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Evaluation of Some Classical Hydrazones of Ketones and 1,2-Diketones as Antileishmanial, Antibacterial and Antifungal Agents

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The paper describes the synthesis and antimicrobial (antileishmanial, antibacterial and antifungal) activity of some classical hydrazones of benzophenones and of 1,2-diketones. *N*-(Diaryl)acyl derivatives of these hydrazones have also been synthesized and evaluated. 4,4,-Demthoxybenzil monohydrazone and 4,4'-dimethoxybenzophenone hydrazone showed significant antileishmanial activity. The effect of substituents on the bioactivity is discussed.

Key words: Hydrazones, *N*-(diaryl)acyl hydrazones, Antileishmanial, Antibacterial, Antifungal

INTRODUCTION

The infectious diseases are the second major cause of death worldwide and third leading cause of death in economically advanced countries (Nathan, 2004). Bacterial strains are getting resistant towards antibiotics in clinical use. The ability of bacteria to deceive any kind of conventional therapy has become apparent and pathogens resistant to one or more antibiotics are emerging and spreading worldwide (Clark et al., 2003). Unnecessary use of antibiotics has further fuelled this problem. The discovery of vancomycin resistant *Staphylococcus aureus* (VRSA) and multiresistant *S. aureus* has generated worldwide concern. It has thus become evident that there is urgent need for novel antibacterial drugs with broader spectrum, less side effects, and without cross-resistance to antibiotics in use.

Leishmaniasis is a tropical disease caused by infection of protozoa, *Leishmania*. It has been recognized by the World Health Organization (WHO) as an increasing health problem (Modabber, 1991). Many parts of Asia and Africa are vulnerable to leishmaniasis. Most of the medicines available currently in the market for treatment of leishmaniasis are costly, have side-effects, and are getting resistant to pathogen after treatment for several weeks. Recent years have witnessed considerable interest in design and development of antileishmanial compounds. Many compounds containing carbon-nitrogen double bond in different structural environments are reported to have promising antileishmanial activity (Croft et al., 2006; Khan et al., 2008). We recently launched a program to synthesize diverse types of nitrogen and sulfur containing compounds and to evaluate them as antileishmanial agents. We observed significant antileishmanial activity in some *N*-substitutes imines from benzaldehydes, cinnamaldehyde, furan-2-carbaldehyde; thiophene-2-carbaldehyde, salicylaldehyde and isatin (Al-Kahraman et al., 2010, 2011). It was therefore considered pertinent to evaluate hydrazones for their antileishmanial, and also antibacterial and antifungal activities. In the present paper, we wish to report the results of antileishmanial, antibacterial and antifungal screening of some hydrazones and *N*-(diaryl)acyl hydrazones derived from benzophenones and benzils that have never been evaluated for their bioactivity.

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MATERIALS AND METHODS

Materials

Melting points (m.p.) have been recorded on a Stuart Scientific melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer-781 IR spectrophotometer using KBr disc of the sample. The NMR spectra were recorded on a Jeol FX 90Q

spectrometer using TMS as an internal standard.

The ketones and hydrazine hydrate were Aldrich products. The 2-diazo-1,2-diarylethanones were synthesized according to the reported method (Nenitzescu and Solomonica, 1950).

Leishmania major was cultured from the blood taken directly from the infected patients at the Bollan Medical College, Quetta, Pakistan, in artificial medium 199. The bacterial and fungal cultures, identified previously by 16S and 18S rRNA, were obtained from the H. E. J. Karachi University, Pakistan.

Synthesis of hydrazones 1-3

To a warmed solution containing appropriate 1,2-diketone (10 mmol) in 20 mL of methanol was added slowly with stirring 15 mmol of hydrazine hydrate (80%, aq.). The solution was heated under reflux for 15 min and then allowed to attain the room temperature. The white solid appeared in the flask that was filtered and washed with cold methanol (Nenitzescu and Solomonica, 1950).

Synthesis of hydrazones 4-6

The solution of appropriate ketone (10 mmol), hydrazine hydrate (15 mmol, 80%, aq.) in 1 mL of n-butanol was heated under reflux for 2-4 h. The hot solution was poured into 5 mL of methanol. The white solid obtained was filtered and recrystallized from methanol (Baltzly et al., 1961).

Synthesis of *N*-(diaryl)acyl hydrazones 7-11

A solution of appropriate hydrazone and 2-diazo-1,2-diarylethanone (10 mmol of each) in 60 mL of dry benzene was refluxed under nitrogen atmosphere for 8 h. The solvent was removed under reduced pressure and the residue obtained was triturated with ethanol to get white crystalline product (Singh et al., 1984; Singh, 2004).

Antileishmanial activity

The title compounds were screened for their antileishmanial activity against the pre-established culture of *L. major*. Parasites were cultured in Medium M199 with 10% foetal bovine serum; 25 mmol HEPES, and 0.22 mg of penicillin and streptomycin, respectively at 24°C in a shaking incubator (Ali et al., 1997).

Each compound to be tested and amphotericin B (as a positive control) were dissolved in DMSO to a concentration of 1 mg/mL. Parasites at log phase were centrifuged at 3000 rpm for 3 min. Parasites were diluted in fresh culture medium to a final density of 2×10^6 cells/mL. In 96-well plates, 180 μL of medium was added in different wells. Experimental compound (20 μL) was added in medium and serially diluted. Parasite culture (100 μL) was added in all wells. In negative controls, DMSO was serially diluted in medium while the positive control contained varying concentrations of standard antileishmanial compound i.e. amphotericin B. The plates were incubated for 72 h at 24°C. The culture was examined microscopically on an improved Neubauer counting chamber and IC_{50} values of compounds possessing antileishmanial activity were calculated. All the assays were run in duplicate. IC_{50} of samples was determined by using the Prism software.

Antibacterial activity

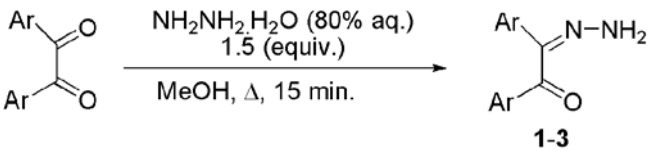
In vitro evaluation of antibacterial activity was carried out by disk diffusion method (Kirby-bauer method) (Cappuccino and Sherman, 1996) against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia* and *Escherichia coli*. The tests were repeated three times and the results are reported as means of least three times. The minimum inhibitory concentrations (MICs) of the compounds were recorded as the lowest concentration of each of the compounds in Petri plates with no turbidity (i.e. no growth) of inoculated bacteria.

Antifungal activity

The antifungal bioassay was performed by the Agar Tube Dilution Method (Choudhary et al., 1995), in which the test Compounds were screened for activity against the following organisms: *Candida albicans, Microsportum canis* and *Fusarium solani* and the percentage inhibition is reported. In all tests, the linear growth in control was 100 mm.

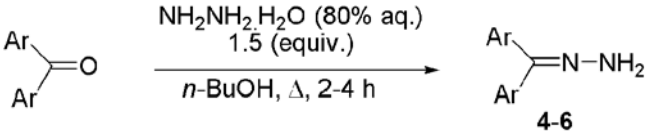
RESULTS

The hydrazones **1-3** were synthesized by refluxing the appropriate 1,2-diketone with hydrazine hydrate in methanol (Scheme 1). The hydrazones **4-6** were prepared by refluxing appropriate ketones with hydrazones in minimum amount of *tert*-butanol (Scheme 2). The *N*-(diaryl)acyl hydrazones were prepared by refluxing equimolar solution of appropriate ketohydrazone and 2-diazo-1,2-diarylethanones **12** (acylating agent) in dry benzene (Scheme 3). The physical data of all the



Ar = **1**. Ph, **2**. 4-MePh, **3**. 4-MeOPh

Scheme 1. Preparation of benzil monohydrazones **1-3**.



Ar = **4**. Ph, 5. 4-MeOPh, **6**. Ph, 4-ClPh

Scheme 2. Preparation of benzophenone hydrazones **4**-**6**.

compounds are shown in Table I.

The results of *in vitro* screening against *L. major* are shown in Table I. All the compounds have been screened for antibacterial and antifungal acitivites and the results are described in Tables II and III, respectively.

DISCUSSION

Chemistry

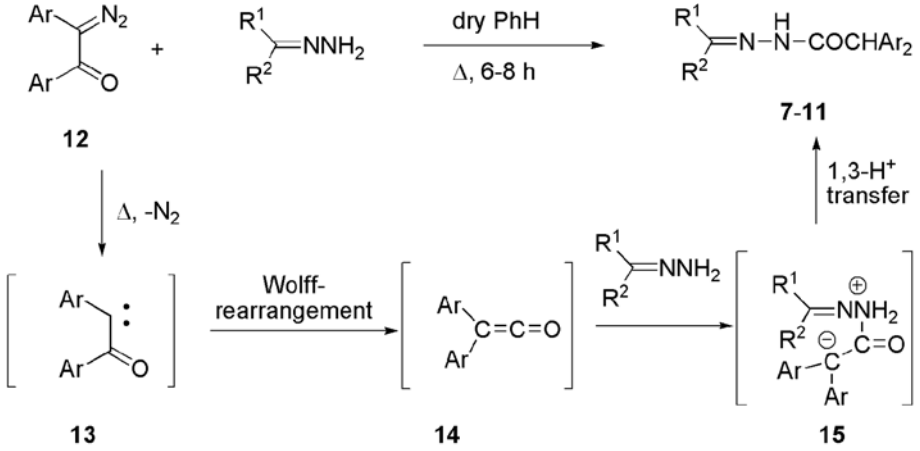
All the hydrazones were characterized on the basis of comparison of m.p. and spectral (IR, and NMR) data with the literature value (Nenitzescu and Solomonica, 1950; Baltzly et al., 1961; Singh et al., 1984; Singh, 2004). Briefly, the IR spectra of hydrazones **1**-**3** exhibited four absorption bands at 3400-3430 cm^{-1}, 3280-3305 cm^{-1}, 1620-1630 cm^{-1}, and 1605-1610 cm^{-1}. The first two bands correspond to the symmetric and asymmetric stretching vibrations of the amino group whereas the latter two bands correspond to the carbonyl group and azomethine linkage, respectively. The IR spectra of hydrazones **4**-**6** showed two high frequency absorption bands - one in the range of 3380-3410 cm^{-1} and other in the range of 3280-3295 cm^{-1} due to amino group. The azomethine linkage in hydrazones **4**, **5** and **6** showed absorptions at 1610 cm^{-1}, 1635 cm^{-1} and 1605 cm^{-1}, respectively. The IR spectra of hydrazones **7**-**11** showed absorptions at 3280 cm^{-1}, 1660 cm^{-1} and 1600 cm^{-1} due to NH, amido carbonyl and azomethine linkage, respectively. The ^{1}H-NMR spectra of hydrazones **7**-**11** displayed the typical singlet signal at around 6.1 ppm corresponding to the methine proton present in the (diaryacyl) group.

Table I. Physical data and antileishmanial activity of hydrazones **1**-**11**

Compds. No.	Yield (%)	m. p. (°C)	Mol Formula*	*L. major**
1	94	149-151	$C_{14}H_{12}N_2O$	0.73 ± 0.03
2	92	141-142	$C_{16}H_{16}N_2O$	0.62 ± 0.08
3	88	145-147	$C_{14}H_{16}N_2O_3$	0.58 ± 0.05
4	85	97-98	$C_{13}H_{12}N_2$	0.62 ± 0.01
5	90	85-86	$C_{15}H_{16}N_2O_2$	0.59 ± 0.01
6	80	103	$C_{13}H_{11}N_2Cl$	0.60 ± 0.02
7	80	147-149	$C_{27}H_{22}N_2O$	0.89 ± 0.04
8	56	119-120	$C_{29}H_{26}N_2O_3$	0.70 ± 0.09
9	52	97-99	$C_{27}H_{21}N_2OCl$	0.90 ± 0.01
10	80	147-148	$C_{29}H_{26}N_2O$	0.59 ± 0.04
11	72	144-146	$C_{29}H_{25}N_2OCl$	0.67 ± 0.02
DMSO as –ve Control				0.99 ± 0.00
Standard Drug IC_{50} (1 μg/mL ± S.D.) (Amphotericin B)				0.56 + 0.06

*Percentage inhibition activity: 0.99 = insignificant; 0.95-0.80 = low; 0.79-0.70 = moderate; 0.69-0.60 = good; < 0.59-0.56 = significant activity.

The formation of *N*-(diaryl)acyl hydrazones **7**-**11** has been explained as shown in Scheme 3 by the reaction of diarylketenes **14**, generated *in situ* by thermal de-



7. $R^1 = R^2$ = Ar = Ph; **8**. $R^1 = R^2$ = 4-MeOPh, Ar = Ph;
9. R^1 = Ph, R^2 = 4-ClPh, Ar = Ph; **10**. $R^1 = R^2$ = Ph, Ar = 4-MePh;
11. R^1 = Ph, R^2 = 4-ClPh, Ar = 4-MePh;

Scheme 3. Preparation of *N*-(diaryl)acyl hydrazones **7**-**11** and mechanism of their formation.

composition of the diazoketones **12** via the Wolff-rearrangement of the initially formed aroyl aryl carbenes **13**, with ketohydrazone leading to formation of the intermediate **15** (Singh, 2004). A 1,3-proton transfer in this intermediate leads to the formation of *N*-(diaryl) acyl hydrazones **7**-**11**.

Antileishmanial activity of hydrazones 1-11

The *N*-unsubstituted hydrazones **3** and **5** bearing strong electron donating methoxy group showed significant activity (0.58 ± 0.05 and 0.59 ± 0.01, respectively) against *L. major* which were almost parallel to the standard drug amphotericin. The hydrazones **1**, **2**, **4**, **6** and **11** showed good activity. The hydrazone **8** showed moderate activity on *L. major*. Thus, the *N*-unsubstituted hydrazones were observed more active than the *N*-substituted series. The high *in vitro* antileishmaniasis activity of these compounds makes them promising leads for development of effective therapeutic agents.

Antibacterial activity of hydrazones 1-11

According to the data shown in Table II, all the hydrazones exhibited good to moderate inhibitory activity against the two Gram (+) strains, *S. aureus* and *B. subtilis* compared to the standard drug imipenem at the tested concentration. Many of them were moderately active against *K. pneumoniae*. Almost all of them were inactive against *E. coli*. The two most active hydrazones against *B. subtilis* are **3** (with 62% inhibition zone) and **11** (with 67% inhibition zone). The MIC of compounds **3** and **11** for this strain was observed as 125 and 500 g/mL, respectively. The hydrazone **9** with 48% inhibition zone (MIC = 500 g/mL) was the most active against *S. aureus*.

Table II. Antibacterial activity of hydrazones **1**-**11**

Compds. No.	Gram (+) strains		Gram (–) strains	
	S. aureus	*B. subtilis*	*K. pneuminiae*	*E. coli*
1	–	5*	6	–
2	6	8	–	–
3	2	11	7	–
4	–	–	2	–
5	8	4	5	–
6	3	6	–	–
7	6	8	2	–
8	1	4	3	–
9	10	7	4	–
10	6	8	2	1
11	9	12	4	–
DMSO as –ve Control	0	0	0	0
Imipenem Standard drug (2 mg/1 mL)	21	18	18	16

*Zone of inhibition (radius, mm); concentration used: 2 mg/1 mL; no activity

Table III. Antifungal activity of hydrazones **1**-**11**

Compds. No.	*C. albicans*	*M. canis*	*F. solani*
1	20*	–	24
2	30	27	16
3	41	32	20
4	–	21	29
5	52	33	38
6	–	30	–
7	38	63	22
8	43	–	27
9	62	34	28
10	31	21	24
11	55	60	20
DMSO as –ve Control	0	0	0
miconazole Standard drug (2 mg/1 mL)	110.8	98.4	73.25

*Percentage inhibition activity: 0-39 = low (insignificant); 40-59 = moderate; 60-69 = good; > 70 = significant activity.

Antifungal activity of hydrazones 1-11

It is evident from the antifungal bioassay that the compound **9** has good activity against *C. albicans* whereas compounds **3**, **5**, **8** and **11** exhibit modearte activity against this fungus. Compounds **7** and **11** have good activity against *Microsportum canis*. All the compounds showed only poor or insignificant activity on *F. solani*.

In conclusion, the study reports for the first time observation of significant antileishmanial activity in some simple hydrazones and good to moderate antibacterial and antifungal activities. The high *in vitro* antileishmaniasis activity of compounds **3** and **5** makes them promising leads for development of effective antileishmanial agents.

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