Synthesis of symmetrically 1,4-disubstituted piperazine-2,5-diones: a new class of antileishmanial agents

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A series of 1,4-diphenyl-2,5-dioxopiperazine derivatives were synthesised in one pot sequence. The compounds demonstrated appreciable cytotoxic activity against *Leishmania donovani* on both forms of the parasite, and the results suggested that some derivatives (**4, 11** and **12**) could be exploited as antileishmanial agents.

Keywords: piperazine-2,5-diones, diketopiperazines, *Leishmania donovani,* antiprotozoals, chloroacetanilides

In recent years, piperazine scaffolds have been prevalent in scientific literature due to the high number of positive hits encountered in the biological evaluation of this heterocycle and its congeners.[1,2] Prominent among these are the diketopiperazine (DKP) derivatives, a family of secondary metabolites, many of which are produced by microorganisms.[3] Being relatively simple compounds, 2,5-diketopiperazines are amongst the most widespread of the small cyclo-dipeptide derivatives found in nature.[4] Their existence was first reported around the year 1900 and many of the simpler members of this family were later synthesised by Emil Fischer.[5] The parent compound, piperazine-2,5-dione, was first synthesised in 1888.[6] Later, innumerable molecules bearing the piperazine-2,5-dione ring have been isolated from cultures and received much attention due to their diverse and interesting biological activities.[7-9] Some of the potent molecules are albonoursin,[10-12] bicyclomycin[13] and glyotoxin.[14] The biosynthetic pathways to the formation of piperazine-2,5-diones in microorganisms, though not properly explored, are believed to be the result of the operation of enzymes like cyclic dipeptide oxidase (CDO) and non-ribosomal peptide synthetase (NRPS).[15] However, the chemical synthesis of piperazine-2,5-diones include the cyclisation of peptide derivatives, intramolecular attack of amines on activated carbonyl groups, cyclisation of *N*-pyruvoyl amino acid amides, intramolecular Diels–Alder reactions, and synthesis of α-haloacyl derivatives of aminoacid esters.[16] Most of the methods employ aminoacid building blocks and involve multistep operations.[17,18] Recently several groups, including ours, have reported the formation of diketopiperazines not from amino acid precursors.[19-24] However, no reports have appeared as yet with regard to their antileishmanial activity. In continuation of our search for antileishmanial agents[25-27] the diverse biological activity of piperazine-2,5-dione derivatives led us to explore the possibility of there being privileged motifs for their antileishmanial activity.

In the present investigation, we disclose the synthesis of symmetrically disubstituted 1,4-piperazine-2,5-diones from α-chlorophenyl acetamides and their differently substituted analogues as model substrates, emphasising the yield of formation, ease of isolation and homogeneity of the product formed, besides an evaluation of their antileishmanial activity against *Leishmania donovani* promastigotes and amastigotes *in vitro*. To the best of our knowledge this is the first report of diketopiperazine derivatives as antileishmanial agents.

Results and discussion

In our synthetic approach, we tried to optimise the reaction conditions for the formation of 1,4-diphenylpiperazine-2,5-dione (**1**) from *N*-phenyl α-chloroacetamide. The starting material was prepared from the acylation of aniline with α-chloroacetyl chloride in dry CH_2Cl_2/benzene. Varying the time and temperature of the reaction, it was observed that exclusive formation of **1** in >90% yield resulted at a temperature of 60 ± 5°C (Table 1) in 1 h when carried out in dry DMSO under dry nitrogen in the presence of NaH. The spectral data of compound **1** fully support the assigned structure. Formation of **1** must have taken place by self-condensation between two molecules of the substrate, the base causing the generation of the anion on the nitrogen atom stabilised by both phenyl and keto groups attached to the nitrogen atom, and subsequently the reaction proceeds *via* a S_N2 pathway (Scheme 1).

To establish the generality of the reaction, differently substituted α-chloroacetanilides were used as substrates. Thus, carrying out the reaction with other substrates under the same conditions as for the 1,4-diphenylpiperazine-2,5-dione (**1**) afforded a variety of substituted phenyl analogues (Table 1). Following the same protocol, 2-chloro-*N*-cyclohexylacetamide furnished 1,4-dicyclohexylpiperazine-2,5-dione (**12**) in 85% yield (Scheme 1). Optimisation of the reaction conditions with respect to time and percentage of yield of the derivatives provided the results summarised in Table 1.

Experimental

Melting points were determined with a capillary melting point apparatus. IR spectra were recorded on a JASCO FTIR (model 410) in KBr pellets; major/significant peaks only are recorded. MALDI-TOF MS (positive) was conducted using Perspective Bio System Voyager DE-STR Mass spectrometer with 2,5-dihydroxybenzoic acid as matrix. ^1H and ^{13}C NMR spectra were taken on a Bruker 300 MHz DPX spectrometer at 300 and 74.99 MHz, respectively, with tetramethylsilane as internal standard, in DMSO-d_6 unless otherwise stated. Sodium hydride, anilines and chloroacetyl chloride were purchased from Aldrich Chemical Ltd (USA). Organic solvents used for the chemical synthesis and for chromatography, acquired from E. Merck (India), were of analytical grade. All chromatographic purification was performed with silica gel (60–120 mesh) and was obtained from SRL (India). TLC was performed on pre-coated silica gel 60 F254 aluminum sheets (E. Merck, Germany) using the solvent system 1-6% MeOH in $CHCl_3$; spots were developed using Liebermann-Burchard solution. Medium 199 was purchased from GIBCO BRL, USA.

Table 1 Optimisation of the reaction conditions of the diketopiperazine analogues (**1–12**)

Compd	Time/h	Yield/%	Compd	Time/h	Yield/%
1	1	90	**7**	2	89
2	1	95	**8**	1.5	93
3	1	94	**9**	2	90
4	2	85	**10**	2	88
5	2	88	**11**	2	90
6	2	80	**12**	1	85

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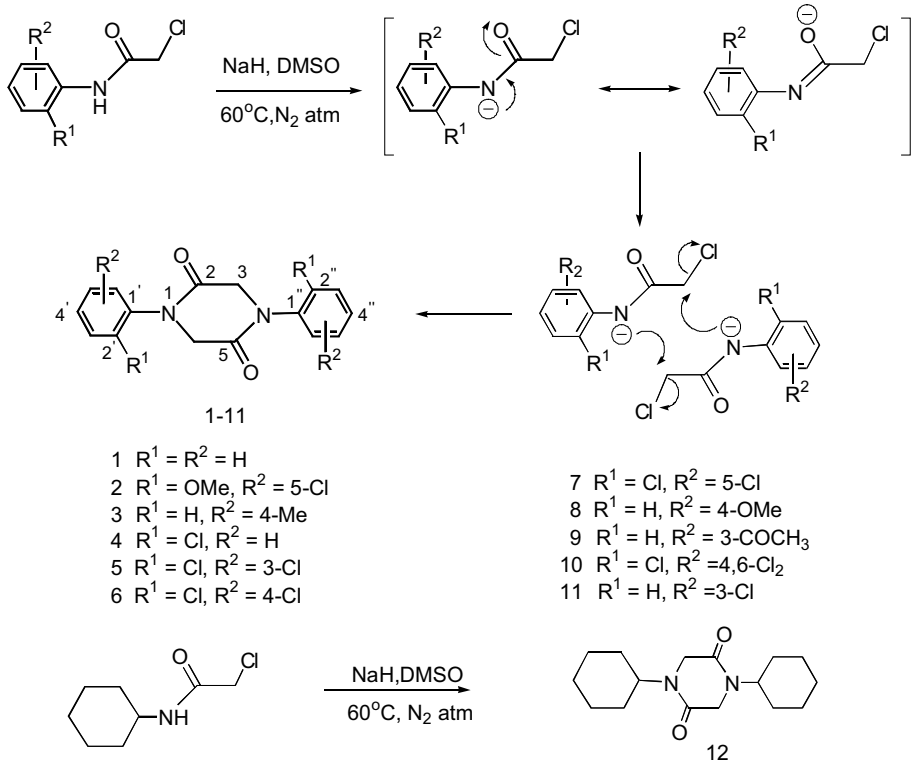

Scheme 1

2,5-Diketopiperazines **1-12**: *general procedure*

A three-necked reaction flask was fitted with a condenser, a dropping funnel and a stopcock connected to a nitrogen source. Sodium hydride (0.5 mol) was taken in the reaction flask and washed free from mineral oils by dry petroleum ether. Dry dimethylsulfoxide (DMSO) was then added to the flask. Heating and stirring was continued until the temperature reached 40–42°C. The chloroacetanilide substrate (0.5 mol) was then added and stirring was continued for 1 h at 60±5°C. The reaction mixture was cooled, poured into ice-cold water and extracted with $CHCl_3$. The extract was washed free from alkali, dried over anhydrous sodium sulfate, concentrated under reduced pressure and crystallised from CH_3OH.

1,4-Diphenylpiperazine-2,5-dione (**1**): M.p. 267°C (lit.[24] 266–267°C). IR: 1658, 1476, 1459 cm^{-1}. NMR: δ_H 4.52 (s, 4H, H-3 and 6), 7.31 (m, 4H, H-2', 2" and H-6', 6"), 7.45 (m, 6H, H-3', 3", 4', 4" and 5', 5"); δ_C 53.6 (t, C-3, 6), 126.1 (d, C-2', 2" and C-6', 6"), 127.6 (d, C-4', 4"), 129.8 (d, C-3', 3" and 5', 5"), 140.9 (s, C-1' and 1"), 164.9 (s, C-2 and 5). MS (MALDI-TOF, positive ion): *m/z* 267 $[M + H]^+$. Anal. Calcd for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52. Found: C, 72.37; H, 5.24; N, 10.36%.

1,4-Bis-(5-chloro-2-methoxyphenyl)piperazine-2,5-dione (**2**): M.p. 300–301°C. IR: 1671, 1497, 1464 cm^{-1}. NMR: δ_H 3.74 (s, 6H, 2"-OMe), 4.32 (s, 4H, H-3 and 6), 6.78 (d, J = 8 Hz, 2H, H-3', 3"), 7.08–7.12 (m, 4H, H-4', 4", and 6', 6"); δ_C 53.5 (t, C-3, 6), 56.0 (q, 2', 2"-OCH_3), 115.6 (d, C-3', 3"), 121.7 (d, C-6', 6"), 125.6 (d, C-4', 4"), 126.4 (s, C-5', 5"), 127.8 (s, C-1', 1"), 152.3 (s, C-2', 2"), 166.9 (s, C-2, 5). MS (MALDI-TOF, positive ion): *m/z* 418 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{16}Cl_2N_2O_4$: C, 54.70; H, 4.08; N, 7.09. Found: C, 54.46; H, 4.21; N, 7.28%.

1,4-Di-p-tolylpiperazine-2,5-dione (**3**): M.p. 233–235°C. IR: 1656, 1514, 1467 cm^{-1}. NMR δ_H 2.3 (s, 6H, 4', 4"-Me), 4.34 (s, 4H, H-3 and 6), 7.10 (d, J = 7.8 Hz, 4H, H-2', 2" and H-6', 6"), 7.38 (d, J = 7.8 Hz, 4H, H-3', 3", and 5', 5"); δ_C 21.2 (q, *p*-Me) 53.7 (t, C-3, 6), 126.6 (d, C-2', 2" and C-6', 6"), 128.1 (s, C-4', 4"), 129.8 (d, C-3', 3" and 5', 5"), 140.7 (s, C-1' and 1"), 164.8 (s, C-2 and 5). MS (MALDI-TOF, positive ion): *m/z* 295.14 $[M + H]^+$and 317.13 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{16}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.74; H, 6.31; N, 9.23%.

1,4-Bis-(2-chlorophenyl)piperazine-2,5-dione (**4**): M.p. 134–136°C. IR: 1694, 1594, 1536, 1445 cm^{-1}. NMR ($CDCl_3$): δ_H 4.34 (s, 4H, H-3 and 6), 7.09 (t, J = 8 Hz, 2H, H-4', 4"), 7.30 (t, J = 8 Hz, 2H, H-5', 5"), 7.38 (d, J = 7.8 Hz, 2H, H-6', 6"), 8.39 (d, J = 7.8 Hz, 2H, H-3', 3"); δ_C 71.9 (t, C-3, 6), 122.0 (d, C-6', 6"), 123.6 (s, C-2', 2"), 125.7 (d, C-4', 4"), 128.2 (d, C-3', 3"), 129.5 (d, C-5', 5"), 133.9 (s, C-1', 1"), 166.4 (s, C-2, 5); MS:(MALDI-TOF, positive ion): *m/z* 373 $[M + K]^+$. Anal. Calcd for $C_{16}H_{12}Cl_2N_2O_2$: C, 57.33; H, 3.61; N, 8.36. Found: C, 57.61; H, 3.43; N, 8.08%.

1,4-Bis-(2,3-dichlorophenyl)piperazine-2,5-dione (**5**): M.p. 163–165ºC. IR: 1697, 1590, 1531, 1407cm^{-1}. NMR ($CDCl_3$): δ_H 4.36 (s, 4H, H-3 and 6), 7.25–7.27 (m, 4H, H-6', 6" and H-4', 4"), 8.35–8.38 (m, 2H, H-5', 5"); δ_C 71.9 (t, C-3, 6), 119.8 (d, C-6', 6"), 122.2 (s, C-2', 2"), 126.4 (d, C-4', 4"), 128.4 (d, C-5', 5"), 133.4 (s, C-3', 3"), 135.5 (s, C-1', 1"), 166.3 (s, C-2, 5); MS (MALDI-TOF, positive ion): *m/z* 443 $[M+K]^+$. Anal. Calcd. for $C_{16}H_{10}Cl_4N_2O_2$: C, 47.56; H, 2.49; N. 6.93. Found: C, 47.42; H, 2.53; N, 6.78 %.

1,4-Bis-(2,4-dichlorophenyl)piperazine-2,5-dione (**6**): M.p. 228–230°C. IR: 1682, 1481, 1447 cm^{-1}. NMR δ_H 4.38 (s, 4H, H-3 and 6), 7.10–7.12 (m, 4H, H-5', 5", and 6', 6"), 7.34 (s, 2H, H-3', 3"); δ_C 53.8 (t, C-3, 6), 123.6 (d, C-6', 6"), 127.1 (s, C-2', 2"), 127.3 (d, C-5', 5"), 129.7 (d, C-3', 3"), 130.6 (s, C-4', 4"), 139.3 (s, C-1' and 1"), 166.8 (s, C-2 & 5). MS (MALDI-TOF, positive ion): *m/z* 443 $[M + K]^+$. Anal. Calcd. for $C_{16}H_{10}Cl_4N_2O_2$: C, 47.56; H, 2.49; N, 6.93. Found: C, 47.39; H, 2.28; N, 7.18%.

1,4-Bis-(2,5-dichlorophenyl)piperazine-2,5-dione (**7**): M.p. 162–164°C. IR: 1691, 1591, 1530, 1453, 1411 cm^{-1}. NMR: δ_H 4.38 (s, 4H, H-3 and 6), δ 7.08 (s, 2H, H-6', 6"), 7.21–7.24 (m, 4H, H-3', 3", and 4', 4"); δ_C 53.1 (t, C-3, 6), 122.6 (d, C-6', 6"), 123.8 (s, C-2', 2"), 125.7 (d, C-4', 4"), 130.6 (d, C-3', 3"), 132.2 (s, C-5', 5"), 142.6 (s, C-1', 1"), 166.6 (s, C-2, 5). MS (MALDI-TOF, positive ion): *m/z* 443 $[M + K]^+$. Anal. Calcd. for $C_{16}H_{10}Cl_4N_2O_2$: C, 47.56; H, 2.49; N, 6.93. Found: C, 47.75; H, 2.61; N, 6.64%.

1,4-Bis-(4-methoxyphenyl)piperazine-2,5-dione (**8**): M.p. 248–250°C. IR: 1651, 1587, 1513, 1466 cm^{-1}. NMR δ_H 3.72 (s, 6H, OCH_3), 4.39 (s, 4H, H-3 and 6), 6.87 (d, J = 8 Hz, 4H, H-3', 3", and 5', 5"), 7.10 (d, J = 7.8 Hz, 4H, H-2', 2" and H-6', 6"); δ_C 53.1 (t, C-3, 6), 55.9 (q, OCH_3), 114.3 (d, C-3', 3" and 5', 5"), 121.6 (d, C-2', 2" and C-6', 6"), 133.2 (s, C-1' and 1"), 157.5 (s, C-4', 4"), 166.8 (s, C-2 & 5). MS (MALDI-TOF, positive ion): *m/z* 349 $[M + Na]^+$. Anal. Calcd. for $C_{18}H_{18}N_2O_4$: C, 66.25; H, 5.56; N, 8.58. Found: C, 65.97; H, 5.83; N, 8.24%.

1,4-Bis-(3-acetylphenyl)piperazine-2,5-dione (**9**): M.p. 180–181°C. IR: 1687, 1657, 1580, 1464 cm^{-1}. NMR ($CDCl_3$): δ_H 2.63 (s, 3H, –Me), 4.58 (s, 4H, H-3 and 6), 7.54–7.62 (m, 4H, H-5', 5"and 6', 6"), 7.90–7.94 (m, 4H, H-2', 2" and 4', 4"); δ_C 27.1 (Me), 53.52 (t, C-3, 6), 124.7 (d, C-2', 2"), 127.8 (d, C-4', 4"), 129.9 (d, C-6', 6"), 130.1 (d, C 5', 5"), 138.7 (s, C-3', 3"), 140.3 (s, C-1', 1"), 164.3 (s, C-2, 5), 197.3 (s, –C=O). MS (MALDI-TOF, positive ion): *m/z* 351 $[M + H]^+$and 373 $[M + Na]^+$. Anal. Calcd. for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.28; H, 5.39; N, 7.83%.

1,4-Bis-(2,4,6-trichlorophenyl)piperazine-2,5-dione(**10**):M.p.128–130°C. IR: 1654, 1591, 1496, 1469 cm^{-1}. NMR: δ_H 4.36 (s, 4H, H-3 and 6), 7.20 (s, 4H, H-3', 3", and 5', 5"); δ_C 52.5 (t, C-3, 6), 127.3 (d, C-3', 3" and 5', 5"), 128.6 (s, C-2', 2" and C-6', 6"), 131.9 (s, C-4', 4"), 139.2 (s, C-1' and 1"), 166.4 (s, C-2, 5). MS (MALDI-TOF, positive ion): *m/z* 473 $[M + H]^+$. Anal. Calcd. for $C_{16}H_8Cl_6N_2O_2$: C, 40.63; H, 1.70; N, 5.92. Found: C, 40.19; H, 1.64; N, 6.04%.

1,4-Bis-(3-chlorophenyl)piperazine-2,5-dione (**11***)*: M.p. 234–236°C. IR: 1671, 1576, 1523 cm^{-1}. NMR: δ_H 4.33 (s, 4H, H-3 and 6), 6.99 (d, *J* = 7.8 Hz, 2H, H-6', 6"), 7.13 (s, *J* = 7.8 Hz, 2H, H-2', 2"), 7.26 (m, 4H, H-4', 4", and 5', 5"); δ_C 53.2 (t, C-3, 6), 118.7 (d, C-6', 6"), 120.8 (d, C-2', 2"), 124.6 (d, C-4', 4"), 130.2 (d, C-5', 5"), 134.1 (s, C-3', 3"), 142.0 (s, C-1', 1"), 166.3 (s, C-2, 5). MS (MALDI-TOF, positive ion): *m/z* 373 $[M + K]^+$. Anal. Calcd. for $C_{16}H_{12}Cl_2N_2O_2$: C, 57.33; H, 3.61; N, 8.36. Found: C, 57.14; H, 3.86; N, 8.62%.

1,4-Dicyclohexylpiperazine-2,5-dione (**12**): M.p. 224–226°C. IR: 1636, 1472, 1450, 1324 cm^{-1}. NMR: δ_H 1.10–1.75 (m, 20H), 3.79-3.83 (m, 2H), 4.99 (s, 4H, H-3 and 6); δ_C 21.9 (t, C-3', 3" and 5', 5"), 27.4 (t, C-4', 4"), 30.2 (t, C-2', 2"and 6', 6"), 46.2 (d, C-1' and 1"), 50.1 (t, C-3, 6), 169.3 (s, C-2, 5); MS (MALDI-TOF, positive ion): *m/z* 317 $[M + K]^+$. Anal. Calcd. for $C_{16}H_{26}N_2O_2$: C, 69.03; H, 9.41; N, 11.49. Found: C, 69.29; H, 9.17; N, 11.58%.

We gratefully acknowledge funding from the Department of Biotechnology, Government of India and Council of Science & Industrial Research for the award of ES to N.P. Sahu and fellowships to A. Hazra and P. Paira. P Palit is thankful to ICMR for his fellowship. We are also thankful to Dr B. Achari, Emeritus Scientist, CSIR, for his helpful suggestions and encouragement.

Received 10 May 2007; accepted 9 July 2007
Paper 07/4641 doi: 10.3184/030823407X228254

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