

SYNTHESIS OF SOME NEW 1,4-DISUBSTITUTED PIPERAZINE-2,3-DIONE DERIVATIVES OF POTENTIAL ANTHELMINTIC ACTIVITY

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تواتر في التراث العلمي أهمية مشتقات البيرازين وفعاليتها البيولوجية المختلفة لذلك تم في هذا البحث تخليق مجموعة جديدة من مشتقات 1,4-ثنائي مستبدل-بيرازين-2,3-دايون من خلال تفاعل الإيثيلين ثنائي الأمين ومركبات الكربونيل المختلفة في وجود سيانوبوروهيدريد الصوديوم للحصول على المركبات الوسيطة (1a-i). ومن خلال تفاعل المركبات الوسيطة (1a-i) مع ثنائي اثيل الأوكسالات فقد تم تحضير المركبات النهائية (2a-i). وقد تم التحقق من التراكيب البنائية لبعض المركبات الوسيطة وجميع المركبات الجديدة من خلال طرق التحاليل الطيفي المختلفة وكذلك التحاليل الدقيقة للعناصر المكونة لها. الى جانب ذلك فقد تم اجراء تحليل مطياف الكتلة لأحد تلك المركبات للتأكد من الوزن الجزيئي له. هذا وقت ثم اختبار فاعلية المركبات الجديدة (2a-i) كمضادات للديدان بالمقارنة بعقار البيرازين هيدرات كدواء مرجعي وأظهرت تلك الدراسة كفاءة تلك المركبات كمضادات للديدان بالمقارنة بالدواء المرجعي.

The purpose of this study based upon design and synthesis of a new series of 1,4-disubstituted piperazine-2,3-dione derivatives through two steps reaction. This protocol involves the formation of *N,N*-Bis-(4-substituted benzyl)-ethane-1,2-diamine and *N,N*-Bis-[1-(4-substituted phenyl)-ethyl]-ethane-1,2-diamine derivatives (1a-i) through reductive alkylation reaction from ethylenediamine and different carbonyl compounds in the presence of sodium cyanoborohydride. The second step involves reaction of compounds (1a-i) with diethyl oxalate affording the target compounds. Consequently, nine new 1,4-disubstituted piperazine-2,3-dione derivatives were synthesized as the target compounds, 1,4-Bis-(4-substituted benzyl)-piperazine-2,3-dione and 1,4-Bis-[1-(4-substituted phenyl)-ethyl]-piperazine-2,3-dione derivatives (2a-i). The structures of the target compounds were elucidated depending upon the data of the different spectral as well as the elemental methods of analyses. In addition, a mass spectrum, for a representative example, was carried out where the expected fragmentation pattern is in accordance with the structure of the considered compound. The lipophilicity of the target compounds as expressed from the ClogP and the measured R_f remarkably supercede that of piperazine. The preliminary anthelmintic activity of the newly synthesized derivatives (2a-i) was investigated *in vitro* against *Enterobius vermicularis* and *Fasciola hepatica*. The tested compounds exhibited, in all cases, considerable inhibitory effects on the growth of the tested parasites in comparison with piperazine hydrate as a reference drug.

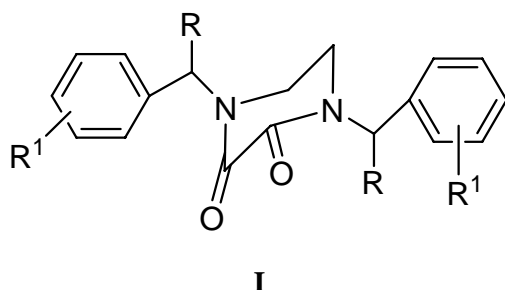
INTRODUCTION

The prevalence of human helminthic infestations is a widespread throughout the globe and represents a major world health problem, particularly in the third world countries.¹ Several classes of chemical are used as anthelmintics and include phenols and derivatives, piperazine and related compounds, antimalarial compounds and natural products.²

On the other hand, a diversity of biological effects is possessed by compounds comprising the piperazine nucleus such as antipsychotic,³ antimalarial,⁴ histamine H1-receptor antagonist,⁵ σ site selective ligands,^{6,7} 5HT3 and 5-HT4 receptor ligands⁸ and δ opioid receptor agonist.⁹ In view of the wide spectrum of useful biological activities of piperazine compounds further exploratory synthesis of

compounds built up on piperazine skeleton was done. On the other hand, it is reported that, the anthelmintic activity of piperazine and related compounds had been suggested to be, based on blockage of the response of the worm muscle to acetylcholine, causing a flaccid paralysis in the worm, which is dislodged from the intestinal wall and expelled in the feces.²

The present study is devoted to the synthesis of some new compounds comprising the piperazine-2,3-dione nucleus, and modulation of the lipophilic properties of the target compounds depending upon the use of different substituents and the degree of branching, structure **I**. Moreover, the biological activity of the target compounds was tested, if any.



EXPERIMENTAL

Melting points were determined on an electrothermal melting point apparatus [Sturat Scientific, UK], and were uncorrected. Precoated silica gel plates (kiesel gel 0.25 mm, 60G F254, Merk) were used for thin layer chromatography. Developing solvent system of chloroform/methanol (5:3) or Hexane/ethyl acetate (3:1) were used and the spots were visualized by ultraviolet light and/or iodine.

The IR spectra (KBr disc or neat) were recorded on IR-470 Shimadzu spectrometer, Japan. The ¹H-NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz) USA. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using CDCl₃ as a solvent. The mass spectra were made on JEOL JMS600 mass spectrometer Japan at Assiut University Central Laboratory, Assiut, Egypt. Elemental analyses

were performed at the Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt. The log P values were computed with a routine method called calculated log P (Clog P) contained in a PC-software package (McLogP 2.0, BioByte Corp., CA, USA). The anthelmintic activity was performed at the Department of Parasitology, Faculty of Medicine, Assiut University, Assiut, Egypt.

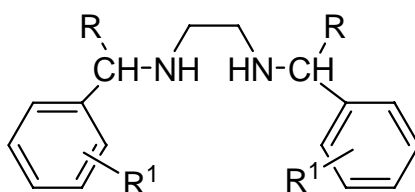
Chemistry

Synthesis of *N,N'*-Bis-(4-substituted benzyl)- and *N,N'*-Bis-[1-(4-substituted phenyl)-ethyl]-ethane-1,2-diamine derivatives (**1a-i**)¹⁰

To a solution of ethylenediamine (30.0 mmole) and the appropriate carbonyl compound (60.0 mmole) in methanol (50 mL) was added sodium cyanoborohydride (48.0 mmole) portionwise at 0°. After stirring for 3 hr at ambient temperature, an additional amount of sodium cyanoborohydride (8.1 mmole) was added and stirring was continued for further **2h**. The reaction mixture was quenched with water, concentrated and extracted with chloroform. The combined organic extract was washed with brine and dried (Na₂SO₄). Concentration and column chromatography (CHCl₃/MeOH) afforded compounds (**1a-i**) as pale yellow oils. Yields and ¹HNMR data are given in Table 1.

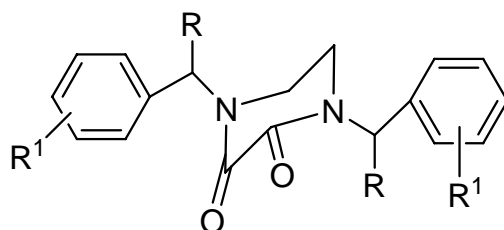
Synthesis of 1,4-Bis-(4-substituted benzyl)- and 1,4-Bis-[1-(4-substituted phenyl)-ethyl]-piperazine-2,3-dione (**2a-i**)

A mixture of *N,N'*-Bis-(4-substituted benzyl)- or *N,N'*-Bis-[1-(4-substituted phenyl)-ethyl]-ethane-1,2-diamine derivatives, compounds **1a-h** (7.5 mmole) and freshly distilled diethyl oxalate (7.5 mmole) in dry ether (10 mL) was stirred at ambient temperature for 1hr. The reaction mixture was left overnight and the separated solid product was obtained by filtration and dried. The crude products were crystallized from a mixture of ethanol and ether affording the target compounds **2a-h**. The physicochemical constants and the spectral data are given in Tables 2 and 3.

Table 1: The yields and the ¹HNMR data of compounds (**1a-i**)*.

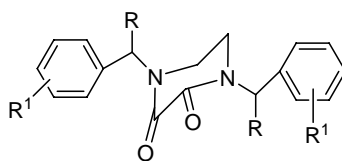
Compd. No.	R	R ¹	Yield %	¹ HNMR data
1a **	H	H	64	1.75 (brs, 2H, 2NH), 2.80 (s, 4H, CH ₂ CH ₂), 3.90 (brs, 4H, 2CH ₂), and 7.20 (s, 10H, 2 Ar-H).
1b	H	<i>p</i> -Br	76	1.80 (brs, 2H, 2NH), 2.67 (s, 4H, CH ₂ CH ₂), 3.64 (brs, 4H, 2CH ₂), and 7.34 (8H, dd, 2 Ar-H).
1c	H	<i>p</i> -Cl	75	1.74 (brs, 2H, 2NH), 2.80 (s, 4H, CH ₂ CH ₂), 3.84 (s, 4H, 2CH ₂), and 7.40 (s, 8H, 2 Ar-H).
1d	H	<i>o</i> -OH	68	1.85 (brs, 2H, 2NH), 2.67 (s, 4H, CH ₂ CH ₂), 3.90 (s, 2H, 2OH), 4.67 (s, 4H, 2CH ₂) and 7.40 (s, 8H, 2 Ar-H).
1e	CH ₃	H	70	1.34 (d, 6H, 2CH ₃), 1.64 (brs, 2H, 2NH), 2.57 (s, 4H, CH ₂ CH ₂), 3.44-4.00 (m, 2H, 2CH), and 7.24 (s, 10H, 2 Ar-H).
1f	CH ₃	<i>p</i> -Br	80	1.30 (d, 6H, 2CH ₃), 1.90 (brs, 2H, 2NH), 2.54 (s, 4H, CH ₂ CH ₂), 3.47-4.00 (m, 2H, 2CH), and 7.40 (dd, 8H, 2 Ar-H).
1g	CH ₃	<i>p</i> -Cl	80	1.27 (d, 6H, 2CH ₃), 1.67 (brs, 2H, 2NH), 2.47 (s, 4H, CH ₂ CH ₂), 3.40-3.87 (m, 2H, 2CH), and 7.24 (s, 8H, 2 Ar-H).
1h	CH ₃	<i>p</i> -CH ₃	75	1.34 (d, 6H, 2CH ₃), 1.70 (brs, 2H, 2NH), 2.34 (s, 6H, 2CH ₃ Ph), 2.60 (s, 4H, 2CH ₂), 3.44-4.00 (m, 2H, 2CH), and 7.30 (s, 8H, 2 Ar-H).
1i	CH ₃	<i>p</i> -OCH ₃	78	1.34 (d, 6H, 2CH ₃), 2.10 (brs, 2H, 2NH), 2.60 (s, 4H, 2CH ₂), 3.87 (s, 6H, 2OCH ₃), and 7.20 (dd, 8H, 2 Ar-H).

*All the compounds are viscous in nature. ** Compounds 1a, 1c, and 1d, are reported¹⁰.

Table 2: 1,4-Disubstituted-piperazine-2,3-dione derivatives (**2a-i**).

Compd. No.	R	R ¹	Mol. Formula	Yield %	MP, °	R _f	Microanalysis			Clog p*
								Calcd. %	Found %	
2a	H	H	C ₁₈ H ₁₈ N ₂ O ₂ (294.14)	55	220-2	0.50	C	73.45	73.53	2.34
							H	6.16	5.95	
							N	9.52	9.65	
2b	H	<i>p</i> -Br	C ₁₈ H ₁₆ Br ₂ N ₂ O ₂ (451.96)	78	215-7	0.56	C	47.82	47.51	3.99
							H	3.57	3.28	
							N	6.20	6.41	
2c	H	<i>p</i> -Cl	C ₁₈ H ₁₆ Cl ₂ N ₂ O ₂ (363.24)	86	207-8	0.51	C	59.52	59.98	3.82
							H	4.44	5.09	
							N	7.71	8.12	
2d	H	<i>o</i> -OH	C ₁₈ H ₁₈ N ₂ O ₄ (326.13)	63	230-1	0.28	C	66.25	65.63	1.56
							H	5.56	5.41	
							N	8.58	8.49	
2e	CH ₃	H	C ₂₀ H ₂₂ N ₂ O ₂ (322.17)	60	190-1	0.52	C	74.51	74.55	2.97
							H	6.88	7.24	
							N	8.69	7.99	
2f	CH ₃	<i>p</i> -Br	C ₂₀ H ₂₀ Br ₂ N ₂ O ₂ (479.99)	85	165-7	0.63	C	50.02	50.33	4.63
							H	4.20	3.99	
							N	5.83	6.10	
2g	CH ₃	<i>p</i> -Cl	C ₂₀ H ₂₀ Cl ₂ N ₂ O ₂ (390.09)	75	135-6	0.52	C	61.39	61.45	4.09
							H	5.15	5.34	
							N	7.16	7.23	
2h	CH ₃	<i>p</i> -CH ₃	C ₂₂ H ₂₆ N ₂ O ₂ (350.20)	70	205-7	0.60	C	75.40	75.43	3.95
							H	7.48	7.55	
							N	7.99	8.10	
2i	CH ₃	<i>p</i> -OCH ₃	C ₂₂ H ₂₆ N ₂ O ₄ (382.19)	69	191-2	0.49	C	69.09	70.10	2.72
							H	6.85	6.75	
							N	7.32	7.42	

* The Clog P for piperazine is -1.13.

Table 3: ¹HNMR chemical shifts of compounds (**2a-i**).

Compd. No.	R	R ¹	¹ HNMR data
2a	H	H	3.50 (s, 4H, CH ₂ CH ₂), 4.70, (s, 4H, 2CH ₂ Ph), and 7.20-7.90 (m, 10H, 2 Ar-H).
2b	H	<i>p</i> -Br	3.47 (s, 4H, CH ₂ CH ₂), 4.70 (s, 4H, 2CH ₂ Ph), and 7.14-7.87 (m, 8H, 2 Ar-H).
2c	H	<i>p</i> -Cl	3.40 (s, 4H, CH ₂ CH ₂), 4.67 (s, 4H, 2CH ₂ Ph), and 7.37 (s, 8H, 2 Ar-H).
2d	H	<i>o</i> -OH	3.57 (s, 4H, 2CH ₂), 4.67 (s, 4H, 2CH ₂ Ph), 6.67-7.70 (m, 8H, 2 Ar-H), and 9.77 (brs, 1H, OH, exchangeable with D ₂ O).
2e	CH ₃	H	1.57 (d, 6H, 2CH ₃), 2.70-3.30 (m, 4H, 2CH ₂), 6.00 (q, 2H, 2CH), and 7.34 (m, 10H, 2 Ar-H).
2f	CH ₃	<i>p</i> -Br	1.54 (d, 6H, 2CH ₃), 2.64-3.70 (s, 4H, 2CH ₂), 6.00 (q, 2H, 2CH), and 7.44 (dd, 8H, 2 Ar-H).
2g	CH ₃	<i>p</i> -Cl	1.50 (d, 6H, 2CH ₃), 2.84 (m, 4H, 2CH ₂), 5.70 (q, 2H, 2CH), and 7.44 (s, 8H, 2 Ar-H).
2h	CH ₃	<i>p</i> -CH ₃	1.40 and 1.57 (d, each 3H, 2CH ₃), 2.40 (s, 6H, 2CH ₃ Ph), 2.77-3.44 (m, 4H, 2CH ₂), 6.14 (q, 2H, 2CH), and 7.37 (s, 8H, 2 Ar-H).
2i	CH ₃	<i>p</i> -OCH ₃	1.40 and 1.57 (d, each 3H, 2CH ₃), 2.64-3.67 (m, 4H, 2CH ₂), 3.70 (s, 6H, 2OCH ₃), 6.10 (q, 2H, 2CH), and 7.20 (dd, 8H, 2 Ar-H).

Calculation of the log P values

The log P values of the target compounds (**2a-i**) as well as piperazine hydrate, were computed with a routine method called calculated log P (Clog P) contained in a PC-software package (McLogP 2.0, BioByte Corp., CA, USA). A representation of the molecular structure where hydrogens are omitted or 'suppressed' (SMILES notation) is entered into the program, which computes the log P based on the fragment method developed by A.J. Leo,¹¹ the results are given in Table 2.

Anthelmintic Activity

The synthesized compounds (**2a-i**) were evaluated *in vitro* for their anthelmintic activities according to a standard protocol mentioned below.^{12,13}

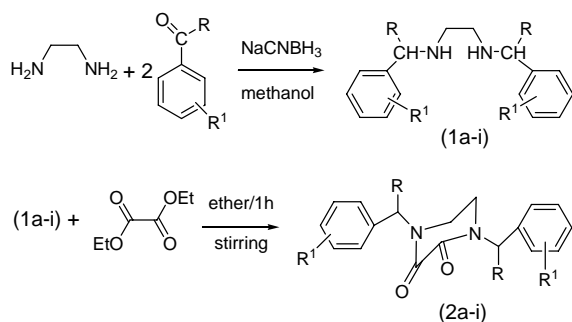
Parasites, culture conditions, and anthelmintic activity

*Entrobium vermicularis*¹² was collected from patients in private lab and transferred in saline immediately to the lab of study. *Fasciola hepatica*¹³ was collected from slaughterhouse and transferred in saline immediately to the lab of study. The parasites were washed several times by saline and put in Petri dishes as living in saline and incubated at 37° for 6 hrs. The solutions of the tested compounds (100 μmole) in dimethyl sulphoxide (5 mL) were added and the numbers of the dead worms are recorded in each solution as well as for the reference drug and a control experiment.

RESULTS AND DISCUSSION

Chemistry

The synthesis of *N,N*¹-disubstituted-piperazine-2,3-dione derivatives has been carried out according to steps in scheme 1.



1 and 2	R	R ¹
a	H	H
b	H	<i>p</i> -Br
c	H	<i>p</i> -Cl
d	H	<i>o</i> -OH
e	CH ₃	H
f	CH ₃	<i>p</i> -Br
g	CH ₃	<i>p</i> -Cl
h	CH ₃	<i>p</i> -CH ₃
i	CH ₃	<i>p</i> -OCH ₃

Scheme 1

In the first step, the appropriate carbonyl compounds were reacted with ethylenediamine in the presence of sodium cyanoborohydride to afford *N,N*¹-Bis-(4-substituted benzyl)-ethane-1,2-diamine and *N,N*¹-Bis-[1-(4-substituted phenyl)-ethyl]-ethane-1,2-diamine derivatives (**1a-i**) through reductive alkylation reaction that are viscous in nature. Compounds (**1a-i**), were subjected to cyclization process through the reaction with diethyl oxalate affording the target compounds, (**2a-i**), with yields varying from 55-86%, Scheme 1. The physicochemical constants of compounds (**2a-i**) are reported in Table 2.

The structures of the synthesized compounds were verified on the bases of spectral as well as elemental methods of analyses. The structures of the intermediate compounds (**1a-i**) are clarified depending upon the IR and the ¹HNMR spectral data and in part with the reported data.¹¹ The IR spectra of the

intermediate compounds (**1a-i**) showed strong broadband at about 3350-3400 (2NH stretching). On the other hand, in the ¹HNMR a common singlet signal for the two methylene groups was observed beside another singlet signal equivalent to four protons of the introduced carbonyl compound in the case of *N,N*¹-Bis-(4-substituted benzyl)-ethane-1,2-diamine or a doublet signal equivalent to six protons of two methyl groups and a quartet signal equivalent to two protons of the 2 methine protons in the case of *N,N*¹-Bis-[1-(4-substituted phenyl)-ethyl]-ethane-1,2-diamine derivatives. Moreover, the presence of a broad signal equivalent to two protons (exchangeable by treatment with D₂O) of the two NH groups and the aromatic protons are other evidences of the structure of such derivatives.

All the spectral data of the target compounds are in accordance with the proposed structures. The IR spectra of compounds **2a-i** show prominent very strong absorption bands around 1653-1671 (2 C=O stretch). In the ¹HNMR spectra, Table 3, a common signal for the two methylene groups of the piperazine-2,3-dione nucleus was observed. The differences in sets and patterns were only attributed to the 1,4-disubstituents, where they give patterns in accordance with the expected structures of the target compounds, Table 3.

Moreover, the structures of the target compounds were additionally clarified on the basis of the fragmentation pattern of a representative example, compounds 2h. The mass spectrum of compound 2h shows a molecular ion peak at *m/z* 350 (32%) corresponding to the molecular weight of the compound and characterized by two prominent peaks at *m/z* 230 (53%), and 119 (100%) due to fragmentation of the N1 or N4 substituents. In addition the data reveal the presence of the very characteristic tropylium cation at *m/z* 91 (15%).

Lipophilicity

The lipophilicity of the target compounds (**2a-i**) as well as piperazine hydrate, is expressed on the term of Clog P values. The values were computed with a routine method called calculated log P (Clog P) contained in a PC-software package as will be mentioned in the experimental section. A program, which computes the log P based on the fragment method developed by A.J. Leo,¹¹ the results are given in Table 2.

As given in Table 2, there is a considerable improvement in the lipophilicity of the target compounds (**2a-i**) in comparison with the reference drug piperazine. Moreover, there is a relationship between the values of the Clog P and those of the R_f measured for these compounds. Thus these new compounds may possess the ability for penetration of various biomembranes that enhances their bioavailability to the site of action.¹⁴

Anthelmintic activities

The anthelmintic activities of the target compounds were tested against *Enterobius vermicularis* and *Fasciola hepatica* according to a reported protocol described in the experimental part.^{12,13} Doses of the tested compounds for anthelmintic activities are given in Table 4, in comparison with the reference drugs.

Table 4: Doses of compounds (**2a-i**) and piperazine hydrate for anthelmintic activity.

Compd. No.	R	R ¹	mg/5ml
2a	H	H	29.4
2b	H	<i>p</i> -Br	45.1
2c	H	<i>p</i> -Cl	36.3
2d	H	<i>o</i> -OH	32.6
2e	CH ₃	H	32.2
2f	CH ₃	<i>p</i> -Br	47.9
2g	CH ₃	<i>p</i> -Cl	39.0
2h	CH ₃	<i>p</i> -CH ₃	35.0
2i	CH ₃	<i>p</i> -OCH ₃	38.2
Piperazine hydrate			19.4

The results are observed as the percentage number of the dead worms in each case. All the tested compounds gave anthelmintic activity comparable to the reference drug used, piperazine hydrate, and the percentage number of the dead worms was 80-90% in each case over the period of observation (6 hrs). As a general pattern, the anthelmintic activities of the tested compounds under the used concentrations are almost independent on variation of the 1,4- side substituents. Accordingly, this series of compounds embedding the piperazine-2,3-dione moiety in its structure can be considered as promising

candidates to be used as anthelmintic agents and consequently intensive *in vitro* and *in vivo* as well as the synthesis of new derivatives are in progress.

Conclusion

We can conclude that, a new series of new 1,4-disubstituted piperazine-2,3-dione derivatives were synthesized as the target compounds. The structures of the target compounds were elucidated depending upon the data of the different spectral as well as the elemental methods of analyses. In addition, a mass spectrum, for representative example, was carried out where the expected fragmentation mode is in accordance with the structures of the considered compounds. The anthelmintic activity of the newly synthesized compounds (**2a-i**) was investigated *in vitro* against *Enterobius vermicularis* and *Fasciola hepatica*. The tested compounds exhibited, in all cases, considerable inhibitory effects on the growth of the tested parasites in comparison with piperazine hydrate as a reference drug. Accordingly, this series of compounds embedding the piperazine-2,3-dione moiety in its structure can be considered as promising candidates to be used as anthelmintic agents and consequently intensive *in vitro* and *in vivo* as well as the synthesis of new derivatives are in progress.

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