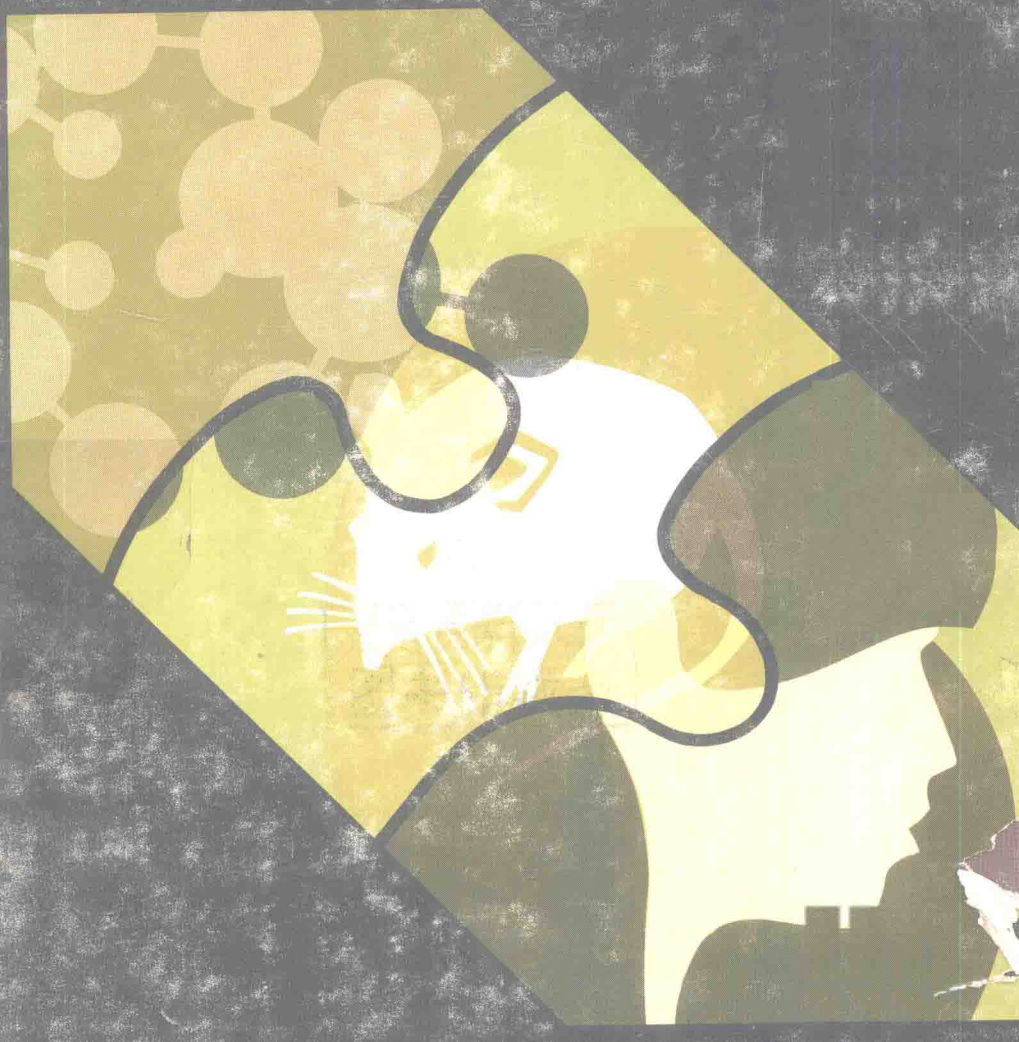
Pharmacological and Biochemical Properties of Drug Substances

Morton E. Goldberg, D.Sc., Editor

American Pharmaceutical Association



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Published by
American Pharmaceutical Association
Academy of Pharmaceutical Sciences
2215 Constitution Avenue, N.W.
Washington, D.C. 20037

International Standard
Book Number: 0-917330-17-X
Library of Congress
Catalog Card Number: 77-88184

American Pharmaceutical Association
2215 Constitution Avenue, N.W.
Washington, D.C. 20037

Printed in the United States of America

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Conducted under the auspices of the Pharmacology and Toxicology Section APhA Academy of Pharmaceutical Sciences

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Preface to Volume 1

Pharmacological and Biochemical Properties of Drug Substances

About two years ago over lunch in the magnificent Squibb restaurant in Princeton, New Jersey, Dr. Klaus Florey convinced me that this volume would serve a valuable function in the literature of therapeutic agents. For several years, Dr. Florey has edited a series on *Analytical Profiles of Drug Substances* which concerns itself largely with chemical properties of drugs, both established and newer therapeutic agents. His facility for convincing me that a companion series describing the biological properties of drugs initially exceeded my abilities to convince appropriate scientists to join me in this endeavor. Undaunted, and aided by a magnificent luncheon sponsored by Squibb, the Editorial Board met in April, 1976, to discuss the ground rules for this volume. After nine different opinions were reduced to a singular philosophy, aided considerably by a delightful beaujolais, we decided that such a series was indeed to be undertaken. The basis for selection of an agent was to be considered in terms of timeliness and potential or actual importance in therapeutics or for use as a diagnostic agent. Initially, we wanted to restrict the selection of agents to those marketed and sold somewhere, although not necessarily restricted to the United States. Later, we felt that such a restriction was unwarranted. What we wish to capture in this series are agents which represent prototypes for the major classes of drugs available, considering the current mood of therapeutics, their selection should exemplify the timeliness which we think is needed to make this series a valuable addition to the armamentarium of books on this subject. Further, we hoped that the authors selected would convey to the reader the philosophy and background which led to its development. To the ends which these goals were attained, your comments on format and future endeavors would be welcomed.

I wish to thank personally the other members of the Editorial Board and all authors who have served unselfishly and without honoraria to provide royalties of this volume to be presented to the Pharmacology and Toxicology Section of the

Academy of Pharmaceutical Sciences. I wish to thank Ms. Helen Stofko of the Squibb Institute for Medical Research and Ms. Linda Bunting for their valuable secretarial work in helping launch this volume. Finally, I would like to thank Klaus Florey, without whose influence this preface need never have been written.

M. E. Goldberg
July 18, 1977
Wilmington, Delaware

MONOGRAPHS

Contents

CLOZAPINE

A. C. Sayers and H. A. Amsler

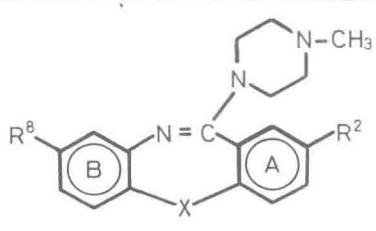
Since the introduction of chlorpromazine in 1952 [1], an ever increasing number of antipsychotic agents have found a place in psychiatric therapy. Although these compounds belong to various structural classes (phenothiazines, thiaxanthenes, butyrophenones, etc.), they have one property in common – the ability to block striatal dopamine(DA)-receptors. This property, until recently thought to be essential for antipsychotic activity [2–4], is responsible for induction of catalepsy and for inhibition of apomorphine- and amphetamine-induced stereotyped behaviour in animals, and also for the development of extrapyramidal symptoms(EPS) in man. EPS have been considered to be unavoidable with antipsychotic agents, and some clinicians [5] have considered the therapeutic dose level of a neuroleptic to be reached only when they appear.

The correlation between DA-receptor blockade, antipsychotic activity and extrapyramidal effects (the so-called DA-hypothesis) has been seriously challenged by the advent of clozapine (Leponex®), a potent antipsychotic agent lacking any appreciable DA-receptor blocking properties and failing to induce the EPS (parkinsonism and tardive dyskinesias) characteristic of classical neuroleptic drugs [6].

CHEMISTRY AND STRUCTURE–ACTIVITY RELATIONSHIPS

Chemistry

Table 1 – Antipsychotic 11-Piperazinyldibenzo-azepines



I

Generic name	X	R^2	R^8
clozapine	NH	H	Cl
clothiapine	S	Cl	H
metiapine	S	Me	H
loxapine	O	Cl	H

Clozapine is a dibasic substance, forming water-soluble salts. Structurally, it belongs to the 11-piperazinyldibenzo-azepines [7, 8, 9] represented by the general formula I. This new tricyclic system has yielded useful new antipsychotic agents (Table 1), antidepressants and sedatives/hypnotics [9, 10].

Clothiapine, metiapine and loxapine are classical neuroleptics, characterized pharmacologically by cataleptic and apomorphine-antagonistic activities and, clinically, by an anti-schizophrenic action which is usually accompanied by more or less pronounced EPS. In contrast, clozapine exhibits no cataleptic or anti-apomorphine effects in the animal, and its antipsychotic action is virtually devoid of EPS. Its 2-chloro-isomer, HF–2046, however, possesses the pharmacological and neurochemical properties of a classical neuroleptic [11–14]. It would appear that the classical-neuroleptic profile is associated with a substituent (R^2) in position 2 of ring A and is not dependent on the nature of the bridging moiety X, and the distinctive pharmacological profile of clozapine appears to be associated with substitution at position 8 in ring B.

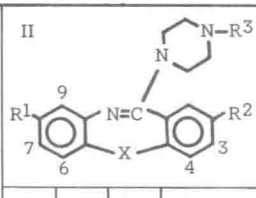
The dibenzo-azepines are tricyclic structures which differ from the phenothiazine and thiaxanthene neuroleptics by having a central 7-membered ring. This results in an unsymmetrical ring system in which the substituent positions in the two aromatic rings are not equivalent, in contrast to those in the symmetrical phenothiazines or thiaxanthenes. This is illustrated by the crystal structures of clozapine on the one hand and of its 2-chloro--isomer HF–2046 and loxapine on the other [15]. The 7-membered central ring of clozapine is folded, with a dihedral angle between the planes of the two benzene rings of 115°. A partial double bond between the tricyclic system and the piperazine ring prevents rotation of the latter which is situated in close proximity to ring A. The plane of the piperazine ring is nearly parallel to the plane of the chlorine-substituted ring B. Loxapine and HF–2046 display molecular conformations practically identical with those of clozapine, so that, in these 2-substituted compounds, the piperazine ring lies close to the chlorine-substituted ring (A) and in a parallel plane with the unsubstituted ring (B). Thus, the clozapine molecule differs from those of the classical neuroleptics loxapine and HF–2046 with respect to the spatial relationship between the piperazine moiety and the benzene ring carrying the chlorine substituent. It is reasonable to expect that molecules showing such structural differences will differ in their affinities for specific receptor sites.

Structure-Activity Relationships

The effects of chlorine substitution in positions 2 or 8 of the dibenzo-thiazepines, -oxazepines, -diazepines and -azepines (general formula II) are compared in Table 2.

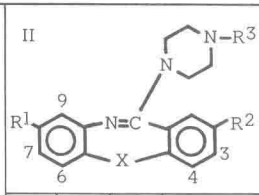
The unsubstituted compounds perlapine, 2, 3 and 4 inhibit locomotor activity, but in the tests for classical neuroleptic activity (catalepsy, apomorphine-antagonism) they are inactive or only marginally active. Striatal DA-metabolism, as measured by DOPAC concentrations, is only weakly affected.

Table 2 – Clozapine: Structure–Activity Relationships

	II 				Locomotor inhibition (mouse)	Arousal inhibition (rabbit)	Extrapyramidal effects (rat)		
							catalepsy	apomorphine antagonism	striatal DOPAC
					ED 50	ED 150	ED 30 sec	ED 50	ED 200
Compound	X	R_1	R_2	R_3	mg/kg po	mg/kg iv	mg/kg po	mg/kg sc	mg/kg po
Perlapine	CH_2	H	H	CH_3	1.7	1.2	32	Ø	13
2	NH	H	H	CH_3	10	Ø	Ø	Ø	100
3	O	H	H	CH_3	2.7	Ø	Ø	13	9
4	S	H	H	CH_3	7.4	3.4	Ø	16	11
5	CH_2	H	Cl	CH_3	0.28	4.3	1	0.16	
6	NH	H	Cl	CH_3	6	Ø	3.5	1.7	5
Loxapine	O	H	Cl	CH_3	0.05	3.2	0.3	0.07	0.07
Clothiapine	S	H	Cl	CH_3	0.6	3.2	0.3	0.27	0.25
9	CH_2	Cl	H	CH_3	8.1	2.3	Ø	Ø	24
Clozapine	NH	Cl	H	CH_3	2.5	1.5	Ø	Ø	56
11	O	Cl	H	CH_3	2.5	3.5	Ø	Ø	80
12	S	Cl	H	CH_3	23	0.9	Ø	Ø	>160
13	NH	Cl	H	$(CH_2)_2OH$	14	Ø	Ø	Ø	103
14	NH	Cl	H	$(CH_2)_3OH$	19	Ø	Ø	Ø	180
15	NH	Cl	Cl	CH_3	2	0.4	8	16	11
16	NH	Cl	F	CH_3	2.2	1.2	Ø	9	28
Haloperidol					0.3	Ø	0.3	0.14	0.47
Chlorpromazine					4	5.8	3.8	11	3.8

Ø = inactive

Table 2 – continued

Compound	II  X	R_1	R_2	R_3	Anticholinergic potency Oxotremorine test-mouse ED 50[a] mg/kg po	³H-QNB assay IC 50 uM	Striatal DA-content (rat) (% ± S.D.) Dose mg/kg po	single treatment	7-day treatment
Perlapine	CH_2	H	H	CH_3	Ø	1.2	100	101±9	103±8
2	NH	H	H	CH_3	15	1	80	100±9	
3	O	H	H	CH_3	Ø	3	32	100±9	
4	S	H	H	CH_3	16	0.2	80	103±7	
5	CH_2	H	Cl	CH_3	not available		not available		
6	NH	H	Cl	CH_3	Ø	0.2	20	92±7[c]	106±5
Loxapine	O	H	Cl	CH_3	16	3	2	82±5[d]	99±4
Clothiapine	S	H	Cl	CH_3	5	2	2.5	87±5[b]	94±5
9	CH_2	Cl	H	CH_3	25	0.3	20	97±7	103±3
							80	102±13	
Clozapine	NH	Cl	H	CH_3	9	0.3	10	104±7	114±5[d]
							80	113±9[c]	119±7[d]
11	O	Cl	H	CH_3	20	1	80	100±9	91±11
12	S	Cl	H	CH_3	38	0.07	20	100±5	116±6[c]
							80	116±11[b]	
13	NH	Cl	H	$(CH_2)_2OH$	>100	9	80	113±5[c]	
14	NH	Cl	H	$(CH_2)_3OH$	100	20	80	113±6[c]	
15	NH	Cl	Cl	CH_3	13	0.3	80	103±7	
16	NH	Cl	F	CH_3	24	0.5	80	103±7	
Haloperidol					Ø	36	0.32	91±5[b]	98±8
							1	71±11[c]	
Chlorpromazine					17	2.6	32	96±7	100±5

[a] For method see [27]. The figures are lower than those in table 7 [method 26] due to slightly different evaluation criteria.

[b] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$ compared with the controls.

Ø = inactive

Chlorine substitution in position 2 (5, 6, loxapine and clothiapine) results in the appearance of a classical neuroleptic profile (induction of catalepsy, apomorphine-antagonism, increase in striatal DOPAC-content, decrease in striatal DA-content after a single dose with development of tolerance on repeated administration). It will be noted that the anticholinergic properties of clothiapine (almost twice as great as clozapine in the oxotremorine test) do not prevent the appearance of this profile.

Compounds substituted with chlorine in position 8 (9, clozapine, 11, 12) again show sedative properties, but are characterized by their lack of effect in tests for classical neuroleptic activity. Of interest is the fact that compound 12, like clozapine, increases striatal DA-content after a single high dose or after repeated lower doses. All four compounds show anticholinergic properties.

Replacement of the methyl group in the piperazinyl side-chain of clozapine by hydroxyethyl or hydroxypropyl (13, 14) reduced the sedative and anticholinergic effects, but not the ability to increase striatal DA-content.

The activities of the dihalogenated compounds 15 and 16 are a mixture of those of the mono-substituted compounds 6 and clozapine. Both dihalogenated derivatives exert an effect on extrapyramidal brain centres, and fail to increase striatal DA-content, thus resembling compound 6. However, like clozapine, both substances antagonize oxotremorine and inhibit the arousal reaction.

ANIMAL PHARMACOLOGY AND TOXICOLOGY

General Activity and Sedative Effects

In the mouse, rat and cat even small oral doses of clozapine produce a reduction in spontaneous activity, with ptosis and muscular relaxation. With increasing doses reactions to acoustic and tactile stimuli decline and disturbances of equilibrium occur. There is no indication of catatonia (catalepsy, rigidity or negativism) in any of the species examined.

The inhibition of locomotor activity induced by clozapine lessens on repeated treatment. In mice, locomotor activity is increased from day 5, reaching a peak on day 12, and then returning to normal. In rats, no increase is observed, and pre-treatment values are reached after 11 days' treatment. On repeated administration of classical neuroleptics, locomotor activity remains depressed [17]. Clozapine(CLOZ), like chlorpromazine(CPZ) and haloperidol(HAL), fails to inhibit the tonic extensor seizure induced by electroshock.

Table 3 – Sedative Effects of Clozapine and Reference Compounds

Test model	CLOZ mg/kg	CPZ mg/kg	HAL mg/kg
Locomotor activity: mouse [16][a] ED 50: po	2.5	4.0	0.3
Locomotor activity: rat [17] ED 50: po	13.0	12.0	1.4
Electrically-induced arousal reaction: rabbit [18,19] ED 150: iv	1.5	5.8	inactive

[a]Reference to methods. Original methods have often been modified.

Effects on Dopamine (DA) Systems

Clozapine fails to induce catalepsy, or to protect against apomorphine- or amphetamine-induced stereotypies. Although theoretically the muscle-relaxing properties of clozapine could mask a weak cataleptic effect, catalepsy was not observed during a 3-week treatment period, during which time tolerance to the muscle-relaxing action develops. Clozapine also fails to induce DA-receptor hypersensitivity, as measured by the circling response to apomorphine in rats with unilateral striatal lesions. However, a single oral dose (2.5–20 mg/kg) reduces the enhanced apomorphine-response in rats treated for 6 days with haloperidol (3 mg/kg po) to the control level [22].

Table 4 – Effects on Striatal DA–Systems

Test model	CLOZ mg/kg po	CPZ mg/kg po	HAL mg/kg po
Induction of catalepsy: rat [20] ED 50	50 Ø	3.8	0.3
Apomorphine gnawing: rat [21] ED 50	40 Ø	13.5	0.24
Amphetamine stereotypies: rat [3] ED 50	40 Ø	8.7	0.21

Ø = inactive

In common with classical neuroleptics clozapine potentiates evoked caudate spindles in the rat [23] and the cat [90], indicating an influence on striatal function. However, this property is not limited to antipsychotic compounds, but is seen with benzodiazepines, beta-adrenoceptor blockers, etc. [unpublished findings, 90].

Clozapine increases striatal DA-content after single high doses or after repeated low doses. An increase in HVA- and DOPAC-content is attained only after high doses of clozapine, with little tolerance to this effect developing after repeated administration. In contrast, classical neuroleptics increase DA-turnover and HVA- and DOPAC-content, and gene-