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Microwave Assisted Synthesis and Evaluation of N-cinnamoyl aryl hydrazones for Cytotoxic and Antioxidant Activities

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ABSTRACT

A series of N-cinnamoyl aryl hydrazones 2a-2i were synthesized in good yields by microwave irradiation technique. The title compounds were formed by nucleophilic condensation of various N^1- substituted benzylidene-2-cyano aceto hydrazides with N,N-dimethyl amino benzaldehyde. The intermediate N^1- substituted benzylidene-2-cyano aceto hydrazide was obtained by condensing various substituted benzaldehydes with cyanoacetohydrazide. The structures of the compounds were characterized by IR, ^{1}H NMR and Mass spectra. The antioxidant activity was studied by reduction of DPPH, scavenging of nitric oxide and hydrogen peroxide methods with ascorbic acid as the standard drug. The compounds were evaluated for cytotoxic activity by BSLT method and their ED_{50} values were compared the standard podophyllotoxin. Among the compounds evaluated, N^1- benzylidene-2-cyano-3-(4-dimethylamino) phenyl acrylo hydrazide (2a) and N^1- (4-methoxy-benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazide (2e) showed good antioxidant activity towards all the three models .The compounds 2a and 2e showed ED_{50} values 3.07 µg/ml and 3.7 µg/ml respectively which were compared against the standard podophyllotoxin (1.64 µg/ml).

Keywords: Cinnamoyl hydrazones, Cyanoacetohydrazide, ED_{50}, BSLT, Cytotoxic,DPPH, Nitric Oxide, Hydrogen Peroxide.

INTRODUCTION

Acyl hydrazone derivatives possessing an azometine -NHN=CH- proton are emerging as a novel class of compounds for new lead development[1-4].They were reported to possess, antimicrobial[5-6], antitubercular[7], antitumour[8-9], antioxidant[10-12], antiinflammatory[13], analgesic[14], antimalarial[15], anti platelet activities[16-17] etc . It was reported that the presence of styryl ketone moiety in the titled compounds were found to be potent scavengers of free radicals. In view of these observations it was considered of interest to synthesise a new class of acyl hydrazones by

incorporating the styryl carbonyl moiety along with aryl hydrazone unit. The aim of present research was to synthesize various N^1-(substituted benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazides and to evaluate for their *in-vitro* antioxidant and cytotoxic activities.

MATERIALS AND METHODS

All the chemicals and solvents used in the present study were purchased from Merck, Hi media, S.D. fine Chemicals limited, Mumbai and Sigma Aldrich, USA. Melting points were determined in an open capillary tube in Thermonik precision melting point cum boiling point (C-PMB) apparatus and are uncorrected. Silica gel G coated on laboratory micro slides prepared by dipping method were used. IR spectra (KBr discs) were confirmed by Shimadzu FT-IR spectrophotometer using KBr pellets technique, ^{1}H NMR spectra were recorded on Bruker 300 MHz NMR spectrometer using DMSO as solvent. Mass spectra were recorded on Apex mass spectrophotometer.

Chemistry

General method of synthesis of compounds (1a-1i)

To 0.01 mol of various substituted benzaldehyde , 0.01 mol of cyanoacetohydrazide was added in few ml of ethanol followed by few drops of glacial acetic acid and irradiated in microoven for 1-3 minutes at 140 watts .The reaction was monitored by TLC and the solid formed was collected and recrystallized from methanol.

General method of synthesis of compounds (2a-2i)

To 0.01 mol of various N^1- substituted benzylidene-2-cyanoacetohydrazides, 0.01mol of N,N-di methylamino benzaldehyde was added in few ml of ethanol followed by few drops of pyridine and irradiated in microoven for 1 -3 minutes at 140 watts. The reaction was monitored by TLC and the solid formed is collected and recrystallized from methanol.

The physical data of compounds 2a-2i was tabulated in table-1.

N^1-benzylidene-2-cyano-3-(4-dimethylamino) phenyl acrylo hydrazide (2a)

Mol. Formula $C_{19}H_{18}ON_4$, Yield : 80 % ; m.p.: 161-162 ^{0}C ; IR (KBr) cm^{-1}: 3209 (N-H), 3075 (Ar-H), 2258 (C≡N), 1671(C=O), 1551 (C=C) . ^{1}H NMR (300 MHz, DMSO-d_6); δ 3.1 (s,6H ,N$(CH3)_2$), 6.8-7.77 (m,9H, Ar-H) , 8.0(s,IH,C=CH), 8.2(s,IH,N=CH),9.7(s 1H,-CONH); Mass: m/z (M±1) 318, $(M+H)^+$ 319

N^1- (3,4-dimethoxy-benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazide (2b)

Mol. Formula $C_{21}H_{22}N_4O_3$, Yield : 95 %; m.p.: 173-176^0 C ; IR (KBr) cm^{-1} : 3307 (N-H) , 3068 (Ar-H), 2253 (C≡N) , 1665(C=O), 1566 (C=C). ^{1}H NMR (300

Table 1: Physical data of N^1-benzylidene-2-cyano-3-(4-dimethylamino)phenyl acrylohydrazides (2a-2i)

Compound code	R_1	R_2	R_3	M.P(^{o}C)	Yield (%)	Molecular Formula
2a	H	H	H	161-162	80	$C_{19}H_{18}N_4O$
2b	OCH_3	OCH_3	H	173-176	85	$C_{21}H_{22}N_4O_3$
2c	OCH_3	OH	H	178-179	84	$C_{20}H_{18}N_4O_2$
2d	OCH_3	OCH_3	OCH_3	161-166	82	$C_{22}H_{24}N_4O_4$
2e	H	OCH_3	H	167-175	89	$C_{20}H_{20}N_4O_2$
2f	H	OH	H	160-162	60	$C_{19}H_{18}N_4O_2$
2g	H	4-CH_3	H	178-179	50	$C_{20}H_{20}N_4O$
2h	H	$N(CH_3)_2$	H	158-160	49	$C_{21}H_{23}N_4O$
2i	3-NO_2	H	H	178-179	76	$C_{19}H_{17}N_5O_3$

MHz,DMSO- d_6); ä 3.0 (s,6H ,$N(CH_3)_2$), 3.7-3.8 (s,6H,-OCH_3), 6.7-7.7 (m,7H, Ar-H), 7.9(s,IH,C=CH), 8.0 (s,IH,N=CH) , 9.6(s 1H,-CONH)

N^1-(3-methoxy,4-hydroxy-benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazide(2c)

Mol. formula $C_{19}H_{18}N_4O_2$, Yield : 94%; m.p.:178-179^{0}C ; IR (KBr) cm^{-1} : 3442 (N-H) , 3078 (Ar-H), 2239 (C≡N), 1653(C=O), 1530 (C=C). ^{1}H NMR (300 MHz,DMSO- d_6) ; δ 3.0 (s,6H ,$N(CH_3)_2$), 3.7 (s,3H,OCH_3), 6.7-6.8 (m,7H,Ar-H) , 7.8 (s,IH,C=CH), 8.0(s,IH,N=CH), 9.6(s ,1H,-CON-H)

N^1- (3,4,5-trimethoxy -benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazide (2d)

Mol. Formula $C_{22}H_{24}N_4O_4$, Yield :92 %; m.p.161-166 ^{0}C;IR-(KBr) cm^{-1} : 3253 (N-H), 3068 (Ar-H), 2319 (C≡N),1660 (C=O) , 1521 (C=C). ^{1}H NMR (300 MHz,DMSO- d_6) ; δ 3.0 (s,6H, $N(CH3)_2$) 3.7-3.8 (s,9H,OCH_3), 6.7-8.0(m,6H,Ar-H), 9.6(s, 1H,-CONH),MASS:m/z (M±1) 408, $(M+H)^+$ 409

N^1- (4-methoxy-benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazide (2e)

Mol. formula $C_{20}H_{20}N_4O_2$, Yield :89 %; m.p.167-175 ^{0} C ; IR (KBr) cm^{-1} : 3213 (N-H) , 3088 (Ar-H), 2320 (C≡N) , 1672 (C=O), 1564 (C=C) .^{1}H NMR (300 MHz,DMSO- d_6) ; δ 3.0 (s,6H ,$N(CH_3)_2$), 3.7 (s,3H,-OCH_3), 6.7-7.6(m, Ar-H), 7.9(s,1H, C=CH) , 8.1(s,1H,N=CH), 9.6(s, 1H,-CONH)

N^1- (4-hydroxy-benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazide (2f)

Mol. formula $C_{19}H_{18}N_4O_2$, Yield :60 %; m.p.160-162 ^{0} C ; IR (KBr) cm^{-1} : 3282 (N-H) , 3095 (Ar-H), 2232 (C≡N), 1628(C=O), 1553 (C=C). ^{1}H NMR (300 MHz,DMSO- d_6) ; δ 3.0 (s,6H ,$N(CH_3)_2$), 6.7-7.5 (m,8H,Ar-H), 7.9(s,1H,C=CH) , 8.1(s,1H,N=CH), 9.6(s ,1H,-CONH)

N^1- (4-methyl-benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazide (2g)

Mol. formula $C_{20}H_{20}N_4O$, Yield : 50 %; m.p.178-179 ^{0} C ; IR (KBr) cm^{-1} : 3260 (N-H) , 3100 (Ar-H), 2321 (C≡N) , 1688(C=O), 1511 (C=C). ^{1}H NMR (300 MHz,DMSO- d_6) ; δ 1.9 (s,1H,CH_3), 3.0 (s,6H ,$N(CH_3)_2$), 6.7-7.7(m,8H,Ar-H), 7.9(s,IH, C=CH), 8.1(s,IH,N=CH) , 9.6(s ,1H,-CONH)

N^1- (4 - dimethylamino -benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazide (2h)

Mol. formula $C_{21}H_{23}N_4O$, Yield :89 %; m.p.158-160 ^{0} C ; IR (KBr) cm^{-1} : 3242(N-H), 3076 (Ar-H), 2325 (C≡N), 1683(C=O), 1543 (C=C). ^{1}H NMR (300 MHz,DMSO- d_6) ; δ 2.9-3.0 (s,12H, $N(CH_3)_2$), 6.7-7.5(m,8H, Ar-H), 7.9(s,IH,C=CH), 8.0(s,IH,N=CH) , 9.6(s ,1H,-CONH)

N^1- (3-nitro -benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazide (2i)

Mol. formula $C_{19}H_{17}N_5O_3$, Yield :89 %; m.p.178-179^0 C ; IR (KBr) cm^{-1} : 3222 (N-H) , 3086 (Ar-H), 2285 (C≡N) , 1628(C=O), 1513 (C=C). ^{1}H- NMR (300 MHz,DMSO- d_6) ; δ 3.0 (s,6H, $N(CH_3)_2$), 6.7-7.7(m,8H,Ar-H), 7.9(s,1H,C=CH), 8.1(s,1H,N=CH) , 9.6(s ,1H,-CONH)

Cytotoxic activity

Brine shrimp lethality test[18]

Brine Shrimp (*Artemia salina*) nauplii were hatched in sterile brine solution (prepared using sea salt 38g/L and adjusted the pH to 8.5 using 1N NaOH) under constant aeration for 48 hr. After hatching,10 nauplii were placed in each vial and added various concentrations of drug solutions in a final volume of 5 mL, maintained at 37°C for 24 h under the light of incandescent lamps and surviving larvae were counted . Each experiment was conducted along with control (vehicle treated), at various concentrations of the test substances. Percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. ED_{50} values were obtained by using Finney probed analysis software .The result for test compound was compared with the positive control podophyllotoxin.

Antioxidant activity

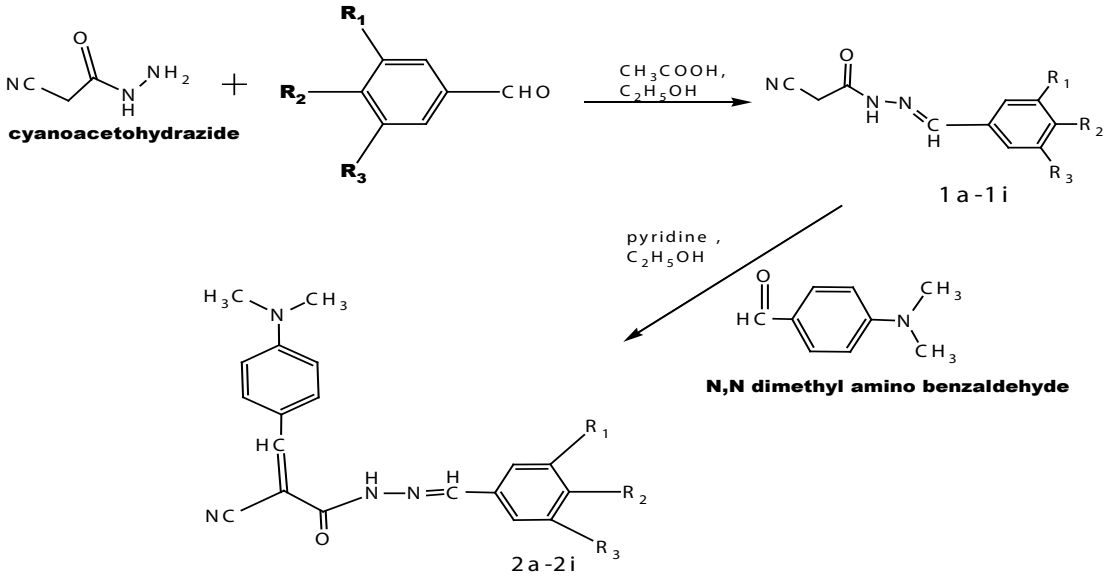
Determination of nitric oxide Scavenging Activity[19]

Nitric oxide scavenging activity of samples was determined by the following procedure. 2ml (10mM) of sodium nitro prusside dissolved in 1.5ml phosphate buffer saline (PH-7.4) and 1 ml of different test samples corresponding to 100µM concentration was added in different test tubes respectively and incubated at 25^{O}C for about 150 min. From this 0.5ml

was taken and 1ml sulphanilic acid reagent (33% in 20% glacial acetic acid) was added and incubated at room temperature for 5min. 1ml of naphthyl ethylene diamine dihydro chloride (0.1% w/v) was added and again incubated at room temperature for 30min, then measured the absorbance at 540 nm in spectrophotometer.

Determination of the effect of samples on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Radical [19]

DPPH scavenging activity was assessed according to the reported method. Solutions of various test samples at 100µM concentration were added to 100µM DPPH in 95 % ethanol and tubes were kept at an ambient temperature for 20-30 min



Scheme

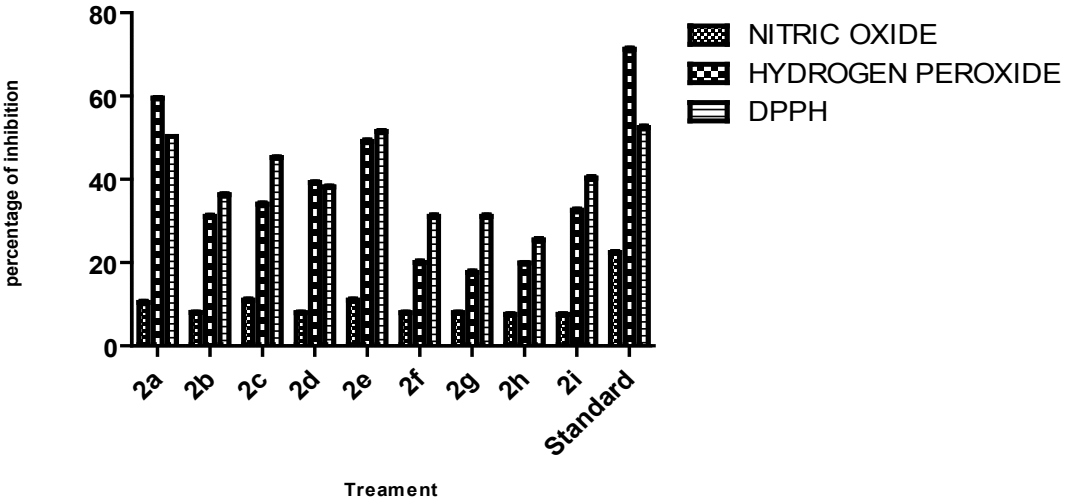


Fig.1 :

Table 2: Antioxidant activity and Cyotoxic activity of compounds (2a-2i)

Compound	% Inhibition of NO	% Inhibition of H_2O_2	% Inhibition of DPPH	ED_{50} (µg/ml)
2a	10	59	50	3.07
2b	8	31	36	5.28
2c	11	34	45	6.37
2d	8	39	38	10.50
2e	11	49	51	3.77
2f	8	20	31	-
2g	8	18	31	-
2h	7	20	25	4.07
2i	7	32	40	-
Ascorbic acid	22	71	52	-
Podopyllotoxin	-	-	-	1.64

and absorbance was measured at 517 nm. Ethanol was used as blank and DPPH solution in ethanol served as the control. The effect of Ascorbic acid on DPPH was also assessed for comparison with that of samples.

Determination of Hydrogen Peroxide Scavenging Activity[20]

4mM solution of H_2O_2 was prepared in phosphate - buffered saline (PBS, pH 7.4). H_2O_2 concentration was determined spectrophotometrically from absorbance at 230 nm using molar absorptivity 81 $M^{-1} cm^{-1}$. 1 ml of different samples corresponding to 100µM concentration were added to 0.6ml hydrogen peroxide- PBS solution respectively and control without sample. Absorbance of H_2O_2 at 230nm was determined 10 minutes later against a blank solution .

RESULTS AND DISCUSSION

Chemistry

A series of N^1-benzylidene-2-cyano-3-(4-dimethylamino)phenyl acrylohydrazides **(2a-2i)** were synthesized by two step procedure .In the first step various N^1-substituted benzylidene-2-cyanoacetohydrazides were synthesized by taking various substituted aromatic aldehydes and cyanoacetohydrazide in few ml of ethanol by adding a few drops of glacial acetic acid and irradiated in microoven for 1 -3 minutes at 140 watts . The free amino group of cyanoacetohydrazide was condensed with carbonyl group of aldehyde to form schiffs linkage. In the second step the various N^1- substituted benzylidene-2-cyanoacetohydrazides are condensed with N,N–dimethylamino benzaldehyde at the electrophilic carbon of cyanoacetohydrazide . The structures of these compounds were established by means of their TLC, IR, ^{1}H NMR and Mass spectra. The synthesized compounds were evaluated for *in-vitro* antioxidant activity. Among the nine compounds synthesized six were evaluated for *in-vitro* cytotoxic activity.

Antioxidant activity

The *in-vitro* antioxidant activity was evaluated by the reported methods of DPPH, nitric oxide and hydrogen peroxide . The results of antioxidant activities of the synthesized compounds were shown in table-2. The unsubstituted (2a) and 4-methoxy derivatives (2e) showed good scavenging activity towards all the three models at 100µM concentration , when compared with the standard ascorbic acid. From the structure activity relationship studies of the compounds **2a-2i** , it was observed that the nature of the substitution on the benzylidene moiety affects the activity. Almost all the compounds showed good to moderate percentage scavenging activity towards DPPH and NO radicals . The moderately electron releasing methoxy derivatives showed good to moderate activity towards H_2O_2

model. The electron releasing like 4-CH_3 and N,N-dimethylamino derivatives showed less scavenging activity towards hydrogen peroxide radical when compared with the standard.

Cytotoxic activity

The brine shrimp lethality test was performed in order to evaluate the cytotoxic nature of the compounds. ED_{50} values were calculated, based on the percentage of larvae survived at different concentrations of test and standard drugs. Compounds **2a** (3.07 µg/ml) and **2e** (3.7 µg/ml) showed good ED_{50} .The results are given in **table-2.** It was observed that the compounds with unsubstituted and 4-methoxy derivatives showed good activity.

CONCLUSION

In the present study we have described the synthesis, *invitro* cytotoxicity screening and antioxidant study of various N^1- (substituted benzylidene)-2-cyano-3-(4-dimethylamino) phenyl acrylohydrazides. From the results it was evident that the further substitution and modification on the benzylidene moiety brings a new lead molecule.

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