ADVANCES IN PHARMACOLOGY AND THERAPEUTICS

Proceedings of the 7th International Congress of Pharmacology

General Editors: J. R. BOISSIER, P. LECHAT & J. FICHELLE

Volume 10

CHEMOTHERAPY

Editor: M. ADOLPHE

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of Pharmacology, Paris 1978

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Paris

PERGAMON PRESS

OXFORD · NEW YORK · TORONTO · SYDNEY · PARIS · FRANKFURT

U.K.	Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England
U.S.A.	Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.
CANADA	Pergamon of Canada, Suite 104, 150 Consumers Road, Willowdale, Ontario M2 J1P9, Canada
AUSTRALIA	Pergamon Press (Aust.) Pty. Ltd., P.O. Box 544, Potts Point, N.S.W. 2011, Australia
FRANCE	Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France
FEDERAL REPUBLIC OF GERMANY	Pergamon Press GmbH, 6242 Kronberg-Taunus, Pferdstrasse 1, Federal Republic of Germany

First edition 1979

British Library Cataloguing in Publication Data

International Congress of Pharmacology, *7th, Paris, 1978*
Advances in pharmacology and therapeutics.
Vol. 10 : Chemotherapy
1. Pharmacology 2. Therapeutics
I. Title II. Boissier, J R III. Lechat, P
IV. Fichelle, J. V. Adolphe, M VI. Chemotherapy
615 RM101 78-41032

ISBN 0-08-023200-0

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Printed in Great Britain by A. Wheaton & Co. Ltd., Exeter

Introduction

The scientific contributions at the 7th International Congress of Pharmacology were of considerable merit. Apart from the sessions organised in advance, more than 2,200 papers were presented, either verbally or in the form of posters, and the abundance of the latter in the congress hall is a good indication that this particular medium of communication is becoming increasingly attractive to research workers, and offers scope for discussions which combine an elaborate, thorough approach with a certain informality.

It would have been preferable to have published the entire congress proceedings within the framework of the reports. That was, however, physically impossible, and the organisers had to adopt a realistic solution by publishing only the main lectures, symposia and methodological seminars. The amount of material presented necessitated the printing of ten volumes, each volume containing congress topics regrouped according to their relevant content and subject areas. This system of division may give rise to criticism on account of its artificiality, and we readily admit that certain texts could have been placed in more than one volume. We are asking the reader to excuse this arbitrariness, which is due to the editors' personal points of view.

I draw attention to the fact that most of the symposia finish with a commentary which the chairmen had the option of including, presenting their personal opinions on one or several points. We think that such an addition will facilitate reflection, discussion, indeed even controversy.

The launching of the scientific programme for this congress began in September 1975 on returning from the last meeting in Helsinki. Long and delicate discussions took place in the Scientific Programme Committee and with the International Advisory Board. Should it be a pioneer, 'avant-garde' congress? Or one laid out like a balance-sheet? Should we restrict the congress to the traditional bounds of pharmacology, or extend the range of papers to cover the finest discipline? The choice was difficult, and the result has been a blend of the two, which each participant will have appreciated in terms of his training, his tastes, and his own research.

A certain number of options, however, were taken deliberately: wide scope was given to toxicology, from different points of view, and to clinical pharmacology, a subject much discussed yet so badly practised; the founding of two symposia devoted

to chemotherapy of parasitic diseases which are still plagues and scourges in certain parts of the world; a modest but firm overture in the field of immunopharmacology, which up until now was something of a poor relation reserved only for clinical physicians; the extension of methodological seminars, in view of the fact that new techniques are indispensable to the development of a discipline.

We have been aware since the beginning that, out of over 4,000 participants who made the journey to Paris, not one could assimilate such a huge body of knowledge. Our wish is that the reading of these reports will allow all of them to become aware of the fantastic evolution of pharmacology in the course of these latter years. If one considers pharmacology as the study of the interactions between a "substance" and a living organism, then there is no other interpretation. Nevertheless, one must admit that there exists a period for describing and analysing a pharmacological effect, and that it is only afterwards that the working mechanism can be specified; a mechanism which will permit these "substances" to be used for the dismantling and breaking down of physiological mechanisms, a process which justifies Claude BERNARD'S term, "chemical scalpel".

The reader will be able to profit equally from more down-to-earth contributions, more applied to therapeutics, and less "noble", perhaps, for the research worker. He will realise then that his work, his research and his creative genius are first and foremost in the service of Man, and will remember this statement from Louis PASTEUR:

> "Let us not share the opinion of these narrow minds who scorn everything in science which does not have an immediate application, but let us not neglect the practical consequences of discovery."

I would like to renew my thanks to my colleagues in the Scientific Programme Committee and also to the members of the International Advisory Board, whose advice has been invaluable. I owe a particular thought to J J BURNS, now the past-president of IUPHAR, who granted me a support which is never discussed, and a staunch, sincere friendship. The Chairmen have effected an admirable achievement in the organisation of their proceedings, and in making a difficult choice from the most qualified speakers. The latter equally deserve our gratitude for having presented papers of such high quality, and for having submitted their manuscripts in good time.

The publisher, Robert MAXWELL, has, as always, put his kindness and efficiency at our service in order to carry out the publication of these reports. But none of it would have been possible without the work and competence of Miss IVIMY, whom I would like to thank personally.

My thanks again to the editors of the volumes who, in the middle of the holiday period, did not hesitate to work on the manuscripts in order to keep to the completion date.

Finally, a big thank you to all my collaborators, research workers, technicians and secretaries who have put their whole hearts into the service of pharmacology. They have contributed to the realisation of our hopes for this 7th International Congress, the great festival of Pharmacology. Make an appointment for the next one, in 1981, in Tokyo.

Jacques R BOISSIER

Chairman

Scientific Programme Committee

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Contents

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Chemotherapy of Schistosomiasis

Recent Advances in the Experimental Chemotherapy of Schistosomiasis[1,3]

L.M. Werbel, E.F. Elslager and D.F. Worth

Warner-Lambert/Parke-Davis Pharmaceutical Research Division
Ann Arbor, Michigan, U.S.A. 48106

The widespread affliction of millions throughout the world with this disease, caused by a small flat worm that utilizes snails as intermediate hosts and man as a final host, should be ample demonstration of the need for expanding efforts in this public health area.

Although the first chemotherapeutic attack on schistosomiasis with antimonials took place in 1917, major advances in drug therapy have been achieved only in the last ten years or so. The difficulties inherent in combatting such an enemy are complex. We must contend with 3 major species (Schistosoma mansoni, S. japonicum, and S. hematobium) of parasite with varying drug susceptibility: with major differences in susceptibility among the multiple strains within these species; with variations of drug effects depending upon the age of the parasite and the drug regimen; with variations in host susceptibility whether for metabolic differences or other unknown reasons; and with a current regulatory climate which decries any hint of toxicity such as mutagenic or tumorigenic effects.

Any new agent of course must have potential advantages over existing types. Key attributes of an ideal drug include: 1) absence of side effects and acute toxicity in man; since many infected people are symptom-free, they will not accept drugs with gastrointestinal or central nervous system (CNS) effects that make them ill, 2) high activity against the three main human schistosomes, 3) efficient when given in a short course of treatment - preferably in a single dose, 4) orally effective, 5) cheap, 6) active against all stages of the parasite in the mammalian host, and 7) physical and chemical stability in oral and parenteral product formulations.

Until very recently, the available alternatives for chemotherapy were the antimonials (severely limited by their toxicity and inconvenient mode of administration), niridazole (1) (limited by CNS toxicity and lack of efficacy against S. mansoni in children and S. japonicum), and hycanthone (2) (which has shown mutagenic liability, limited oral efficacy and lack of activity against S. japonicum).

More recently have appeared oxamniquine (3) - which may be the best currently

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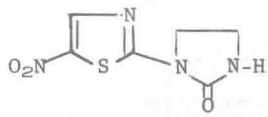
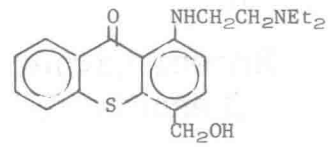
[1] Partial support of this work by the Edna McConnell Clark Foundation is gratefully acknowledged.

available agent against S. mansoni, although apparently not equally effective against all geographical strains: the Ciba-Geigy isothiocyanate (4); metrifonate (5) - which may be the current drug of choice against S. hematobium; and most recently praziquantel (6) (from Bayer-Merck) and the novel pyrazine (7) from Rhone-Poulenc. Whether any of these will offer that combination of properties to make it the ideal schistosomicide remains to be seen.



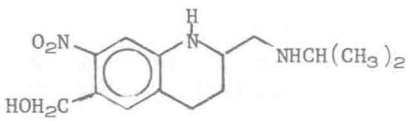
Niridazole

1



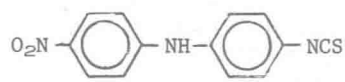
Hycanthone

2



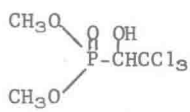
Oxamniquine

3



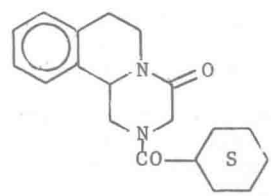
Ciba-Geigy

4



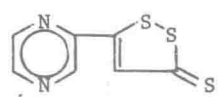
Metrifonate

5



Praziquantel

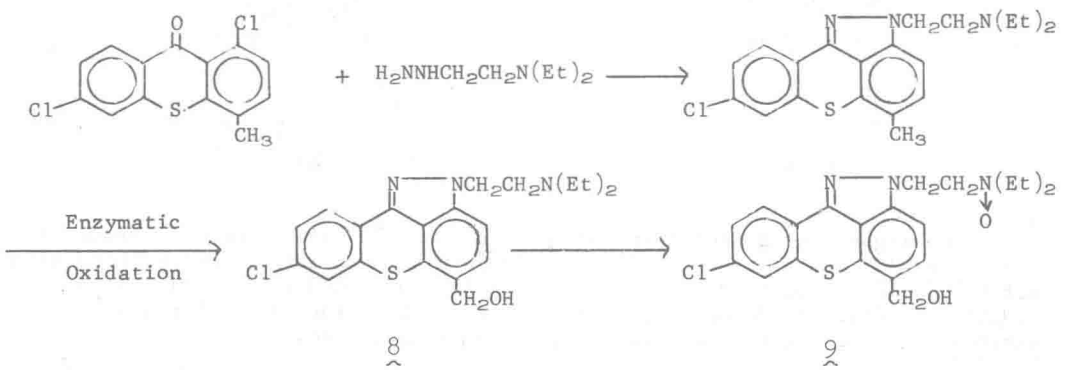
6



Rhone-Poulenc

7

The efforts which I will review here were initiated in our laboratory some time ago, based essentially on the desire to improve upon hycanthone. A unique class of compounds emerged - the benzothiopyrano[4,3,2-cd]indazoles. The synthetic scheme (Fig. 1) involves a biological oxidation of an aromatic methyl group - a process also utilized in the production of hycanthone and oxamniquine. Although an extensive amount of work was done with the indazoles themselves, I will concentrate in this communication primarily on the more exciting results achieved with the N-oxide 9.



8 9

Fig. 1 Synthesis of benzothiopyrano[4,3,2-cd]indazoles

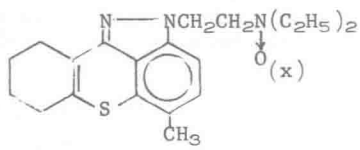
Early data obtained with the benzothiopyrano[4,3,2-cd]indazoles were impressive. Against a Puerto Rican strain of S. mansoni in mice the des N-oxide 8 for example, upon gavage dosing for 5 days at 100 mg/kg/day, resulted in a 74% reduction of live worm burden compared to an 81% reduction with hycanthone at the same dose and regimen. This level of potency achieved after such a striking modification of the hycanthone structure stimulated rapid follow-up of this lead. The hydroxymethyl compound 8 was substantially more effective than either the analogous methyl or corresponding aldehyde.

Initial data with the N-oxide 9 were equally impressive. This compound administered in a single oral dose to mice infected with a Puerto Rican strain of S. mansoni was completely curative at 60 mg/kg and provided a 96% reduction of live worm burden at 40 mg/kg. In this test system this level of activity was significantly superior to those obtained with hycanthone, oxamniquine and praziquantel (which provided a % reduction of live worm burden at a mg/kg dose of 66%-80 mg/kg, 43%-100 mg/kg, and 74%-100 mg/kg respectively).

Compound 9, or IA-4-N-oxide as it became known, was also shown to be effective against S. mansoni (Puerto Rican) infections in a single I.M. dose both in the hamster (100% cures at 80 mg/kg) and mice (88% reduction at 212 mg/kg).

More recently, the examination of some even more far-reaching structural modifications of this system have been undertaken. The 7,8,9,10-tetrahydro analogs of 8 and 9, i.e. 10a,b and 11a,b, were prepared by a scheme similar to that depicted in Fig. 1. While 10a administered in a single oral dose to S. mansoni infected mice resulted only in a moderate reduction of live worm burden (62%) even at a very high dose (500 mg/kg), the corresponding hydroxymethyl analog 11a provided a substantially stronger, though variable, effect with a maximum of 60% cures at 75 mg/kg. The N-oxide of the methyl compound 10b did afford a

substantial increase in activity (99% reduction at 150 mg/kg) but surprisingly the corresponding hydroxymethyl analog 11b did not, and in neither case was the level of activity superior to that of IA-4-N-oxide (9).

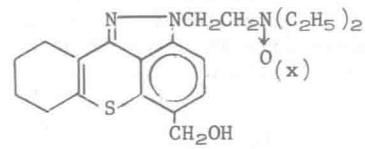


10

X

a) 0

b) 1



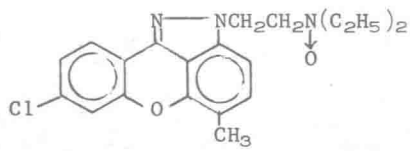
11

X

a) 0

b) 1

The oxygen analog (benzopyrano[4,3,2-cd]indazole) of the methyl precursor to 8, i.e. 12, was also prepared. Since it exhibited a lower order of biological activity, i.e. a 40% reduction in live worm burden upon administration in a single oral dose of 250 mg/kg to mice infected with a Puerto Rican strain of S. mansoni, further efforts in this series were deemphasized.



12

Finally it was of interest to remove one ring completely from the benzothiopyranoindazoles. The synthetic route to the resulting thiopyrano[4,3,2-cd]-indazoles is shown in Fig. 2. Representative biological data for these compounds is shown in TABLE 1. Clearly there is some activity in this series, but these analogs suffer by comparison with the IA-4 types, i.e. 9.

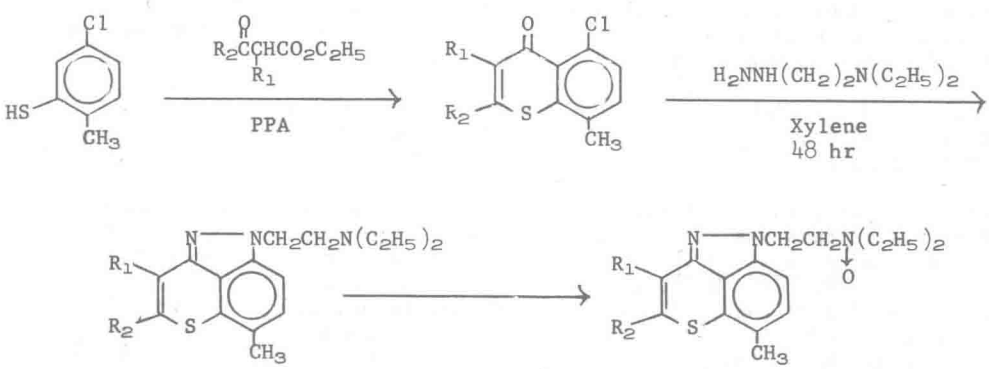
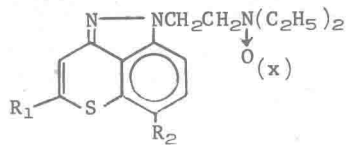


Fig. 2

TABLE 1 Oral Effects of Thiopyrano[4,3,2-cd]Indazoles Vs. Mature S. Mansoni (P.R./Mich.) In Mice



R_1	R_2	x	Single Oral Dose mg/kg	% Reduction Live Worms	% Cure
CH_3	CH_3	0	100	69	0
C_6H_5	CH_3	0	500	19	0
CH_3	CHO	0	250	80	14
CH_3	CH_2OH	0	500	0	0
CH_3	CH_3	1	150	30	0
CH_3	CH_3 (7,8-dihydro)	1	250	29	0
CH_3	CH_2OH	1	75	75	0

Thus, structure modifications accomplished to date have been unsuccessful in improving upon the activity of the benzothiopyrano[4,3,2-cd]indazole-N-oxides. Further evaluation of one representative of this class, i.e. IA-4-N-oxide (9), continues to show promise. This agent (TABLE 2) shows broad activity against several strains of S. mansoni, and in limited direct comparison with oxamniquine compares quite favorably. Studies thus far in primates have also been quite encouraging. Thus, in the rhesus monkey infected with S. mansoni (Walter Reed/ Puerto Rican) cures were obtained upon either oral or intramuscular administration of one to two doses of 100 mg/kg. A similar infection in the Cebus monkey appeared to be somewhat less susceptible although a reduction of live worm burden of greater than 90% was achieved upon administration of a single oral dose of 50 mg/kg.

Moreover, comparative mutagenic studies of IA-4-N-oxide, hycanthone and oxamniquine[2] indicated only very low (and essentially equivalent) levels of mutagenicity for IA-4-N-oxide and oxamniquine significantly below those of hycanthone.

Thus, of the variations explored within this structural class, IA-4-N-oxide (9) clearly remains unchallenged. This compound compares favorably in a variety of laboratory models with clinically used schistosomicidal drugs. Final evaluation as to its ultimate utility must be obtained in man. We are hopeful of having such studies initiated in the near future.

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[2] R. P. Batzinger and E. Bueding, J. Pharm. Exp. Therap., 200, 1 (1977).

TABLE 2 Comparative Antischistosomal Effects of IA-4-N-oxide and Oxamniquine in a Single Oral Dose to Mice Infected With Varying Strains of S. Mansoni

Strain	Compound	Single Oral Dose mg/kg	% Reduction Live Worms	% Cures
P.R./Mich.	IA-4-N → O	80	100	100
		60	100	100
		40	96	82
	Oxamniquine	100	43	20
		50	71	40
Brazilian	IA-4-N → O	40	100	100
		20	99	90
	Oxamniquine	50	90	25
		30	90	75
Liberian	IA-4-N → O	60	100	100
	Oxamniquine	100	65	0
P.R./S.W.	IA-4-N → O	80	100	100
		40	100	100
St. Lucia	IA-4-N → O	80	100	100
		40	93	---

[3] Biological data contained herein has been provided by Dr. Paul E. Thompson (Warner-Lambert/Parke Davis), Dr. Ernest Bueding (Johns Hopkins University), and Dr. John Bruce (University of Lowell).

Antischistosomal Activity of a Nitrodiphenylaminoisothiocyanate (C 9333-Go/CGP 4540) against *Schistosoma Japonicum* (Philippine Strain) in Mice and Rabbits

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SUMMARY

A new schistosomicidal compound, 4-isothiocyanato-4'-nitrodiphenylamine (C9333-Go/CGP 4540), was lethal to Schistosoma japonicum in vitro at a concentration of 0.015 µg/ml for 120 hrs. In mice experimentally infected with a Philippine strain of S. japonicum, the drug administered as a single oral dose at the level of 20mg/kg, produced a complete parasitological cure. More than a 95% reduction in adult worms was obtained when treated with a single oral dose as low as 5mg/kg. Mice treated with a curative dose of the drug tended to gain weight after treatment, while untreated control mice lost weight. Although no schistosomicidal effect was seen against 1 week juvenile schistosomula, there was no apparent difference in the susceptibility of the parasites to the drug when the age of infection varied between 3 and 5 weeks. The efficacy of the drug was likely to be slightly higher with a diluted form (0.8mg of the active ingredient per ml of vehicle) than with a concentrated form (40mg of the active ingredient per ml of vehicle).

In rabbits, the drug caused a worm reduction of 93.2-100% when treated with a singlw oral dose of 20mg/kg. No indication of a higher susceptibility of female worms than of males was observed in S. japonicum infected rabbits.

INTRODUCTION

Although a number of drugs are available for the treatment of schistosomiasis, most, if not all, clinically used antischistosomal drugs are not satisfactory for large scale chemotherapy on account of toxicity or prolonged course of treatment. A recent critical review of the status of chemotherapy of schistosomiasis and of related research strategy (Hoffman, 1975) indicates that a drug suitable for the improved treatment of S. japonicum and for use in its mass chemotherapy needs to be developed to replace the antimonials. The results in recent years of treating experimental animals infected with S. haematobium, S. mansoni and S. japonicum (Striebel, 1976; Bueding et al., 1976) indicate that 4-isothiocyanato-4'-nitrodiphenylamine (C9333-Go/CGPz4540) (subsequently referred to as CGP 4540) has high antischistosomal activity when administered as a single oral dose. These findings stimulated the authors to test this compound in mice and rabbits infected with S. japonicum.

MATERIALS AND METHODS