H NMR (CDCl_3) δ 2.3 (3 H, s, COCH_3), 5.1 (2 H, s, OCH_2Ph), 5.90 (1 H, s, $\text{N}(\text{OAc})\text{HCO}$). Compound 33 was treated with AlCl_3 and anisole as in method D, giving 34.

7-Chloro-1,3-dihydro-5-(2-fluorophenyl)-3-hydroxy-1-(1-methyl-4-piperidinyl)-2H-1,4-benzodiazepin-2-one (35). To a stirred solution of 7-chloro-1,3-dihydro-5-(2-fluorophenyl)-3-hydroxy-1-(4-piperidinyl)-2H-1,4-benzodiazepin-2-one (34b; 900 mg, 2.32 mmol) in CH_3CN (270 mL) were added K_2CO_3 (321 mg, 2.32 mmol) and a solution of $(\text{CH}_3)_2\text{SO}_4$ (0.22 mL, 2.32 mmol) in CH_3CN (30 mL), and the mixture was stirred at room temperature for 0.5 h. The precipitate was filtered off and the filtrate was

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concentrated in vacuo. Ice and concentrated NH_4OH were added, and the mixture was extracted with CH_2Cl_2 . The extract was purified on SiO_2 (10 g, CH_2Cl_2 and CH_3Cl_2 containing 10% MeOH). Crystallization of the eluate gave 35 (400 mg).

Antagonism of Tetrabenazine-Induced Ptosis in Mice. Male DS strain mice, weighing 20–27 g, were randomly assigned to groups of five each. Test compounds were dissolved in physiological saline or suspended in arabic gum solution and orally or subcutaneously administered at 0.1 mL/10 g of body weight. A dose of 50 mg/kg of tetrabenazine (TBZ) was subcutaneously injected 1, 4, or 24 h after the test compounds. The animals were scored for ptosis 1 h after TBZ administration according to the following scale: normal = 1, slightly closed = 2, half closed = 3, 100% closed = 4. Observed scores were subjected to riddit transformation¹⁴ and doses causing 50% prevention of the response were calculated by regression analysis.

Antagonism of Pentylenetetrazol-Induced Convulsion in Mice. Eight male ddY strain mice, weighing 20–24 g, were challenged with a dose of 125 mg/kg sc of pentylenetetrazol (PTZ) 1 h after oral or subcutaneous administration of the test compounds. The dose required to prevent tonic convulsion and death in 50% of the animals during a 2-h observation period was calculated by the probit method.¹⁵

Anticonflict Effect in Rats. To more directly evaluate rat antianxiety activities, their anticonflict behavior was observed with selected compounds by using the method described by Geller and Seifter.¹⁶ Groups of five to eight Wistar rats were trained to press a lever for a food reward on a variable interval of a 60-s schedule. When the lever pressing was established, a tone stimulus of 3-min duration was presented every 15 min as a signal informing every fifth lever pressing would be rewarded with a food pellet. After this behavioral contingency was established, conflict was introduced by punishment with a 60-Hz electroshock through the grid floor at every fifth lever response during the tone period. A dose was determined as positive when the number of shocks exceeded 12 or the shock ratio was more than 4 during four consecutive tone periods. Doses for 50% suppression of the conflict behavior were calculated by the probit method.

Acute Toxicity in Mice. Male DS strain mice, weighing 20–26 g, were employed to assess the oral LD_{50} values for selected

compounds. The mortality was determined with groups of 10 mice on the next day after the treatment. LD_{50} values were calculated by the probit method or the up and down method of Brownlee et al.¹⁷

Uptake Inhibition of [^3H]Norepinephrine into Rat Brain Synaptosome. The procedure was mainly based on the method of Randrup and Braestrup.⁹ Synaptosomal P2 fractions were prepared from the cerebral cortex of male Wistar rats. The fraction was incubated with [^3H]norepinephrine (NE) at 37 °C for 20 min in the presence of a test compound in five to six different concentrations. The reaction was terminated by dilution with Krebs-Henseleit solution and then by filtration with Whatman glass fiber filter (GF/C). The obtained radioactive sample was dissolved in scintillation fluid and the radioactivity was counted with a scintillation counter. IC_{50} values were calculated as the concentration of the test compound inhibiting the uptake of [^3H]NE by 50%.

Displacing Potential to [^3H]Diazepam Binding. The cerebral cortex homogenates of Wistar strain rats were used for the binding assay. Our procedure was mostly based on the method of Möhler and Okada.¹⁸ Tissue homogenate, a test compound, and 2 nM [^3H]diazepam (DZ) were incubated at 0 °C for 60 min. After termination of the incubation, the radioactive sample, trapped on Whatman glass filter (GF/F), was dissolved in scintillation fluid and the radioactivity was measured with a scintillation counter. K_i values were calculated from the following equation: $K_i = \text{IC}_{50}/(1 + (L/K_d))$, where IC_{50} is the concentration of the test compound causing 50% inhibition of specific binding of the radioactive ligand and L and K_d are the concentration and the dissociation constant of the ligand, respectively.

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Supplementary Material Available: Summarized spectral data characteristic for the structures of intermediates 13, 14, and 16–29 and melting point and combustion data for crystalline intermediates and byproduct 15 (9 pages). Ordering information is given on any current masthead page.

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1968

L. Capuano und M. Zander

Liebigs Ann. Chem. 712, 73-78 (1968)

Über Reaktionen der Diazoalkane, II¹⁾

Darstellung von α -[2-Alkoxycarbonyl-anilino]- und α -[2-Acyl-anilino]-carbonsäureamiden

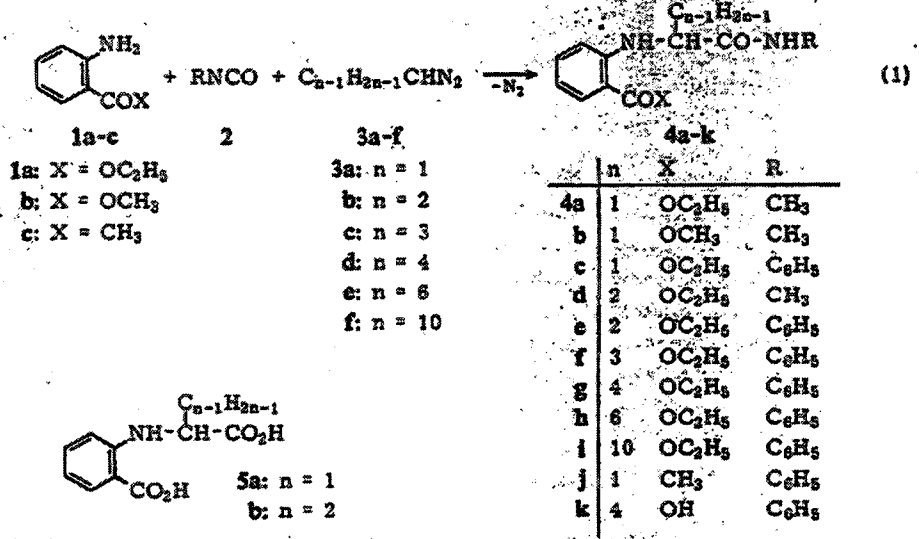
von Lilly Capuano *) und Mechthild Zander *) 2)

Aus dem Institut für Organische Chemie der Universität des Saarlandes, Saarbrücken

Eingegangen am 26. Juli 1967

Anthranilester (1a,b) reagieren mit Isocyanaten (2) in Gegenwart von Diazoalkanen (3a-f) zu α -[2-Alkoxycarbonyl-anilino]-carbonsäureamiden (4a-i) [Gl. (1)]. Milde Verseifung dieser Produkte führt zu den α -[2-Carboxy-anilino]-carbonsäureamiden, energische Verseifung zu den α -[2-Carboxy-anilino]-carbonsäuren. Nebenprodukte der Reaktion sind *o*-Alkoxycarbonyl-phenylharnstoffe (8) und substituierte 2,4-Dioxo-1,2,3,4-tetrahydro-chinazoline (9). — *o*-Amino-acetophenon (1c) bildet ähnlich mit Phenylisocyanat und Diazomethan 2-Acetyl-anilino-essigsäure-anilid (4j), das durch Schmelzen mit Natriumhydroxid in Skatol-carbonsäure-(2) übergeführt wurde.

In einer früheren Mitteilung¹⁾ hatten wir berichtet, daß *o*-Aminoaryl-carbonsäure-ester (1a,b und Benzologe) mit Methyl- bzw. Phenylisocyanat (2) und Diazomethan (3a) unter Bildung der 2-Alkoxycarbonyl-anilino-essigsäure-methylamide und -anilide (4a-c) bzw. ihrer Benzologen reagieren [Gl. (1)].



*) Herrn Prof. Dr. Bernd Eistert zum 65. Geburtstag gewidmet.
¹⁾ I. Mitteilung: L. Capuano und M. Zander, Chem. Ber. 89, 3085 (1966).
²⁾ Aus der Dissertation M. Zander, Univ. Saarbrücken 1967.

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Durch Übertragung dieser Reaktion auf die Homologen des Diazomethans bis zum Diazodecan gelang es uns, eine allgemeine Darstellungsmethode für die bisher noch nicht beschriebenen homologen α -[2-Alkoxycarbonyl-anilino]-carbonsäure-methylamide und -anilide der allgemeinen Struktur 4 zu ermitteln. Diese Amide entstehen aus Anthranilsäureestern (1a,b) mit 1–2.5 Mol-Äquivv. 2 und 2–3 Mol-Äquivv. 3 in ätherischer Lösung bei Raumtemperatur. Die auf die eingesetzte Menge Ester berechneten Ausbeuten an 4 streuen um 50–70%; die besten Ausbeuten wurden bei Anwendung von 2 Mol 2 auf 1 Mol Ester erzielt. Unter diesen Bedingungen konnte auch die frühere¹⁾ Ausbeute an 4c verbessert werden (s. Tab. 1).

Tabelle 1. Produkte der Reaktion von Anthranilsäureäthylester und *o*-Amino-acetophenon mit Isocyanaten 2 und Diazoalkanen 3 (2–3 Mol-Äquivv.)

1 Mol-Äquiv.	2 (Mol-Äquivv.)	3	Reakt.- Produkt	Ausbeute
Anthranilsäure- äthylester (1a)	C ₆ H ₅ NCO (1.2)	CH ₂ N ₂	4c	nach 1 Tag ^{a)} : 50%
	C ₆ H ₅ NCO (2.0)	CH ₂ N ₂	4c	3 Tagen: 72
	CH ₃ NCO (1.0)	CH ₃ CHN ₂	4d	3 Tagen: 45
	C ₆ H ₅ NCO (2.0)	CH ₃ CHN ₂	4e	3 Tagen: 71
	C ₆ H ₅ NCO (2.0)	C ₂ H ₅ CHN ₂	4f	3 Tagen: 70
	C ₆ H ₅ NCO (2.0)	C ₃ H ₇ CHN ₂	4g	2 Tagen: 61
	C ₆ H ₅ NCO (1.0)	C ₅ H ₁₁ CHN ₂	4h	5 Tagen: 30
	C ₆ H ₅ NCO (2.0)	C ₉ H ₁₉ CHN ₂	4i	3 Tagen: 49
<i>o</i> -Amino- acetophenon (1c)	C ₆ H ₅ NCO (1.25)	CH ₂ N ₂	4j	3–4 Stdn.: 41

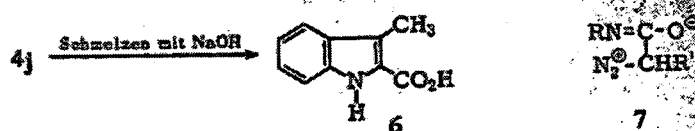
a) Frühere Vorschrift Lit.¹⁾.

Milde Verseifung mit 1*n* NaOH bei 60° greift nur die Estergruppe an: So wurde α -[2-Äthoxycarbonyl-anilino]-valeriansäure-anilid (4g) in das noch nicht beschriebene α -[2-Carboxy-anilino]-valeriansäure-anilid (4k) übergeführt. Energischere Verseifung durch Kochen mit 2*n* NaOH liefert die α -[2-Carboxy-anilino]-carbonsäuren 5: Unter diesen Versuchsbedingungen waren schon früher¹⁾ die *o*-Methoxycarbonyl- und *o*-Äthoxycarbonyl-anilino-essigsäure-methylamide (4a,b) zu Phenylglycin-*o*-carbonsäure (5a) verseift worden; [2-Äthoxycarbonyl-anilino]-essigsäure-anilid (4e) bildet ähnlich die bisher noch unbekannte α -Phenylalanin-*o*-carbonsäure (5b), deren Identität mit der aus Anthranilsäure und α -Chlorpropionsäure³⁾ hergestellten Säure als Beweis für die Konstitution von 4e angesehen werden kann.

³⁾ In Analogie zur Darstellung von 5a: H. E. Fierz-David und L. Blangey, Grundlegende Operationen der Farbenchemie, 5. Aufl., S. 306, Springer-Verlag, Wien 1943; zit. in Beilsteins Handbuch der Organischen Chemie, 4. Aufl., Bd. 14, II. Erg.-Werk, S. 225, Springer-Verlag, Berlin 1951.

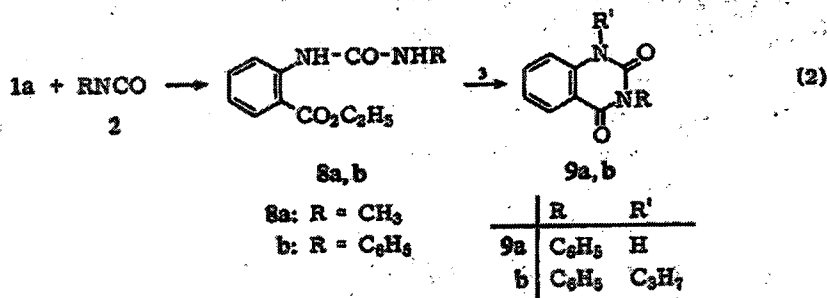
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In Analogie zu den *o*-Aminocarbonsäureestern reagiert *o*-Amino-acetophenon (1c) mit Phenylisocyanat und Diazomethan unter Bildung des bisher noch nicht beschriebenen 2-Acetyl-anilino-essigsäure-anilids (4j), dessen Konstitution durch Überführung in Skatol-carbonsäure-(2) (6) beim Schmelzen mit Natriumhydroxid gesichert wurde.



Wie bereits für die Umsetzungen mit Diazomethan erwogen wurde¹⁾, könnte der erste Schritt der Reaktion (1) der nucleophile Angriff des Diazoalkans an das Isocyanat-C sein. Vielleicht entsteht ein Isocyanat-Diazoalkan-Komplex 7, dessen Angriff durch den Anthranilester am Diazoalkan-C zur Bildung von 4 führen könnte. Wir hatten indessen früher¹⁾ festgestellt, daß Anthranilester mit Isocyanaten direkt zu den Harnstoffen 8 reagiert, und daß diese unter *Katalyse* von Diazoalkanen zu den entsprechenden Chinazolin 9 cyclisieren [Gl. (2)].

Zwecks Prüfung auf etwaige Konkurrenzreaktionen untersuchten wir daher die Nebenprodukte der Umsetzung von 1a mit Phenylisocyanat und Diazopropan. Neben 2,4-Dioxo-3-phenyl-1.2.3.4-tetrahydro-chinazolin (9a) konnten wir wenig *N*-Phenyl-*N'*-(2-äthoxycarbonyl-phenyl)-harnstoff (8b) und das noch nicht beschriebene 2,4-Dioxo-1-propyl-3-phenyl-1.2.3.4-tetrahydro-chinazolin (9b) isolieren. Erwartungsgemäß findet demnach bei Umsetzung von 1a mit 2 und 3 neben dem nucleophilen Angriff von 3 an 2 gemäß Gl. (1) zu 4 ein nucleophiler Angriff von 1a an 2 gemäß Gl. (2) unter Bildung von 8 statt, das mit Diazoalkanen unter gleichzeitiger Alkylierung cyclisiert. Wie aus der Konzentrationsabhängigkeit der Ausbeuten an Haupt- und Nebenprodukten hervorgeht, wird (2) durch hohe Konzentrationen an 1a bevorzugt.



Herrn Prof. Dr. B. Elstert danken wir für Förderung der Arbeit durch Sachmittel, den Herren Dipl.-Chem. W. Marks und K. Schäfer für die Elementaranalysen, Fräulein R. Zander danken wir für ihre ausgezeichnete Mitarbeit.

Beschreibung der Versuche

Die Schmelzpunkte wurden im Heizblock bestimmt und sind unkorrigiert. — Die Elementaranalysen wurden nach dem Verfahren von Walisch⁴⁾ durchgeführt.

1) α -[2-Äthoxycarbonyl-anilino]-essigsäure-anilid (4c) (verbessertes Verfahren). — Eine Lösung von 2 g Anthranilsäureäthylester (1a) in 5 ccm absol. Äther wurde mit 3.0 g Phenylisocyanat (ca. 2 Mol-Äquiv.) und äther. Diazomethan aus 5 g Nitrosomethylharnstoff⁵⁾ (2.75 Mol-Äquiv. Diazomethan) versetzt. Es setzte sofort lebhaftes N₂-Entwicklung ein. Nach 3 Tagen wurde i. Vak. eingedampft und der ölige Rückstand 3 mal mit je 10 ccm Petroläther verrieben. Aus dem ungelösten Rückstand kristallisierten auf Zusatz von etwas Äther 1.3 g 4c, aus der mit den Petroläther-Auszügen vereinigten Mutterlauge in der Kälte noch einmal 1.3 g 4c (Gesamtausbeute 72%); Schmp. und Mischprobe mit authent. 4c¹⁾ 166°.

2) α -[2-Äthoxycarbonyl-anilino]-propionsäure-methylamid (4d). — Wie bei 1) mit 0.70 g Methylisocyanat (ca. 1 Mol-Äquiv.) und äther. Diazoäthan aus 5 g Nitrosoäthylharnstoff⁵⁾. Gesamtausbeute nach 3 Tagen 1.35 g (44%) 4d, die nach mehrmaligem Umkristallisieren, erst aus Petroläther, dann aus Äthanol/Wasser bei 145° schmolzen.

C₁₃H₁₈N₂O₃ (250.3) Ber. C 62.38 H 7.25 N 11.2 Gef. C 61.3 H 6.97 N 11.7

3) α -[2-Äthoxycarbonyl-anilino]-propionsäure-anilid (4e). — Wie bei 1) mit äther. Diazoäthan aus 3.5 g Nitrosoäthylharnstoff. Ausbeute nach 3 Tagen 2.7 g (71%) 4e, die nach Umkristallisieren aus Petroläther, dann aus Äthanol/Wasser, bei 158° schmolzen.

C₁₈H₂₀N₂O₃ (312.4) Ber. C 69.21 H 6.45 N 8.97 Gef. C 68.6 H 6.37 N 8.8

α -[2-Carboxy-anilino]-propionsäure (5b). — a) Aus den Natriumsalzen der Anthranilsäure und der α -Chlorpropionsäure in wäbr. Lösung entstand in 4 Tagen bei 40° und 4 Stdn. bei 100° 5b vom Schmp. 212° (aus Wasser/Kohle).

C₁₀H₁₁NO₄ (209.2) Ber. C 57.41 H 5.30 N 6.70 Gef. C 57.5 H 5.35 N 6.8

b) 0.5 g 4e wurden mit 20 ccm 2n NaOH 3 Stdn. unter Rückfluß gekocht. Nach Erkalten wurde 2 mal mit Äther ausgezogen, die wäbr. Phase mit 2n HCl angesäuert und mit Äther extrahiert. Die Ätherextrakte hinterließen nach Eindampfen i. Vak. 0.33 g 5b (quantitativ); Schmp. und Mischprobe 212° (aus Äthanol/Wasser).

4) α -[2-Äthoxycarbonyl-anilino]-buttersäure-anilid (4f). — Wie bei 1) mit äther. Diazo-propan aus 7 g Nitrosopropylharnstoff⁵⁾. Gesamtausbeute nach 3 Tagen 2.75 g (70%) 4f, das nach Umkristallisation aus Petroläther, dann aus Äthanol/Wasser, bei 136° schmolz.

C₁₉H₂₂N₂O₃ (326.4) Ber. C 69.92 H 6.79 N 8.58 Gef. C 69.5 H 6.63 N 8.6

N-Phenyl-N'-[2-äthoxycarbonyl-phenyl]-harnstoff (8b), 2,4-Dioxo-3-phenyl-1,2,3,4-tetrahydro-chinazolin (9a) und 2,4-Dioxo-1-propyl-3-phenyl-1,2,3,4-tetrahydro-chinazolin (9b). — 2 g unverdünntes 1a wurden mit 1.5 g Phenylisocyanat (1 Mol-Äquiv.) und äther. Diazo-propan aus 7.5 g Nitrosopropylharnstoff versetzt. Nach 3 Tagen wurde i. Vak. eingedampft;

⁴⁾ W. Walisch, Chem. Ber. 94, 2314 (1961).

⁵⁾ Allgemeine Arbeitsvorschrift nach F. Arndt, Org. Syntheses, Coll. Vol. II, 165 (1943).

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der ölige Rückstand lieferte beim Verreiben mit kaltem Petroläther 2 g Kristalle. Aus dem Petroläther-Filtrat kristallisierte nach mehreren Stdn. 9b, das nach Umkristallisieren aus Petroläther, dann aus Äthanol/Wasser, bei 182° schmolz.

$C_{17}H_{16}N_2O_2$ (280.3) Ber. C 72.85 H 5.75 N 10.00 Gef. C 71.8 H 5.68 N 10.0

Die in kaltem Petroläther unlöslichen Kristalle lösten sich nur teilweise in heißem Petroläther; beim Erkalten kristallisierte eine geringe Menge 8b, Schmp. und Mischprobe mit authent. 8b¹⁾ 145°. — Der in heißem Petroläther ungelöste Rückstand zeigte nach Umkristallisieren aus Äthanol den Schmp. und Misch-Schmp. mit authent. 9a⁶⁾ 283°. — 4f konnte unter diesen Bedingungen nicht gefaßt werden.

5) α -[2-Äthoxycarbonyl-anilino]-valeriansäure-anilid (4g). — Wie bei 1) mit äther. Diazobutan aus 8 g Nitrosobutylharnstoff⁵⁾. Gesamtausbeute nach 2 Tagen 2.5 g (60%) 4g, die nach Umkristallisation zuerst aus Petroläther, dann aus Äthanol/Wasser, bei 140° schmolzen.

$C_{20}H_{24}N_2O_3$ (340.4) Ber. C 70.57 H 7.11 N 8.23 Gef. C 70.0 H 6.96 N 8.3

α -[2-Carboxy-anilino]-valeriansäure-anilid (4k). — 0.5 g 4g wurden in Äthanol gelöst und mit 20 ccm 1n NaOH 2.5 Stdn. auf 60° erwärmt. Nach Ansäuern mit 2n HCl wurde mit Äther extrahiert und i. Vak. eingedampft: 0.45 g (98%) 4k vom Schmp. 234° (aus Äthanol).

$C_{18}H_{20}N_2O_3$ (312.4) Ber. C 69.21 H 6.45 N 8.97 Gef. C 68.7 H 6.46 N 8.8

6) N-Nitroso-hexylharnstoff. — Durch Nitrosierung von Hexylharnstoff mit NaNO₂ in essigsaurer Lösung⁷⁾; Schmp. 73–75° (aus Methanol).

$C_7H_{15}N_3O_2$ (173.2) Ber. C 48.54 H 8.93 N 24.26 Gef. C 48.5 H 8.76 N 24.1

α -[2-Äthoxycarbonyl-anilino]-önanthsäure-anilid (4h). — 1.5 g 1a in 3.5 ccm absol. Äther wurde mit 1 g Phenylisocyanat (ca. 1 Mol-Äquiv.) und äther. Diazohexan aus 6 g Nitrosohexylharnstoff versetzt. Nach 5 Tagen wurde i. Vak. eingedampft. Aus dem öligen Rückstand kristallisierte bei 4° nach 3 Wochen 1 g (30%) 4h vom Schmp. 117° (nach Abpressen auf Ton und Umkristallisieren aus Äthanol/Wasser).

$C_{22}H_{28}N_2O_3$ (368.5) Ber. C 71.71 H 7.66 N 7.60 Gef. C 71.5 H 7.55 N 7.4

7) N-Nitroso-decylharnstoff. — Wie bei 6), Schmp. 72° (aus Methanol).

$C_{11}H_{23}N_3O_2$ (229.3) Ber. C 57.62 H 10.11 N 18.33 Gef. C 57.3 H 9.96 N 18.2

α -[2-Äthoxycarbonyl-anilino]-undecansäure-anilid (4i). — 1 g 1a in 2.5 ccm Äther wurde mit 1.5 g Phenylisocyanat (2.0 Mol-Äquiv.) und äther. Diazodecan aus 3.5 g Nitrosodecylharnstoff versetzt. Nach 3 Tagen wurde ein geringer flockiger Niederschlag abfiltriert und i. Vak. eingedampft. Der ölige Rückstand wurde in Petroläther aufgenommen, von etwas ungelöstem Diphenylharnstoff abfiltriert und der Petroläther i. Vak. entfernt. Nach 1–2tägigem Aufbewahren im Kühlschrank und Anreiben mit wenig Äthanol wurden 1.25 g (48%) 4i vom Schmp. 52° (aus Äthanol/Wasser) gewonnen.

$C_{26}H_{36}N_2O_3$ (424.6) Ber. C 73.55 H 8.55 N 6.60 Gef. C 73.4 H 9.53 N 6.7

⁶⁾ B. Taub und J. B. Hino, J. org. Chemistry 26, 5238 (1961).

⁷⁾ In Analogie zur Darstellung von N-Nitroso-cyclohexylharnstoff: K. Heyns und A. Heyns, Liebigs Ann. Chem. 634, 29 (1960), und zwar S. 44.

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2-Acetyl-anilino-essigsäure-anilid (4j). — 2 g o-Amino-acetophenon (1c) in 4 ccm Äther wurden mit 2.2 g Phenylisocyanat (ca. 1.25 Mol.-Äquivv.) und äther. Diazomethan (aus 4 g Nitroso-methylharnstoff) versetzt. Unter N₂-Entwicklung setzte beinahe sofort Ausscheidung von Kristallen ein. Nach 3—4 Stdn. wurden 1.6 g (41 %) 4j abgesaugt, die nach Auswaschen mit wenig Äther und 2maligem Umkristallisieren aus Äthanol/Kohle blaßgelbe Nadeln vom Schmp. 161° darstellten.

C₁₆H₁₆N₂O₂ (268.3) Ber. C 71.63 H 6.01 N 10.44 Gef. C 71.8 H 6.12 N 10.4

Skatol-carbonsäure-(2) (6). — Beim Schmelzen von 4j mit ca. der doppelten Menge Natriumhydroxid wurde heftiger Skatol-Geruch wahrnehmbar. Die erkaltete Schmelze wurde in Wasser gelöst, mit HCl angesäuert und ausgeäthert. Aus den Ätherauszügen kristallisierte nach Eindunsten 6; Schmp. und Mischprobe mit authent. 6⁸⁾ 165° (aus Benzol/Petroläther).

⁸⁾ R. Camps, Ber. dtsch. chem. Ges. 32, 3228 (1899).

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D 8

Reaktionen von Cyclooctin mit Nickel-Verbindungen

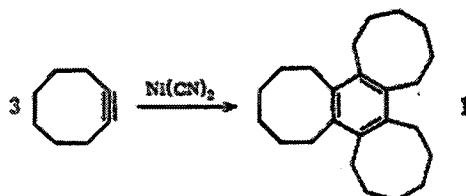
von Georg Wittig*) und Peter Fritze

Aus dem Institut für Organische Chemie der Universität Heidelberg

Eingegangen am 14. Juli 1967

Von Umsetzungen des Cyclooctins mit Nickel-Verbindungen erwartete man Hinweise auf den Mechanismus der Cyclooctatetraen-Synthese. Während mit Nickelcyanid nur das trimere Cyclooctin 1 resultierte, lieferte die Reaktion mit Nickelacetylacetonat die Addukte 2 und 3. Mit Nickelbromid und -jodid erhielt man außer 1 die Cyclobutadien-Komplexe 4 und 5, deren Stabilität es unwahrscheinlich erscheinen ließ, daß sie Zwischenstufen der Trimerisierung darstellen. Während mit Nickeltetracarbonyl bei Raumtemperatur eine CO-Einschiebung zu 6 erfolgte, konnte bei 0° ein Primärkomplex 8 gefaßt werden, dessen Reaktivität Hinweise auf den Mechanismus der Trimerisation gab.

Der Mechanismus der Cyclooctatetraen-Synthese aus Acetylen und Nickel(II)-Verbindungen, die erstmals von Reppe¹⁾ durchgeführt wurde, konnte bis heute noch nicht restlos aufgeklärt werden²⁾. Unklar blieb ferner, warum nichtsubstituierte Acetylene die entsprechende Reaktion eingehen³⁾. Da man im Cyclooctin ein besonders reaktives substituiertes Alkin⁴⁾ in Händen hatte, erschien es lohnend, diese Problematik erneut zu untersuchen.



Man setzte Cyclooctin mit den gebräuchlichen Cyclooctatetraen-Katalysatoren um — in der Hoffnung, auch Zwischenstufen isolieren zu können. Alle Reaktionen

*) Herrn Professor Dr., Dr. h. c. Adolf Steinhöfer zu seinem 60. Geburtstag in freundschaftlicher Verbundenheit gewidmet.

¹⁾ W. Reppe, O. Schlichting, K. Klager und T. Toepel, Liebigs Ann. Chem. 560, 1 (1948).

²⁾ H. C. Longuet-Higgins und L. E. Orgel, J. chem. Soc. [London] 1956, 1969; G. N. Schrauzer, P. Glockner und S. Eichler, Angew. Chem. 76, 28 (1964).

³⁾ A. C. Cope und R. M. Pike, J. Amer. chem. Soc. 75, 3220 (1953); vgl. J. R. Leto und M. F. Leto, ebenda 83, 2944 (1961).

⁴⁾ G. Wittig und H.-L. Dorsch, Liebigs Ann. Chem. 711, 46 (1968).

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Sci. Pharm., 39 (1971), 4, 267-292

Aus der Forschung und Entwicklung der Biochemie Kundl Ges. m. b. H.

Zur Reaktion von Penicillinen

Teil 1: Verhalten gegen Penicillinase

Von

E. Brandl

Eingelangt am 27. September 1971

Einführung

Das Verhalten von Penicillinen gegenüber dem Enzym Penicillinase ist von wesentlicher, praktischer Bedeutung. Seit der Einführung des Penicillin G in die Therapie wußte man, daß die an sich empfindlichen Krankheitskeime, die

Penicillinase bilden, gegenüber dem Antibiotikum resistent sind. Auch für das säurestabile Penicillin V trafen diese Beobachtungen zu. Jedoch erst im Laufe der immer stärkeren Verbreitung des Penicillins entstand daraus das Problem der Resistenz. Die Eröffnung des technischen Zuganges zur 6-Aminopenicillinsäure (6-APA, siehe Teil 2), der Schlüsselsubstanz der halbsynthetischen Penicilline, bot die Möglichkeit zur systematischen Suche nach neuen, penicillinasestabilen Penicillinen.

Die Resistenz ist ein der Chemotherapie ganz allgemein inhärentes Phänomen. Man unterscheidet drei Typen:

1. die natürliche Spezies- oder Rassenresistenz;
2. die primäre und sekundäre „erworbene“ Resistenz;
3. die infektiöse, übertragbare Resistenz.

Die natürliche Resistenz ist einfach die Wirkungslücke im antibakteriellen Spektrum eines Chemotherapeutikums und ist genetisch fixiert: entweder als Stabilität im Metabolismus gegen das Agens, als Penetrationsbarriere, als Möglichkeit, das Chemotherapeutikum zu inaktivieren, usw.

Als primäre und sekundäre (erworbene) Resistenz bezeichnet man das Auftreten resistenter Varianten in Populationen von Mikroorganismen, die normalerweise gegenüber dem betreffenden Wirkstoff empfindlich sind.

Primär resistent sind einzelne Organismen, die ohne vorherigen Kontakt mit dem Antibiotikum dagegen unempfindlich sind. Die sekundäre Resistenz entwickelt sich während des Kontaktes mit einem Antibiotikum.

In beiden Fällen handelt es sich um mutagene Veränderungen, die nicht als eine Abwehrreaktion gegen das Chemotherapeutikum aufzufassen sind. Dieses spielt lediglich die Rolle des Selektionsmechanismus. Primäre und sekundäre Resistenz unterscheiden sich daher nur dadurch, daß die erstere das Endprodukt einer Mutationsentwicklung ist, während die letztere in ihrer Entwicklung beobachtet werden kann. Die Häufigkeit der Mutation ist sehr gering, sie entspricht einfach der natürlichen Mutationsrate und die liegt in der Größenordnung von $1 : 10^6$ bis $1 : 10^{13}$ Keimen. Die Bedeutung für die Therapie kann allerdings enorm sein: Unter dem Selektionsdruck des Chemotherapeutikums überleben nur die resistenten Keime und deren Nachkommen, so daß schließlich eine resistente Population die Infektion in Gang hält. Begünstigt wird eine solche Entwicklung besonders beim Penicillin durch zu niedrige Dosierung — denn Resistenz ist ja ein relativer, dosisabhängiger Begriff — und durch zu kurze Medikation. Auf Grund von Beobachtungen bei der sekundären Resistenzentwicklung lassen sich verschiedene Resistenzentwicklungstypen ableiten, die vom Chemotherapeutikum und seinem Wirkungsmechanismus abhängig sind:

a) One Step Mutations,

die nach einmaligem oder höchstens 2- bis 4-maligem Kontakt mit dem Antibiotikum erfolgen und hohe, von der Antibiotikumkonzentration unabhängige Resistenzgrade aufweisen (Streptomycintyp der Resistenzentwicklung).

b) Multiple Step Mutations,

wobei die Resistenzentwicklung langsam und stufenweise erfolgt, weil offenbar zur Ausprägung des Resistenzmerkmals mehrere aufeinanderfolgende Mutationsschritte erforderlich sind. Die Selektion höherer Resistenzgrade ist ab-

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hängig von der jeweiligen Chemotherapeutikumkonzentration (Penicillintyp der Resistenzentwicklung).

Schließlich noch die übertragbare Resistenz, die auf der Übertragung genetischen Materials von einer Zelle auf die andere beruht.

Drei Mechanismen werden dabei unterschieden:

Transformation:

Übertragung von DNS von einer Zelle auf die andere, nur in vitro bekannt.

Transduktion:

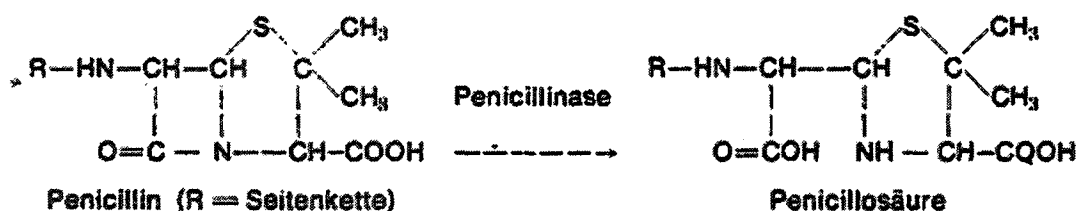
Übertragung größerer oder kleinerer Genomteile durch Phagen. Diese Form der Resistenzübertragung ist nur wirksam, solange der Phage lysogen bleibt und hat nur eine geringe Frequenz.

Konjugation:

Dabei wird durch den sexuellen Vorgang eines Zell-zu-Zell-Kontaktes, z. B. über Plasmabrücken, Genmaterial übertragen. Hierdurch können Mehrfachresistenzen induziert werden. Auch die episomale Resistenzübertragung, die eigentliche, infektiöse Resistenz, fällt darunter, bei welcher Resistenzfaktoren von gramnegativen Bakterien innerhalb und zwischen den Spezies übertragen werden können. Auf diesen Mechanismus ist die große Zahl mehrfach resistenter Enterobakterien zurückzuführen. Neuerdings hat man bei Staphylokokken eine ähnliche Form der infektiösen Resistenz beobachtet, wobei die Information zur Penicillinasebildung mittels extrachromosomaler Partikel, den Plasmiden, übertragen wird. Ein Lichtblick bei dieser infektiösen Resistenz ist, daß sie sich nach mehreren Generationen wieder von selbst verlieren kann.

Bei den Resistenztypen 1 und 3 kann das Enzym Penicillinase eine Rolle spielen. Für den „modernen“ Hospitalismus haben Infektionen mit resistenten Staphylokokken eine besondere Bedeutung.

Die Penicillinase – exakter als Penicillin- β -Lactamase E. C. 3.5.2.6. bezeichnet – ist ein primär intrazelluläres Enzym, das von verschiedenen Bakterien gebildet wird. Die Menge der gebildeten Penicillinase ist von Stamm zu Stamm verschieden (bei Staphylokokken zum Beispiel 6 E–460 E Enzym pro mg Trockengewicht). Das Enzym öffnet hydrolytisch den β -Lactamring des Penicillins, wobei unter Bildung einer neuen Carboxylgruppe die inaktive Penicillosäure entsteht.



Die verschiedenen Penicillinasen aus *B. cereus*, *Staph. aureus* sowie *E. coli* sind unter anderem in ihrer Reaktionsweise und -geschwindigkeit gegenüber Penicillin nicht einheitlich. Methicillin zum Beispiel schützt Penicillin G vor der Zerstörung durch Penicillinase aus *B. cereus*, jedoch nicht vor der durch Staphylokokken².

Ampicillin wird durch Staphylokokkenpenicillinase, weniger jedoch durch *E. coli*-Penicillinase inaktiviert³. Methicillin und Oxacillin sind nicht nur weitgehend resistent gegenüber dem Enzym, sondern zeigen auch eine Hemmwirkung dagegen⁴.

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Das Enzym Penicillinase ist bei potentiellen Penicillinasebildnern nur in Spuren vorhanden; seine Bildung in Bakterien wird unter Penicillineinfluß induziert. Das Induktionsvermögen der verschiedenen Penicilline ist unterschiedlich, jedoch ist die Enzyminduktion nur bei potentiellen Penicillinasebildnern möglich, nicht aber bei den gegen Penicillin empfindlichen penicillinase-negativen Keimarten.

Das zweite Enzym, das zu einer Inaktivierung des Penicillins führt, ist die Penicillinamidase oder Penicillinacylase E. C. 3.5.1.11. Es kommt in Pilzen und gramnegativen Bakterien vor. Seine Rolle bei der Penicillinresistenz ist noch nicht sicher abgeklärt⁵. Die technische Bedeutung der Penicillinamidase ist jedoch äußerst groß, denn sie führt, ausgehend von Penicillin G oder V, durch Abspaltung der Seitenketten zu 6-Aminopenicillansäure, aus der durch Umsetzung mit organischen Säuren eine große Anzahl neuer semisynthetischer Penicilline hergestellt wurde.

Das Ziel der vorliegenden Arbeit war es, an einer Reihe von neuen, biosynthetischen und halbsynthetischen Penicillinen* die Zusammenhänge zwischen der Struktur der Seitenkette — als den für die Eigenschaften des jeweiligen Penicillins verantwortlichen Molekülteil — und dem Verhalten gegenüber den Enzymen Penicillinase bzw. Penicillinamidase näher zu beleuchten. Darüber sollen die Teile 1 bzw. 2 handeln. Im Teil 3 schließlich wird über Versuche berichtet, das gegenüber dem Penicillinaseenzym empfindliche Penicillin G durch den Zusatz von penicillinasestabilen Penicillinen oder deren Seitenketten gegen die Inaktivierung durch das Enzym zu stabilisieren. Weiters sollte versucht werden, gesetzmäßige Beziehungen zwischen der biologischen Wirksamkeit der Penicilline in Abhängigkeit von der eingeführten Seitenkette zu erfassen.

Methoden

Die Inaktivierung von Penicillin durch das Enzym Penicillinase verläuft unter bestimmten Bedingungen wie Temperatur und pH-Konstanz sowie Substratüberschuß nach einer Reaktion nullter Ordnung. Unter Berücksichtigung dieser Gegebenheiten wurde die Methode nach zahlreichen Vorversuchen, wobei Substratkonzentration, pH und Enzymmenge variiert wurden, wie folgt festgelegt: Auf Grund der bekannten jodometrischen Aktivität des zu untersuchenden Penicillins wurde eine Einwaage durchgeführt, so daß nach Auflösen in 50 ml sterilem Puffer von pH 7,0 eine Lösung mit einer Konzentration von 3000 E/ml entstand. Nach Zugabe der Penicillinase wurde der Ansatz auf einzelne Röhrchen aufgeteilt und bei 37° C bebrütet. Außer zum Zeitpunkt des Versuchsbeginnes erfolgte nach einer halben, einer, eineinhalb, zwei, drei, fünf und sieben Stunden die Bestimmung des Penicillingehaltes auf üblichem, jodometrischem Wege⁶, wobei jedesmal ein Röhrchen entnommen wurde. Die Blindwerte der Jodometrie waren besonders bei den 5- und 7-Stunden-Werten leicht erhöht, was auf die Anwesenheit geringer Mengen anderer Jod verbrauchender Substanzen neben Penicillin hinweist. Die gesamte Manipulation wurde zur Vermeidung von Penicillinzerstörung durch eingeschleppte, penicillinasebildende Keime unter sterilen Kautelen durchgeführt. Die Enzymkonzentration wurde so gewählt, daß ein stets als Vergleich mitlaufendes Penicillin G nach zwei bis vier Stunden zu 50% inaktiviert war.

* Die Synthese der halbsynthetischen Penicilline erfolgte durch die Herren A. Frank, F. Gapp und K. Riedl in der Chemischen Abteilung der Kundler Forschung unter der Leitung des am 3. 2. 1968 verstorbenen H. Magreiter.

Zur Ermittlung einer Kennziffer der Penicillinastabilität wurde wie folgt vorgegangen: Aus der Differenz des zwischen zwei aufeinanderfolgenden Bestimmungen vorhandenen Penicillingehaltes wurde das in dem zwischen den beiden Bestimmungen liegenden Zeitintervall zerstörte Penicillin in Einheiten/ml ermittelt und daraus der Quotient zwischen Penicillin G und dem zu untersuchenden Penicillin errechnet.

$$Q_t = \frac{\text{in der Zeit t zerstörtes Penicillin Na-G (E/ml)}}{\text{in der Zeit t zerstörtes zu untersuchendes Penicillin (E/ml)}}$$

Das Mittel aller Q_t -Werte ergibt die gesuchte Kennziffer. Bei der Titration gefundene Werte unter 500 E wurden wegen der Ungenauigkeit der jodometrischen Penicillinbestimmung in diesem Bereich nicht berücksichtigt. Die Kennziffer wurde für Cereuspenicillinase als Q_C^G bezeichnet, wobei der Index G darauf hindeutet, daß Penicillin G als Vergleichsbasis zur Ermittlung der Relation führte; der Buchstabe C steht für Cereuspenicillinase. Wenn Staphylokokkenpenicillinase eingesetzt wurde, lautet der Kennzifferausdruck Q_{St}^G . Da definitionsgemäß die Kennziffer für Penicillin G 1,0 gesetzt wurde, bedeuten Werte über 1,0 erhöhte Stabilität des zu untersuchenden Penicillins gegenüber der betreffenden Penicillinase. Bei stabilen Penicillinen (Q_C^G , sehr sehr groß) müßte die Penicillinasekonzentration wesentlich erhöht werden, um eine Zerstörungsgeschwindigkeit zu bekommen, die Differenzbeträge zwischen den aufeinanderfolgenden Bestimmungen liefert, welche außerhalb der Fehlerbreite der Methode liegen. Der Vergleich mit Penicillin G wäre aber dann nicht möglich, da dieses unter solchen Bedingungen zu rasch inaktiviert würde. Es wurde daher die zur Zerstörung durch Penicillin G geeignete Penicillinasedosierung in allen Fällen beibehalten und die Abnahme der verglichenen, stabilen Penicilline, die unter diesen Bedingungen sehr gering ist und infolge der Fehlerbreite der jodometrischen Bestimmung nicht genau errechnet werden kann, graphisch ausgewertet bzw. nach Ermittlung der Regressionsgeraden im Einheiten-Zeit-Diagramm die auf diese Weise korrigierten Werte zur Berechnung der Kennziffer verwendet.

Cereuspenicillinase:

Als Cereuspenicillinase diente ein steriles, standardisiertes, käufliches Penicillinasepräparat.

Staphylokokkenpenicillinase:

Die Staphylokokkenpenicillinase wurde in Abänderung einer von S. Ortel⁷ modifizierten Methode nach G. J. Harper⁸ aus einem penicillinasebildenden Staphylokokkenstamm isoliert.

mg-Aktivität:

Die Bestimmung der biologischen mg-Aktivität erfolgte nach der Lochplattenmethode gegen Staph. aureus SG 511 als Testorganismus und Penicillin G als Bezugsstandard.

Chromatographie:

Die chromatographischen Analysen wurden in Anlehnung an die Methode von R. R. Goodall und A. A. Levi⁹ mit wassergesättigtem Äther als Laufmittel absteigend durchgeführt, die Hemmzonen durch Auflegen der Streifen auf Staph. aureus SG 511-Platten bioautographisch entwickelt und die R_x -Werte dadurch ermittelt, daß die Wanderungsstrecke des betreffenden Peni-

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cillins durch die Wanderungsstrecke des mitgelaufenen Penicillin G dividiert wurde.

Die R_f -Werte sollten vor allem als Kriterium der Einheitlichkeit und Reinheit der eingesetzten Penicilline dienen.

Ergebnisse und Diskussion

Die Tabelle 1 bietet eine Übersicht über die untersuchten Penicilline, wobei die Strukturformeln der Seitenketten R festgehalten sind. Die Penicilline wurden dabei nach steigenden Q_C^G -Werten geordnet. Daneben finden sich die zugehörigen Q_S^G -Werte, die biologische mg-Aktivität sowie die R_f -Werte. Die laufende Nummer und die Substanznummer sollen bei weiteren Tabellen und der Diskussion die Übersichtlichkeit erleichtern.

Bei der Nomenklatur wurde der einfacheren Übersichtlichkeit halber in manchen Fällen nicht streng nach den Nomenklaturregeln vorgegangen.

Bei dem in dieser Tabelle angestellten Vergleich zwischen den Kennziffern Q_C^G (Cereuspenicillinasestabilität) und den Kennziffern Q_S^G (Staphylokokkenpenicillinasestabilität) wird deutlich, daß zwar keine gleichsinnige Beziehung im strengen Sinne vorliegt, dennoch aber, wenn auch mit gewissen Einschränkungen, eine gleichlaufende Tendenz zu beobachten ist.

Es zeigt sich aber auch, daß die Q_C^G -Werte schneller ansteigen als die Q_S^G -Werte; d. h. es gibt eine wesentlich größere Zahl von Penicillinen, die gegenüber Cereuspenicillinase resistent sind als gegenüber Penicillinase aus Staphylokokken. Andererseits weisen die Penicilline mit erhöhter Resistenz gegen Staphylokokkenpenicillinase immer auch eine erhöhte Stabilität gegenüber Cereuspenicillinase auf.

Die biologische Aktivität steht nur insofern in Zusammenhang mit dem Verhalten gegenüber den untersuchten Enzymen, als hohe Stabilität gegen Penicillinase mit hoher Aktivität unvereinbar zu sein scheint. Die R_f -Werte deuten darauf hin, daß die untersuchten Substanzen in reiner Form vorlagen, wobei in manchen Fällen allerdings keine biologische Wirksamkeit zu beobachten war.

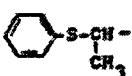
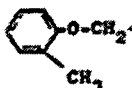
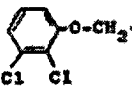
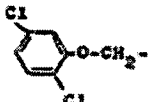
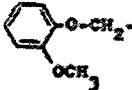
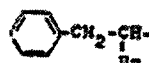
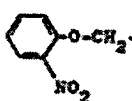
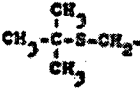
In der Tabelle 2 sind Penicillin V und die im Kern substituierten Phenoxyessigsäurederivate nach chemischer Ähnlichkeit zusammengefaßt. Ein völlig eindeutiger Zusammenhang mit der Stellung des Substituenten im Aromaten ist vor allem für die Staphylokokkenpenicillinase-Kennziffern nicht festzustellen. Die biologische Aktivität gegen den verwendeten Testkeim ist bei der m-Substitution eindeutig am höchsten. Die Art des Substituenten ist bei den Kennziffern von untergeordneter Bedeutung.

Dies geht auch beim Vergleich der Penicilline mit halogenierten Seitenketten aus der Tabelle 3 hervor. Lediglich die mg-Aktivität nimmt mit steigendem Atomgewicht der Halogene eindeutig zu.

Eine Reihe weiterer p-substituierter Phenoxyethylpenicilline sind in Tabelle 4 zusammengefaßt. Ein eindeutiger Einfluß im Sinne einer Stabilisierung gegenüber der Cereuspenicillinase geht lediglich vom großvolumigen elektronegativen Phenylrest aus, wobei die ortho-Stellung wesentlich wirksamer ist (Ild. Nrn. 93 und 102). Die Q_S^G -Werte werden jedoch kaum verändert. Durch die p-Acetamidogruppe wird die Stabilität besonders gegenüber Cereuspenicillinase verringert (Ild. Nr. 2). Daß die p-Substitution höhere mg-Aktivität

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Tabelle 1: Übersicht

Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx-Wert
				Q_C^G	Q_{St}^G		
1	508	Anilino-		0,46	--	56	1,80
2	10027	p-Acetamino-phenoxy-methyl-		0,50	0,72	625	2,60
3	450	α-Phenylmercapto-äthyl-		0,59	--	992	2,30
4	10013	p-Propyloxycarbonyl-phenoxy-methyl-		0,64	1,00	790	2,70
5	417	o-Tolyloxymethyl-		0,65	1,04	2150	2,40
6	10012	p-Methoxycarbonyl-phenoxy-methyl-		0,66	1,00	700	1,00
7	10017	2,3-Dichlorphenoxy-methyl-		0,68	0,58	2400	2,90
8	10018	2,5-Dichlorphenoxy-methyl-		0,68	0,80	2265	3,20
9	449	o-Methoxyphenoxy-methyl-		0,69	0,64	601	1,00
10	585	α-Brom-β-phenyl-äthyl-		0,69	--	--	--
11	453	o-Nitrophenoxy-methyl-		0,69	0,45	1744	1,00
12	10010	p-Acetylphenoxy-methyl-		0,76	0,77	910	0,30
13	556	Tertiärbutylmercaptomethyl-		0,76	--	3100	2,60

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Lfd. Nr.	Subst. Nr.	Penicillin- Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx- Wert
				Q_C^G	Q_{St}^G		
14	669	p-Nitrophenylmercaptomethyl-		0,76	—	3350	1,43
15	672	3-Methylenecyclobutyl-		0,77	—	540	0,58
16	960	Phenylmercaptomethyl-		0,78	—	3185	—
17	462	2,4-Dichlorphenoxy-methyl-		0,79	0,57	3455	3,30
18	10002	m-Nitrophenoxy-methyl-		0,80	0,80	3100	1,00
19	V	Phenoxymethyl		0,82	0,80	1530	2,10
20	574	Benzylaminomethyl-		0,82	—	—	0,00
21	464	Phenylen-1,4-bis(2-oxaäthyl)-		0,83	0,94	279	0,00
22	512	2,3-Dimethoxy-phenoxy-methyl-		0,83	0,80	474	1,00
23	10011	p-Propionylphenoxy-methyl-		0,87	1,15	650	1,00
24	10044	p-Nitrobenzyl-		0,88	1,50	1640	1,00
25	225	p-Chlorphenoxy-methyl-		0,88	1,00	2630	5,46
26	227	p-Jodphenoxy-methyl-		0,89	1,00	3046	7,72
27	466	α-Naphthoxymethyl-		0,89	0,72	2834	2,50
28	468	α-Phenoxy-β-brom-äthyl-		0,89	0,70	963	4,20
29	223	p-Tolyloxymethyl-		0,90	0,80	2270	4,47

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Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx-Wert
				Q_C^G	Q_{St}^G		
30	10003	p-Nitrophenoxy-methyl-		0,90	0,80	2290	1,00
31	10001	p-Cyanophenoxy-methyl-		0,90	0,90	1420	0,60
32	10029	p-Benzoyloxyphenoxy-methyl-		0,92	0,92	1400	2,70
33	566	Dibenzylamino-methyl-		0,92	—	—	2,92
34	226	p-Bromphenoxy-methyl-		0,93	1,00	2835	2,10
35	479	Thymoxymethyl-		0,95	—	—	5,10
36	10030	p-Methoxyphenoxy-methyl-		0,95	0,92	1340	1,40
37	10021	3,5-Dichlorphenoxy-methyl-		0,96	1,01	1945	2,50
38	10043	p-Methoxybenzyl-		0,99	1,14	1790	0,77
39	684	α-Äthoxycarbonyl-amino-β-3-indolyl-äthyl-		0,99	—	—	1,00
40	307	p-Fluorphenoxy-methyl-		0,99	—	1665	1,90
41	G	Benzyl-		1,00	1,00	1670	1,00
42	10005	m-Tolyloxymethyl-		1,00	1,00	3780	2,60

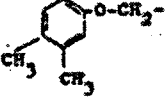
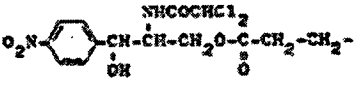
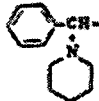
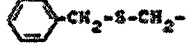
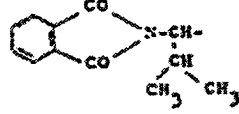
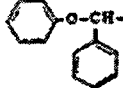
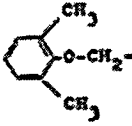
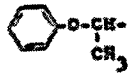
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Lfd. Nr.	Subst. Nr.	Penicillin- Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	R _x - Wert
				Q _C ^G	Q _{St} ^G		
43	10023	2,5-Dimethylphenoxy- methyl-		1,00	0,84	1270	2,40
44	10032	m-Chlorphenyl-		1,00	0,44	538	1,80
45	673	α-Phthalimido-γ- methyl-mercapto- propyl-		1,05	—	118	1,00
46	10026	3,5-Dimethylphenoxy- methyl-		1,08	0,96	1120	3,00
47	577	α-[3-Phenylacetyl- aminoazetidinon-(2)- yl-1]-isobutyl-		1,08	—	30	1,00
48	592	Acridin-9-		1,09	—	30	1,01
49	10025	2,3-Dimethylphenoxy- methyl-		1,11	0,57	1210	3,30
50	677	1-Benzylloxycarbonyl- piperidyl-(4)-		1,11	—	476	1,00
51	676	1-Benzylloxycarbonyl- piperidyl-(3)-		1,16	—	172	2,51
52	229	n-Pentyloxymethyl-	$\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-}$	1,18	—	1070	—
53	F	Penten-(2-)yl-(1)-	$\text{CH}_3\text{-CH}_2\text{-CH=CH-CH}_2\text{-}$	1,19	—	1280	1,66
54	10014	p-Tertiärbutyl- phenoxymethyl-		1,20	1,00	880	3,90
55	10004	2,4-Dinitrophenoxy- methyl-		1,20	1,20	990	1,00

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Lfd. Nr.	Subst. Nr.	Penicillin- Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx- Wert
				Q_C^G	Q_{St}^G		
56	584	4-Thia-1-azabicyclo- [3.2.0]-heptan-3,3- dimethyl-7-oxo-6- (phenoxyacetamido)- yl-2-		1,21	—	1809	—
57	416	Isopropyliden- aminoxymethyl-		1,22	—	215	0,20
58	273	n-Hexyloxymethyl-	$CH_3CH_2CH_2CH_2CH_2CH_2OCH_2-$	1,23	—	1700	9,80
59	10009	p-Methoxyphenyl-		1,26	0,74	225	1,10
60	689	8-Methoxy-2-chinoly-		1,28	—	35	1,00
61	593	Phenylsulfenyl- methyl-		1,31	—	30	1,00
62	228	n-Butoxymethyl-	$CH_3-CH_2-CH_2-CH_2-O-CH_2-$	1,35	—	1585	2,42
63	254	n-Propoxymethyl-	$CH_3-CH_2-CH_2-O-CH_2-$	1,37	—	1500	1,00
64	10042	m-Methoxybenzyl-		1,37	0,70	1640	1,00
65	10033	p-Chlorphenyl-		1,38	0,57	300	2,20
66	10036	p-Methylphenyl-		1,38	0,87	420	2,00
67	10038	m-Nitrophenyl-		1,39	0,41	400	0,64
68	10020	3,4-Dichlorphenoxy- methyl-		1,40	1,00	2160	2,70
69	595	Cyclopropyl-		1,41	—	35	1,00
70	4	Isobutoxymethyl-		1,43	—	1700	3,20
71	10008	m-Methoxyphenyl-		1,45	0,60	250	1,30

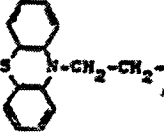
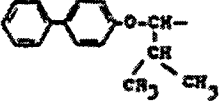
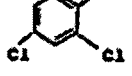
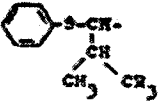
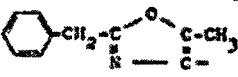
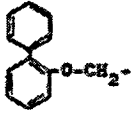
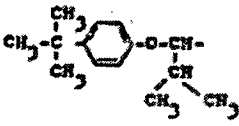
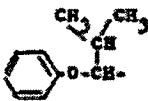
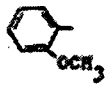
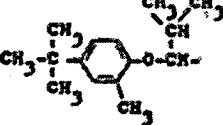
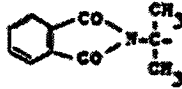
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Lfd. Nr.	Subst. Nr.	Penicillin- Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	R _x - Wert
				Q _C ^G	Q _{St} ^G		
72	10035	m-Methylphenyl-		1,49	0,62	370	2,10
73	10006	3,4-Dimethylphenoxy- methyl-		1,50	0,80	3790	3,89
74	516	β-[1-p-Nitrophenyl-1- hydroxy-2-dichlor- acetamido-propyl-(3)- oxycarbonyl]-äthyl-		1,50	—	40	0,50'
75	569	α-Piperidinobenzyl-		1,51	—	70	0,72
76	654	Benzylmercapto- methyl-		1,55	—	2400	2,80
77	10019	2,6-Dichlorphenoxy- methyl-		1,56	0,64	1290	3,30
78	674	α-Phthalimido- isobutyl-		1,58	—	90	2,57
79	454	β-Phenoxybenzyl-		1,58	—	341	4,30
80	10039	p-Nitrophenyl-		1,61	0,97	1260	1,40
81	10022	2,6-Dimethylphenoxy- methyl-		1,62	0,47	1270	2,50
82	10041	3,4-Dichlorphenyl-		1,64	0,76	570	2,20
83	436	α-Phenoxyäthyl-		1,66	—	1026	2,50
84	10024	2,4-Dimethylphenoxy- methyl-		1,70	0,73	2005	2,30

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Lfd. Nr.	Subst. Nr.	Penicillin- Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx- Wert
				Q _C ^G	Q _{St} ^G		
85	570	D-(-)-Formamino- benzyl-		1,78	—	20	1,00
86	482	2,6-Dimethoxy- phenoxy-methyl-		1,85	—	—	1,88
87	496	2,4,6-Trichlor- phenoxy-		1,85	—	10	—
88	499	Thymoxy-		1,87	—	—	3,52
89	493	o-Methoxyphenoxy-		1,90	—	45	3,40
90	675	3-Dimethylamino- phenyl-		1,94	—	90	2,46
91	559	2,6-Diisopropyl- phenoxy-methyl-		1,99	0,49	1300	4,95
92	591	Menthyloxymethyl-		2,04	2,50	730	2,94
93	10015	p-Phenylphenoxy- methyl-		2,15	1,08	2120	3,00
94	590	2-Methoxy-3-oxo-4- methylpentadien- (1,4)-yl-1-		2,17	—	0	1,00
95	10028	2-Benzoyl-4-chlor- phenoxy-methyl-		2,25	0,72	225	2,60
96	576	α-Anilino-isobutyl-		2,34	—	15	1,00

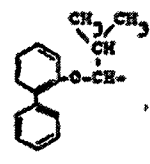
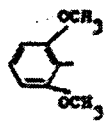
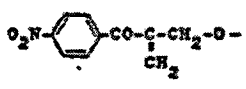
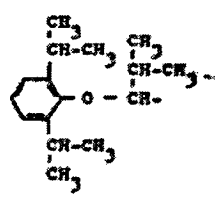
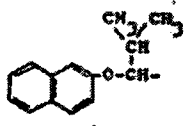
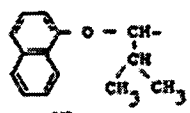
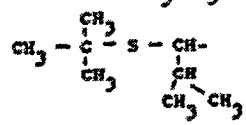
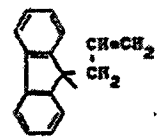
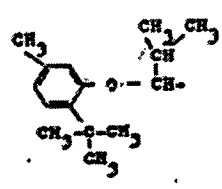
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Lfd. Nr.	Subst. Nr.	Penicillin- Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx- Wert
				Q _C ^G	Q _{St} ^G		
97	557	β-(10-Phenothiazin- yl)-äthyl-		2,88	—	1360	2,95
98	535	α-(4-Cyclohexyl- phenoxy)-isobutyl-		2,96	4,10	115	4,35
99	10040	2,4-Dichlorphenyl-		3,24	1,50	234	1,00
100	537	α-Phenylmer- captoisobutyl-		3,56	—	405	4,60
101	717	2-Benzyl-5-methyl- oxazolyl-(4)-		4,30	1,26	—	4,90
102	10016	o-Phenylphenoxy- methyl-		4,76	0,86	858	3,60
103	534	α-(p-Tertiärbutyl- phenoxy)-isobutyl-		5,26	6,20	145	3,80
104	543	D-α-(Phenoxy)-iso- butyl-		5,28	5,67	270	2,86
105	10007	o-Methoxyphenyl-		5,50	0,90	74	1,30
106	536	α-(2-Methyl-4-tertiär- butyl-phenoxy)-iso- butyl-		5,68	5,20	190	6,35
107	691	α-Phthalimido-iso- propyl-		5,79	8,14	1000	—
108	10034	o-Methylphenyl-		5,80	0,93	363	1,90
109	10031	o-Chlorphenyl-		6,34	1,50	450	1,10

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Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx-Wert
				Q _C ^G	Q _{St} ^G		
110	456	α-Phenoxyisopropyl-		6,63	2,50	380	3,80
111	531	α-o-Tolyloxy-isobutyl-		6,83	2,60	260	3,04
112	573	3-(p-Methoxy-phenyl)-propen-(1)-on-(3)-yl-(1)		7,71	3,95	0	—
113	527	α-Thymoxy-isobutyl-		7,80	8,90	80	3,39
114	690	5-Methyl-3-phenyl-4-isoxazoli- (Oxacillin)		8,73	∞	—	2,28
115	542	D,L-α-(4-Methyl-6-tertiärbutylphenoxy)-isobutyl-		8,79	5,24	100	4,60
116	655	α,α'-Diphenyl-β-bromäthyl-		9,04	—	370	1,00
117	530	α-(o-Methoxy-phenoxy)-isobutyl-		9,38	2,50	96	2,65
118	539	α-(2,4-Dichlor-phenoxy)-isobutyl-		10,22	3,58	420	—
119	10037	o-Nitrophenyl-		10,60	9,83	140	0,45

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Lfd. Nr.	Subst. Nr.	Penicillin- Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	R _x - Wert
				Q _C ^a	Q _{St} ^a		
120	541	D,L-α-(2-Phenyl- phenoxy)-isobutyl-		11,80	3,24	120	3,50
121	487	2,6-Dimethoxyphenyl- (Methicillin)		20,80	∞	200	1,00
122	558	2-p-Nitrobenzoyl-pro- pan-(2)-yloxy-(1)-		∞	∞	10	0,00
123	560	α-(2,6-Diisopropyl- phenoxy)-isobutyl-		∞	∞	70	5,28
124	561	α-(β-Naphthoxy)-iso- butyl-		∞	5,25	405	4,75
125	562	α-(α'-Naphthoxy)-iso- butyl-		∞	3,70	390	4,30
126	563	α-Tertiärbutylmer- captoisobutyl-		∞	3,98	90	4,30
127	596	9-Allyl-fluorenyl-(9)-		∞	2,95	90	4,14
128	533	α-(3-Methyl-6-tertiär- butyl-phenoxy)-iso- butyl-		∞	∞	90	5,40

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Tabelle 2: Ortho-, meta-, para-substituierte Phenoxyethylpenicilline

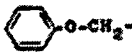
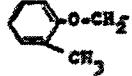
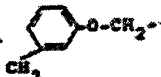
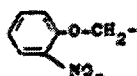
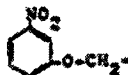
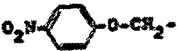
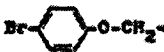
Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx-Wert
				Q _C ^G	Q _{St} ^G		
19	V	Phenoxyethyl-		0,82	0,80	1530	2,10
5	417	o-Tolyloxyethyl-		0,65	1,04	2150	2,40
42	10005	m-Tolyloxyethyl-		1,00	1,00	3780	2,60
29	223	p-Tolyloxyethyl-		0,90	0,80	2270	4,47
11	453	o-Nitrophenoxyethyl-		0,69	0,45	1744	1,00
18	10002	m-Nitrophenoxyethyl-		0,80	0,80	3100	1,00
30	10003	p-Nitrophenoxyethyl-		0,90	0,80	2290	1,00

Tabelle 3: p-Halogenierte Phenoxyethylpenicilline

Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx-Wert
				Q _C ^G	Q _{St} ^G		
26	227	p-Jodphenoxyethyl-		0,89	1,00	3046	7,72
34	226	p-Bromphenoxyethyl-		0,93	1,00	2835	2,10
25	225	p-Chlorphenoxyethyl-		0,88	1,00	2630	5,46
40	307	p-Fluorphenoxyethyl-		0,99	—	1665	1,90

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Tabelle 4: Verschiedene p-substituierte Phenoxyethylpenicilline

Lfd. Nr.	Subst. Nr.	Penicillin-Seltenkette (R)	Strukturformel der Seltenkette	Kennziffern		Aktiv. E/mg	Rx-Wert
				Q _C ^G	Q _{St} ^G		
31	10001	p-Cyanophenoxy-methyl-		0,90	0,90	1420	0,60
12	10010	p-Acetylphenoxy-methyl-		0,76	0,77	910	0,30
23	10011	p-Propionylphenoxy-methyl-		0,87	1,15	650	1,00
6	10012	p-Methoxycarbonyl-phenoxy-methyl-		0,66	1,00	700	1,00
4	10013	p-Propyloxycarbonyl-phenoxy-methyl-		0,64	1,00	790	2,70
54	10014	p-Tertiärbutyl-phenoxy-methyl-		1,20	1,00	880	3,90
93	10015	p-Phenylphenoxy-methyl-		2,15	1,08	2120	3,00
102	10016	o-Phenylphenoxy-methyl-		4,76	0,86	858	3,60
2	10027	p-Acetamino-phenoxy-methyl-		0,50	0,72	625	2,60
32	10029	p-Benzoyloxyphenoxy-methyl-		0,92	0,92	1400	2,70
38	10030	p-Methoxyphenoxy-methyl-		0,95	0,92	1340	1,40

zur Folge hat als diejenige in ortho-Stellung, zeigt der Vergleich zwischen den Substanzen der laufenden Nummern 93 und 102.

Verschiedene disubstituierte Phenoxyethylpenicilline sind in Tabelle 5 zusammengestellt. Der vorher erhobene Befund, daß die Phenylsubstitution in o-Position den Q_C^G am stärksten anhebt, wird durch die hohen Kennziffern von 1,56 beim 2,6-Dichlorphenoxyethylpenicillin (lfd. Nr. 77) und von 1,62 beim 2,6-Dimethylphenoxyethylpenicillin (lfd. Nr. 81) bestätigt. Auch die 2,4 und 3,4 Substitution führt zu einem gleichsinnigen Effekt (lfd. Nrn. 84, 55 und 68).

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Tabelle 5: Disubstituierte Phenoxy-methylpenicilline

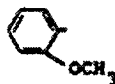
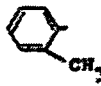
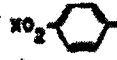
Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	R _x -Wert
				Q _C ^G	Q _{St} ^G		
7	10017	2,3-Dichlorphenoxy-methyl-		0,68	0,58	2400	2,90
8	10018	2,5-Dichlorphenoxy-methyl-		0,68	0,80	2265	3,20
77	10019	2,6-Dichlorphenoxy-methyl-		1,56	0,64	1290	3,30
68	10020	3,4-Dichlorphenoxy-methyl-		1,40	1,00	2160	2,70
37	10021	3,5-Dichlorphenoxy-methyl-		0,96	1,01	1945	2,50
49	10025	2,3-Dimethylphenoxy-methyl-		1,11	0,57	1210	3,30
84	10024	2,4-Dimethylphenoxy-methyl-		1,70	0,73	2005	2,30
43	10023	2,5-Dimethylphenoxy-methyl-		1,00	0,84	1270	2,40
81	10022	2,6-Dimethylphenoxy-methyl-		1,62	0,47	1270	2,50
46	10026	3,5-Dimethylphenoxy-methyl-		1,08	0,96	1120	3,00
95	10028	2-Benzoyl-4-chlor-phenoxy-methyl-		2,25	0,72	225	2,60
55	10004	2,4-Dinitrophenoxy-methyl-		1,20	1,20	990	1,00

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Die Substitution in Position 2,3 (lfd. Nrn. 7, 49) sowie 2,5 (lfd. Nrn. 8, 43) und 3,5 (lfd. Nrn. 37, 46) ergibt keine Stabilisierung gegenüber Cereuspenicillinase.

Das Verhalten gegen Staphylokokkenpenicillinase zeigt in diesen Kennzifferbereichen keine Relation zu demjenigen gegenüber Cereuspenicillinase.

Tabelle 6: Ortho-, meta-, para-substituierte Phenylpenicilline

Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	R _x -Wert
				Q _C ^G	Q _{SI} ^G		
105	10007	o-Methoxyphenyl-		5,50	0,90	74	1,30
71	10008	m-Methoxyphenyl-		1,45	0,60	250	1,30
59	10009	p-Methoxyphenyl-		1,26	0,74	225	1,10
109	10031	o-Chlorphenyl-		6,34	1,50	450	1,10
44	10032	m-Chlorphenyl-		1,00	0,44	538	1,80
65	10033	p-Chlorphenyl-		1,38	0,57	300	2,20
108	10034	o-Methylphenyl-		5,80	0,93	363	1,90
72	10035	m-Methylphenyl-		1,49	0,62	370	2,10
66	10036	p-Methylphenyl-		1,38	0,87	420	2,00
119	10037	o-Nitrophenyl-		10,60	9,83	140	0,45
67	10038	m-Nitrophenyl-		1,39	0,41	400	0,64
80	10039	p-Nitrophenyl-		1,61	0,97	1260	1,40

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Auffallend ist die Erniedrigung der Q_{St}^G -Werte bei den 2,3 und 2,6 disubstituierten Phenoxymethylpenicillinen (lfd. Nrn. 7, 49 und 77, 81).

Eine sehr klare Aussage und damit eine Bestätigung verschiedener, schon beim Phenoxymethylpenicillin angedeuteter Befunde, erlauben die Ergebnisse der o-, m- und p-Substitution für Phenylpenicilline der Tabelle 6: Substituenten in der o-Stellung machen das Molekül weitgehend unempfindlich gegen den Angriff der Cereuspenicillinase (lfd. Nrn. 105, 109, 108, 119). m- und p-Substitution tun dies in wesentlich geringerem Ausmaße und unterscheiden sich untereinander praktisch nicht.

Derselbe Trend gilt – wenn auch wesentlich abgeschwächt – auch für das Verhalten gegenüber Staphylokokkenpenicillinase. Lediglich das o-Nitrophenylpenicillin (lfd. Nr. 119) zeigt eine unerwartet hohe Stabilität gegen dieses Enzym. Die Art des Substituenten spielt auch hier wieder nur eine untergeordnete Rolle.

Tabelle 7: Disubstituierte Phenylpenicilline

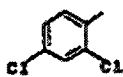
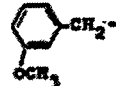
Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx-Wert
				Q_C^G	Q_{St}^G		
99	10040	2,4-Dichlorphenyl-		3,24	1,50	234	1,00
82	10041	3,4-Dichlorphenyl-		1,64	0,76	570	2,20

Tabelle 8: Verschiedene substituierte Benzylpenicilline

Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx-Wert
				Q_C^G	Q_{St}^G		
41	G	Benzyl-		1,00	1,00	1670	1,00
64	10042	m-Methoxybenzyl-		1,37	0,70	1640	1,00
38	10043	p-Methoxybenzyl-		0,99	1,14	1790	0,77
24	10044	p-Nitrobenzyl-		0,88	1,50	1640	1,00

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Disubstitution mit Chlor (Tabelle 7) ergibt für die 2,4 Position einen wesentlich stärkeren Anstieg der Kennziffern auch bei der Staphylokokken-penicillinase als für die 3,4 Position.

Was die Benzylpenicilline angeht, kann, wie aus Tabelle 8 ersichtlich, für die Methoxygruppe bei m-Substitution stärkere Stabilisierung gegen Cereus-penicillinase erzielt werden als durch Einführung eines Substituenten in p-Position. Dabei bewegen sich allerdings die Q_{St}^G -Werte gegenläufig. Auffallend ist der für das p-Nitrobenzylpenicillin relativ hohe Q_{St}^G -Wert von 1,50 gegenüber einem solchen für Q_C^G von 0,88.

Tabelle 9: Mercaptopenicilline

Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	R _x -Wert
				Q_C^G	Q_{St}^G		
3	450	α-Phenylmercapto-äthyl-		0,59	—	992	2,30
13	556	Tertiärbutylmercaptomethyl-		0,76	—	3100	2,60
16	960	Phenylmercaptomethyl-		0,78	—	3185	—
14	669	p-Nitrophenylmercaptomethyl-		0,76	—	3350	1,43
45	673	α-Phthalimido-γ-methyl-mercapto-propyl-		1,05	—	118	1,00
61	593	Phenylsulfonylmethyl-		1,31	—	30	1,00
76	654	Benzylmercaptomethyl-		1,55	—	2400	2,80
100	537	α-Phenylmercapto-isobutyl-		3,56	—	405	4,60
126	563	α-Tertiärbutylmercaptolisobutyl-		∞	3,98	90	4,30

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In Tabelle 9 sind die Mercaptopenicilline einander gegenübergestellt. Verglichen mit dem Benzylpenicillin bringt die Einführung eines Schwefelatoms ebenso wie diejenige des Sauerstoffatoms eine leichte Reduzierung der Stabilität gegenüber Cereuspenicillinase; auch das p-Nitrophenylmercaptomethylpenicillin (lfd. Nr. 14) deutet in diese Richtung. Hingegen erfährt die

Tabelle 10: Aminopenicilline

Lfd. Nr.	Subst. Nr.	Penicillin-Seitenkette (R)	Strukturformel der Seitenkette	Kennziffern		Aktiv. E/mg	Rx-Wert
				Q_C^G	Q_{St}^G		
20	574	Benzylaminomethyl-		0,82	—	—	0,00
33	566	Dibenzylaminomethyl-		0,92	—	—	2,92
1	508	Anilino-		0,46	—	56	1,80
96	576	α-Anilino-isobutyl-		2,34	—	15	1,00
85	570	D-(-)-Formamino-benzyl-		1,78	—	20	1,00
107	691	α-Phthalimido-iso-propyl-		5,79	8,14	1000	—
78	674	α-Phthalimido-iso-butyl-		1,58	—	90	2,57
45	673	α-Phthalimido-γ-methyl-mercapto-propyl-		1,05	—	118	1,00
39	684	α-Athoxycarbonyl-amino-β-3-indolyl-äthyl-		0,99	—	—	1,00
90	675	3-Dimethylamino-phenyl-		1,94	—	90	2,46

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biologische Aktivität gegen den verwendeten Testkeim eine Erhöhung. Eine Stabilisierung gegenüber dem Angriff durch die Cereuspenicillinase wird erreicht durch die Einführung zusätzlicher Methylgruppen, wie dies in den Substanzen mit den laufenden Nummern 76, 100, 126 erfolgt ist; gleichzeitig wird dadurch die biologische Aktivität verringert. Daß die Stellung der Methylgruppen dabei ausschlaggebend ist, zeigt das Tertärbutylmercaptomethylpenicillin (laufende Nummer 13) mit seinem sehr niedrigen Q_C^G -Wert von 0,76. Der Einfluß, den die Einführung einer Aminogruppe in die Penicillin-Seitenkette auf das betrachtete Verhalten nach sich zieht, geht aus Tabelle 10 hervor.

Danach führt auch die Aminogruppe zu einer niedrigeren Kennziffer, ähnlich wie Sauerstoff und die Mercaptogruppe. Das Anilinopenicillin (Ifd. Nr. 1) ist mit einem Q_C^G -Wert von 0,46 das instabilste Penicillin gegenüber Cereuspenicillinase. Die Einführung von stabilisierenden Methylgruppen ist hier weniger wirksam, wie folgende Vergleiche zeigen: Benzylaminomethylpenicillin Q_C^G 0,82 – Benzylmercaptomethylpenicillin Q_C^G 1,55 – α -Anilino-isobutylpenicillin Q_C^G 2,34 – α -Phenylmercaptoisobutylpenicillin Q_C^G 3,56.

Von den weiteren untersuchten Aminopenicillinen ist vor allem das Phthalimido-isopropylpenicillin zu erwähnen, dessen Q_S^G -Wert mit 8,14 deutlich über dem Q_C^G -Wert von 5,79 liegt.

Auffallend ist bei diesen Penicillinen die niedrige biologische Aktivität.

Bei einer Überschau der Tabelle 1 sieht man, daß die Zunahme der Penicillinase-Stabilität im allgemeinen bei Strukturen auftritt, die großvolumige Substituenten in Nähe der Seitenkettenamidbindung tragen. Damit im Einklang steht der bereits oben mehrfach erhobene Befund, wonach ortho-Substitution mehr zur Stabilisierung beiträgt als meta- oder para-Substitution. Diese Beobachtung wird gefestigt durch die Tatsache, daß Phenylpenicilline stabiler gegen Cereuspenicillinase sind als die analogen Phenoxy-methylpenicilline, wie der Vergleich der Tabellen 2 und 6 zeigt. Im 2,6-Dimethoxyphenylpenicillin (Ifd. Nr. 121), bei dem beide o-Stellen besetzt sind, wird nicht nur die Stabilität gegen Cereuspenicillinase, sondern auch diejenige gegen Staphylokokkenpenicillinase sehr stark erhöht.

Bei Phenoxypenicillinen scheinen voluminöse Gruppen in o-Position noch keine ausreichende Resistenz zu bedingen. Hierzu ist noch eine zusätzliche Substitution des α -C-Atoms der Seitenkette erforderlich. Dies geht aus dem Vergleich der Substanzen Ifd. Nr. 91 und 123 klar hervor; letztere ist auch gegen Staphylokokkenpenicillinase stabil. Noch deutlicher ist dieser stabilisierende Effekt zu erkennen, wenn man das α -Naphthoxy-methylpenicillin (laufende Nummer 27) mit seinem α -Propylhomologen (laufende Nummer 125) vergleicht.

Auch die alleinige Einführung einer voluminösen Gruppe in α -Position der Seitenkette erhöht die Kennziffern der Penicillinase-Stabilität sehr stark, wie das etwa bei der Substanz Ifd. Nr. 104 der Fall ist.

Diese Beobachtungen decken sich mit den Feststellungen in der Literatur^{10, 11, 12}, daß bei penicillinase-stabilen Penicillinen sterische Gründe dafür verantwortlich sind, daß der Angriff durch das Enzym gehemmt wird. Auch die Befunde über Penicilline mit Doppelbindungen in der Aralkylseitenkette, wie das 3-(p-Methoxyphenyl)-propen-(1)-yl-(1)-penicillin (laufende Nummer 112) und das 2-p-Nitrobenzoyl-propen-(2)-yloxy-(1)-penicillin (laufende Nummer

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122) sowie in abgeschwächtem Maße auch das Alkylpenicillin der laufenden Nummer 94, stimmen damit überein. Bei dem geradkettigen Penicillin F (laufend Nummer 53) und dem α -Phenoxy- β -bromäthylenpenicillin (laufende Nummer 26) verliert sich dieser Effekt. Offenbar ist die ungehinderte Ausbildung des Enzym-Substrat-Komplexes, wenn eine niedervolumige Seitenkette vorliegt, auch trotz teilweisen Verlustes der freien Drehbarkeit möglich.

Für die ganze Gruppe der gegen Penicillinase hochstabilen Penicilline ist eine reduzierte mikrobielle Aktivität charakteristisch.

Zusammenfassung

Bei einer Vielzahl von biosynthetischen und halbsynthetischen Penicillinen wurde die Stabilität gegenüber Cereuspenicillinase und Staphylokokkenpenicillinase, die antibakterielle Wirkung gegen Penicillinase negative Staphylokokken sowie der R_x -Wert bestimmt.

Auf mögliche Zusammenhänge zwischen der Struktur der Seitenkette und dem geprüften Verhalten wurde hingewiesen.

Verhalten gegen Penicillinase:

Stabilität gegen Cereuspenicillinase tritt sehr viel häufiger auf als gegenüber Staphylokokkenpenicillinase.

Penicilline mit Resistenz gegen Staphylokokkenpenicillinase waren immer auch stabil gegen Cereuspenicillinase. Großvolumige Substituenten in der Seitenkette sind Stabilisatoren; dabei ist die Entfernung von der Amidbindung bedeutsam.

Hinderung der freien Drehbarkeit durch eine Doppelbindung wirkt in ähnlicher Weise stabilisierend.

Einführung von Sauerstoff, einer Mercapto- oder einer Aminogruppe in die Seitenkette erhöht die Reaktionsgeschwindigkeit des Penicillins mit Penicillinase.

Antimikrobielle Aktivität gegen sensible Staphylokokken:

Halogene sowie Methyl- und Nitrogruppen führen besonders in meta- und para-Stellung zu Aktivitätssteigerung.

Die Einführung einer Mercaptogruppe erhöht gleichfalls die biologische Wirkung.

Aminogruppen verringern die Aktivität ebenso wie großvolumige Substituenten im Kern und am α -C-Atom der Seitenkette.

Hohe Stabilität gegen Penicillinase und hohe antimikrobielle Aktivität scheinen inkompatibel zu sein.

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★
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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Re: **P1.-GLAXO GROUP LIMITED.-Solicitud de Patente de Invención**
para "DERIVADOS DE ARIL CETONA COMO INHIBIDORES DE
LA TRANSCRIPTASA REVERSA".-Clase A.61.-Expediente número
00-065.946.-

1. Me refiero al auto dictado por ese Despacho y notificado por fijación en lista de 8 de Marzo de 2007.

2. Atentamente ruego a usted concederme un plazo de treinta (30) días para dar respuesta al auto antes mencionado, cuyo término vence el próximo **6 de Junio de 2007.**

3. Anexo acompaño recibo número 07-39.884, que acredita el pago de los derechos que dicho plazo causa.

4. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Superintendente,

GUSTAVO TAMAYO ARANGO
T.P. 37.172