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Short communication

Synthesis of diphenyl bisamidines as potential amoebicides*

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Summary — Various substituted diphenyl bisamidines were prepared and tested for antiamoebic activity in Golden hamsters and Wistar rats. Compounds **10** showed in vitro activity at 100 μg/mL and displayed excellent in vivo activity against hepatic infection in the hamster. Its in vivo activity against intestinal amoebiasis in the experimental rat was superior to metronidazole and was comparable to diloxanide furoate. The less pronounced in vitro activity, and comparable in vivo activity of compound **10** with respect to the standard drugs, may be due to some of its metabolites.

antiamoebic activity / diphenyl bisamidine / Liroldine / *Entamoeba histolytica*

Introduction

Amoebiasis is one of the major health problems of developing and underdeveloped countries [1]. Though the 5-nitroimidazoles [2] play a pivotal role in management of the disease, they are not free from side effects. They can cause nausea, headache, a dry mouth and a bitter taste. Moreover, they can induce carcinogenesis in rodents and mutagenic effects in bacteria [3]. Hence the need for a lead compound with activity against hepatic and intestinal amoebiasis becomes essential. In connection with our programme to find novel amoebicides [4–7], we were interested in synthesizing the bisamidines of diphenyls, a lead derived from the polycyclic bisamidine derivatives [8].

Chemistry

The substituted 2-pyrrolidones were prepared as shown in scheme 1. Michael addition of the carbanion, generated from nitro alkanes with acrylate in the presence of Triton B, gave the adducts which underwent catalytic reduction in the presence of Raney nickel in ethanol followed by heating at 50 °C, to yield the substituted pyrrolidones **A–D** [9–11]. Reaction of the substituted benzidines with pyrrolidines **A–D** in the presence of $POCl_3$ gave the corresponding bisamidines **1–26**. The physical data of all the compounds described herein are given in table I.

Results and discussion

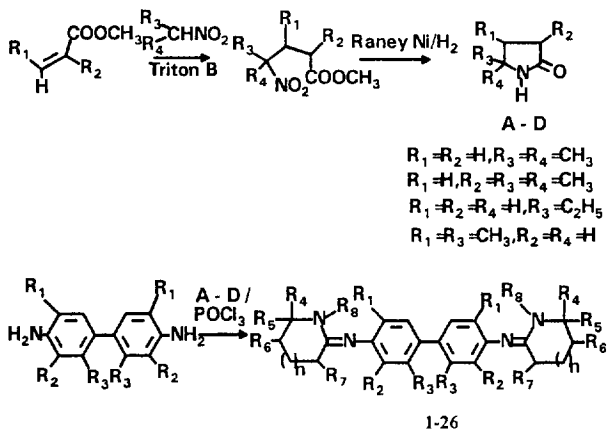
In vitro study

The compounds **1–26** were initially tested for their in vitro activity against *Entamoeba histolytica* using a polyxenic culture (table II). Seventeen compounds displayed in vitro activity in the range of 75–200 μg/mL. Standard antiamoebic compounds such as metronidazole and diloxanide furoate showed in vitro activities in the ranges of 5 and 2.5 μg/mL respectively in the above test.

Animal study

All the 17 compounds with in vitro activity were tested against extraintestinal and intestinal models using Golden hamsters and Wistar rats [12] respectively. All the compounds were tested at 100 mg/kg × 4 for extraintestinal (hepatic) amoebiasis and at 200 mg/kg × 4 for intestinal (caecal) amoebiasis. If the compounds were found to be active, the tests were carried out at lower dosages and their ED_{50} values are

*Dedicated to the memory of the late Dr BS Bajwa



Scheme 1.

listed in table II. Out of the 17 compounds tested, only six showed antiamoebic activity against hepatic and intestinal amoebiasis in animal studies. Compounds **10**, **12** and **20** displayed excellent in vivo activity compared to the standard compounds. The ED_{50} value of compound **10** was comparable with that of metronidazole with respect to both hepatic and caecal amoebiasis.

In conclusion, the in vitro activity of these derivatives against *E histolytica* was comparatively less pronounced than that of metronidazole and diloxanide furoate (table II). However a number of derivatives displayed in vivo activity, of which compound **10** was the best. As compared with standard drugs like metronidazole and diloxanide furoate in both the models (hamster for hepatic infection and rat for intestinal infection), compound **10** showed the best activity profile. It is possible that some of the metabolites of compound **10** are more active than the compound itself, however this aspect has not been included in the present study. Details of the biological activity are being published elsewhere. A generic name, Liroldine, has been registered for this compound.

Experimental protocols

Chemistry

Melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 157 Spectrophotometer. Chemical shifts (δ) are in parts per million relative to tetramethylsilane. Coupling constants (J values) are in hertz (Hz). ^{1}H-NMR spectra were run on a Varian T-60 Spectrometer. Analyses were performed on a Hereus microelemental analyser.

3,5,5-Trimethyl, 4,5-dimethyl, 5,5-dimethyl, 5-ethyl and (4-phenyl pyrrolidin-2-one were prepared as reported in the literature [9–11].

General procedure for the preparation of diphenyl bisamidines 3,3'-Dichloro-4,4'-di-(5-methylpyrro-2-ylidene amino)diphenyl ***21.*** Phosphorous oxychloride (0.75 g, 0.46 mL, 5 mmol) was added dropwise to 0.5 g (5 mmol) of 5-methyl-2-pyrrolidone at 0 °C. The reaction mixture was stirred for an additional 1 h at 0 °C. Dichlorobenzidine (0.58 g, 2.3 mmol) was added and the reaction mixture was heated at 100 °C for 16 h. The reaction mixture was poured into water and neutralized with ammonia. The white solid thus precipitated was filtered, washed with water, dried and recrystallized from chloroform/pet ether to give 0.5 g (52%) of 3,3'-dichloro-4,4'-di(5-methylpyrro-2-ylidine amino)diphenyl **21** as a white solid, mp 220–222 °C; ^{1}H-NMR ($CDCl_3$) δ 1.2 (d, J = 6 Hz, 6H, CH_3), 2.85 (m, 4H, CH_2), 2.5 (t, J = 6 Hz, 4H, CH_2), 3.8 (m, 2H, CH), 4.9 (bs, 2H, NH), 7.0 (d, J = 8 Hz, 2H, ArH), 7.15 (d, J = 8 Hz, 2H, ArH), 7.35 (dd, J = 8 Hz, 2H, ArH). Anal calc for $C_{22}H_{24}C_{12}N_4$; C, 63.60; H, 5.82; Cl, 17.09; N 13.49; found C, 63.35; H, 5.69; Cl, 17.31; N, 13.29%.

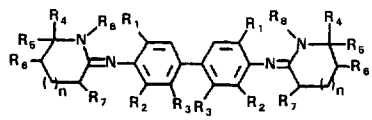
Biological evaluation

Materials and methods

The minimum inhibitory concentration (MIC) was determined using a polyxenic culture of *E histolytica* BY 80 strain in Jones medium [13]. Compounds were dissolved in DMSO (0.2%) and serial dilutions were made. An appropriate concentration of DMSO alone (0.2%) was taken during the experiments as DMSO control. The highest concentration of the test compounds was 200 μg/mL and the lowest was 25 μg/mL, prepared by serial dilution. If the compounds showed activity at 25 μg/mL, further dilutions were made and tested. The MIC was determined by microscopic examination of ten fields for motile *E histolytica* from the sediments of the culture tube after thorough mixing with 0.5 mL of fresh medium. Two sets of controls were maintained, one without any test material and another with metronidazole or diloxanide furoate as the standard antiamoebic compound.

For in vivo studies, at least eight animals were used per dose and two experiments were performed. For in vivo drug administration, compounds were prepared as a suspension with 0.5% aqueous carboxy methyl cellulose (tylose) and drenched orally by a stomach tube to the animals. For the hepatic infection study, the compounds were administered 2 h before infection, followed by a second dose 2 h after infection and then one dose on each of the two consecutive days. Autopsy was performed on the sixth day post infection and smears from the liver tissues of the treated hamsters were examined. A compound was considered inactive when motile trophozites of *E histolytica* were detected by such examination. The intestinal amoebiasis was studied in weanling Wistar rats. The rats were inoculated with 40 000 trophozites in the caecum through the ileocaecal junction after brief ether anaesthesia. The first dose of the test compounds was administered 24 h post infection followed by three more doses, one each day for three consecutive days. Autopsy was performed on the sixth day post infection and the caecum was examined for amoebae, first microscopically and, if negative, then by culture of the affected tissues. In control groups, metronidazole-treated animals were considered as active drug controls for hepatic study. Similarly, diloxanide furoate-treated animals were kept as active controls for intesti

Table I. Diphenyl bisamidines.



Comp no	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	*n*	*Mp (°C)*	*Solv*[a]	*Yield (%)*
1	Cl	H	H	H	H	H	H	H	0	237–39	A	30
2	Cl	H	H	H	H	H	H	H	1	238–41	A	48
3	OCH_3	H	H	H	H	H	H	H	0	223–26	B	16
4	OCH_3	H	H	H	H	H	H	H	1	208–10	B	25
5	CH_3	H	H	H	H	H	H	H	0	231–34	B	25
6	CH_3	H	H	H	H	H	H	H	1	225–28	B	13
7	Br	H	H	H	H	H	H	H	0	245–47	A	36
8	Br	H	H	H	H	H	H	H	1	244–46	A	40
9	Br	H	H	H	H	H	H	H	2	252–54	B	20
10	F	H	H	H	H	H	H	H	0	272–75	A	30
11	F	H	H	H	H	H	H	H	1	268–71	A	11
12	F	H	H	CH_3	H	H	H	H	0	251–53	B	15
13	F	H	H	Ph	H	H	H	H	0	230–32	B	11
14	F	H	H	CH_3	CH_3	H	H	H	0	> 300	B	9
15	F	H	H	CH_3	CH_3	H	CH_3	H	0	222–24	B	36
16	F	H	H	H	H	H	H	CH_3	0	168–70	B	38
17	F	H	H	C_2H_5	H	H	H	H	0	238–40	B	10
18	F	H	H	CH_3	H	CH_3	H	H	0	224–26	B	37
19	Cl	H	H	C_3H_5	H	H	H	H	0	218–20	B	16
20	NO_2	H	H	H	H	H	H	H	0	222–24	A	30
21	Cl	H	H	CH_3	H	H	H	H	0	220–22	B	52
22	H	H	F	H	H	H	H	H	0	215–18	A	14
23	Cl	Cl	H	H	H	H	H	H	0	275–77	B	18
24	Cl	Cl	H	H	H	H	H	H	1	272–75	B	31
25	CH_3	CH_3	H	H	H	H	H	H	0	258–60	B	29
26	CH_3	CH_3	H	H	H	H	H	H	1	242–44	B	25

[a]Solvent-A: $CHCl_3/CH_3OH$/pet ether (60–80 °C); B: $CHCl_3$/pet ether (60–80 °C).

nal amoebiasis. Every experiment had infected but untreated animals as controls for comparison. Details of the methodologies for the in vitro and in vivo tests have been published elsewhere [12].

Acknowledgment

Our thanks are due to Dr Inamdar for the spectral and analytical data.

Table II. Antiamoebic activity of diphenyl bisamidines.

Compound no	*Molecular formula*[a]	*In vitro MIC (μg/mL)*	*In vivo*	
			Extra intestinal (hepatic) (mg/kg × 4 per os)	*Intestinal (caecal) (mg/kg × 4 per os)*
1	$C_{20}H_{20}N_4Cl_2 \cdot 1/2H_2O$	75	82[c]	200[d]
2	$C_{22}H_{24}N_4Cl_2 \cdot 1/2H_2O$	200	100[c]	100[d]
3	$C_{22}H_{26}N_4Cl_2 \cdot 1/2H_2O$	200	150[b]	NT
4	$C_{24}H_{30}N_4O_2 \cdot 1/2H_2O$	200	150[b]	NT
5	$C_{22}H_{26}N_4 \cdot 1/2H_2O$	200[b]	NT	NT
6	$C_{24}H_{30}N_4 \cdot 1/2H_2O$	200	150[b]	NT
7	$C_{20}H_{20}Br_2N_4$	100	150[b]	300
8	$C_{22}H_{24}Br_2N_4$	200	150[b]	300[b]
9	$C_{24}H_{28}Br_2N_4$	200	150[b]	NT
10	$C_{20}H_{20}F_2N_2$	100	28[c] (25.4–30.2)	125[c] (107–145)
11	$C_{22}H_{24}F_2N_4 \cdot 1/2H_2O$	200[b]	NT	NT
12	$C_{22}H_{24}F_2N_4$	200	31[c]	150[c]
13	$C_{32}H_{28}F_2N_4$	200[b]	NT	NT
14	$C_{24}H_{28}F_2N_4$	200[b]	NT	NT
15	$C_{26}H_{32}F_2N_4 \cdot 1/2H_2O$	100	150[b]	NT
16	$C_{22}H_{24}F_2N_4$	200[b]	NT	NT
17	$C_{24}H_{28}F_2N_4$	100	39[c]	200[d]
18	$C_{24}H_{28}F_2N_4 \cdot 1/2H_2O$	200[b]	NT	NT
19	$C_{24}H_{28}Cl_2N_4$	200[b]	NT	NT
20	$C_{20}H_{20}N_6O_4$	100	37[c]	150[d]
21	$C_{22}H_{24}Cl_2N_4$	100	100[b]	NT
22	$C_{20}H_{20}F_2N_4$	200	100[b]	NT
23	$C_{20}H_{18}Cl_4N_4 \cdot 1/2H_2O$	200	100[b]	NT
24	$C_{22}H_{22}Cl_4N_4 \cdot 1/2H_2O$	200[b]	NT	NT
25	$C_{24}H_{30}N_4 \cdot 1/2H_2O$	200[b]	NT	NT
26	$C_{26}H_{34}N_4$	200	100[b]	NT
	Metronidazole	5	26[c] (22.0–30.8)	230[c] (199–267)
	Diloxanide furoate	2.5	400[b]	81[c]

[a]All the compounds were satisfactorily characterized by spectroscopic methods and by elemental analysis.
[b]Highest dose tested and was inactive;[c]approximate ED_{50}; [b]100% effective dose; NT: not tested.

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