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Research Article

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Synthesis and Evaluation of n-acyl aryl Hydrazones for Antimicrobial and Anthelminthic Activities

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ABSTRACT

A series of N-acyl aryl hydrazones (1a-10a) were synthesized by refluxing 2-naphthaldehyde with various substituted cyanoacetohydrazones (1-10). The substituted cyanoacetohydrazones were prepared by treating various substituted aromatic aldehydes with cyanoacetohydrazide. The chemical structures of synthesized compounds were confirmed on the basis of their spectral data. The synthesized compounds were evaluated for antimicrobial activity against bacterial strains Staphylococcus aureus (gram positive), Escherichia coli (gram negative) and fungal strains Penicillium chrysogenum, Penicillium notatum and Aspergillus niger by diffusion assay method. The compounds were also evaluated for anthelminthic activity using earthworms (Pheretima posthuma). Among the derivatives 4-cinnamyl (7a) and 4-pyridyl (8a) showed good potent antimicrobial and antihelminthic activities when compared against their standard drugs oxytetracycline, fluconazole and albendazole respectively.

Keywords: N Acyl hydrazones; Cyanoacetohydrazide; Anti-microbial activity; Antihelmintic activity

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INTRODUCTION

Hydrazone derivatives of carbonyl compounds which possess the structure R_1R_2C=NNHCOR constitute an important class of biologically active compounds [1,2]. Literature studies on hydrazones have shown that these derivatives possess a wide variety of biological activities such as antimicrobial [3,4], antitubercular [5], antitumor [6,7], antioxidant [8-11], anti- inflammatory[12], analgesic[13], antimalarial[14] and antiprotozoal [15] etc. From the literature studies it was revealed that various substitutions on the acyl carbon and imine carbon were significantly effect the reactivity of hydrazone moiety. Again in the search of better pharmacophore, it was observed that the napthalene has occupied a central place among medicinally important compounds due their diverse and interesting antibiotic properities with minimum toxicity. Napthalene derivatives were identified as potent antimicrobials effective against wide range of human pathogens [16]. In view of these observations it was considered of interest to synthesize a new class of acyl hydrazones, by incorporating napthy-2-lidene on acyl moiety and bring various aryl and heteroaryl substitutions on imine carbon and to study the possible contribution of both the functionalized units by evaluating for antimicrobial and antihelminthic activities.

EXPERIMENTAL SECTION

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) Bruker analyzers were confirmed by Shimadzu FT-IR Spectrophotometer using KBr pellets technique, 1H NMR spectra were recorded on Bruker 300 MHz NMR spectrometer using DMSO as solvent. Mass spectra were recorded on Apex mass spectrophotometer.

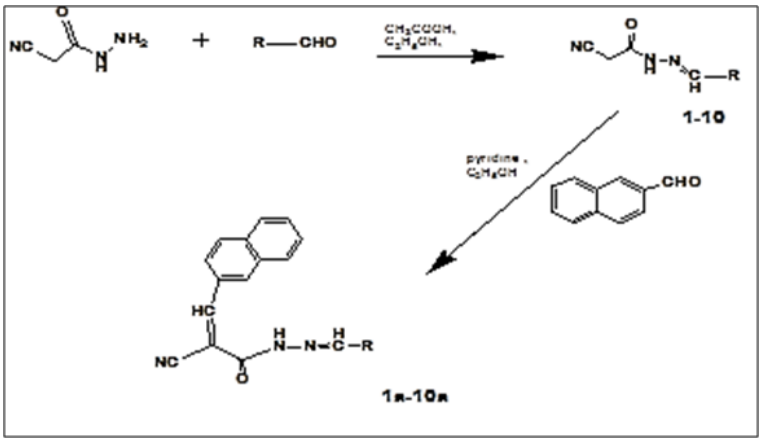
Chemistry

General method of synthesis of compounds (1-10):

To 0.01 mol of benzaldehyde, 0.01 mol of cyanoacetohydrazide was added in few ml of ethanol followed by few drops of glacial acetic acid and stirred for 4 hours. The reaction was monitored by TLC and the solid formed was collected, washed with water and recrystallized from methanol. The various N^1- substituted benzylidene-2-cyanoacetohydrazides were prepared by similar procedure.

General method of synthesis of compounds (1a-10a):

To 0.01 mol of various N^1- substituted benzylidene-2-cyanoacetohydrazides, 0.01 mol of naphthaldehyde was added in few ml of ethanol followed by few drops of pyridine and refluxed for one hour at 60°C. The reaction was monitored by TLC and the solid formed is collected, washed with water and recrystallized from methanol.



Scheme

N^1-benzylidene-2-cyano-3-(2-naphthyl) phenyl acrylo hydrazide (1a):

Yield: 80 %; M.P:167-168°C; IR (KBr) cm^{-1}: 3411(N-H), 2255(C≡N), 1661(C=O), 1591 (C=N), 1537 (C=C). ^{1}H NMR (300 MHz, DMSO-d6): δ 7.4-7.77 (m, 12H, Ar-H), 8.5(s,IH,C=CH), 8.9(s, IH, N=CH),10.4(s 1H, CONH);

Mass: m/z (M±1) 325, $(M-H)^-$324

N^1-(3-methoxy, 4-hydroxy-benzylidene)-2-cyano-3-(2-naphthyl) phenyl acrylohydrazide (2a):

Yield: 90%; M.P:182-184 °C; IR (KBr) cm^{-1}: 3430 (N-H), 2255 (C≡N), 1644(C=O), 1609 (C=N), 1503 (C=C). ^{1}H NMR (300 MHz,DMSO- d6); 3.8(s,3H,OCH_3), 8.1-8.2(m,10,Ar-H), 8.3 (s,IH,C=CH), 8.9(s, IH, N=CH), 9.6(s, 1H, CON- H)

N^1-(3,4,5-trimethoxy -benzylidene)-2-cyano-3-(2-naphthyl) phenyl acrylohydrazide (3a):

Yield: 82%; M.P:192- 198 °C; IR-(KBr) cm^{-1}: 3432 (N-H), 2251(C≡N), 1663 (C=O), 1573 (C=N), 1504 (C=C). ^{1}H NMR (300 MHz,DMSO- d_6) ; 3.7-3.8 (s, 9H, OCH_3), 7.6-8.3(m, 9H, Ar-H), 8.4 (s, IH, C=CH), 8.9 (s, IH, N=CH), 9.6(s, 1H,-CONH)

N^1- (4-methoxy-benzylidene)-2-cyano-3-(2-naphthyl) phenyl acrylohydrazide (4a):

Yield: 86%;M.P :185-186 °C; IR (KBr) cm^{-1}: 3436(N-H), 2255 (C≡N), 1671 (C=O), 1605 (C=N), 1561 (C=C) .^{1}H NMR (300 MHz,DMSO- d6); δ 3.8 (s,3H,-OCH_3), 7.0-7.9(m,Ar-H), 8.4(s,1H,C=CH) , 8.9(s,1H,N=CH) , 9.6(s, 1H,-CONH)

N^1- (3-nitro-benzylidene)-2-cyano-3-(2-naphthyl) phenyl acrylohydrazide (5a):

Yield: 85 %; M.P: 210-213 °C; IR (KBr) cm^{-1}: 3432(N-H), 2254 (C≡N), 1693(C=O), 1610 (C=N), 1525 (C=C) .^{1}H-

NMR (300 MHz,DMSO- d6) ; δ 7.6-8.3 (m,11H,Ar-H), 8.6 (s,1H,C=CH) , 8.9 (s,1H,N=CH-Ar) ,10.4 (s 1H,-CONH) Mass: m/z (M±1) 370, $(M-H)^-$369,$(M+H)^+$371

N^1-(4-cyano-benzylidene)-2-cyano-3-(2-naphthyl) phenyl acrylohydrazide (6a):

Yield: 89%; M.P212-214°C; IR (KBr) cm^{-1}: 3436 (N-H), 2255 (C≡N), 1688 (C=O), 1615 (C=N), 1511 (C=C). ^{1}H NMR (300 MHz,DMSO-d6); δ7.6-8.0(m,8H,Ar-H), 8.5(s,IH,C=CH) , 9.1(s,IH,N=CH) , 10.4(s ,1H,-CONH)

N^1- cinnamyl-2-cyano-3-(2-naphthyl) phenyl acrylohydrazide (7a):

Yield: 80%; M.P: 186-188°C; IR (KBr) cm^{-1}: 3307 (N-H), 2253 (C≡N), 1665(C=O), 1601 (C=N), 1566 (C=C). ^{1}H NMR (300 MHz,DMSO- d6) ; δ 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- (4-pyridyl)-2-cyano-3-(2-naphthyl) phenyl acrylohydrazide (8a):

Yield: 82%; M.P195-199°C; IR (KBr) cm^{-1}: 3432 (N-H), 2232 (C≡N) , 1628(C=O), 1648 (C=N), 1553 (C=C) . ^{1}H NMR (300 MHz,DMSO- d6) ; δ 6.7-7.5(m,11H,Ar-H), 7.9(s,1H,C=CH) , 8.1(s,1H,N=CH) , 9.6(s ,1H,-CONH)

N^1- (2 -thiophenyl)-2-cyano-3-(2-naphthyl) phenyl acrylohydrazide (9a):

Yield: 80 %; M.P 220-222°C; IR (KBr) cm^{-1}: 3432(N-H), 2325 (C≡N) , 1683(C=O), 1610 (C=N), 1543 (C=C). ^{1} H NMR (300 MHz,DMSO- d6) ; δ 7.6-8.0(m,10H, Ar-H), 8.5(s,IH,C=CH) , 9.1(s,IH,N=CH) , 10.4(s,1H,-CONH)

N^1- (3-indolyl)-2-cyano-3-(2-naphthyl) phenyl acrylohydrazide (10a):

Yield: 80%; M.P:172-173°C; IR (KBr) cm^{-1}: 3372(N-H), 2325 (C≡N) , 1683(C=O), 1653 (C=N), 1543 (C=C). ^{1}H NMR (300 MHz,DMSO- d6) ; δ 6.7-7.5(m,13H, Ar-H), 7.9(s,IH,C=CH) , 8.0(s,IH,N=CH), 9.6(s ,1H,-CONH), 11.22 (1H, indole-NH)

Biological evaluation

Antimicrobial activity:

All the synthesized compounds were examined for invitro antibacterial activity against two bacteria strains *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) and three different fungal strains *Penicillium chrysogenum* , *Penicillium notatum* and *Aspergillus niger* by cup plate method using nutrient agar medium[17-18]. The hole of 8mm diameter were punched carefully using a sterile cork borer and these were filled with test solutions of different concentrations corresponding to 50 µg/ml, 100 µg/ml, 200 µg/ml in one petri plate and in another plate cups were made for standard and control. The plates thus prepared were left for 90 minutes in a refrigerator for diffusion. After incubation for 24 hours at 37℃ the plates were examined for inhibition zones

Antihelmintic activity:

Indian adult earthworms of the genus and species, *Pheritima posthuma* (family: megascolecidae), were used to study the antihelmintic activity [19]. The earthworms were collected from the water logged areas of soils in Vijayawada (Andhra Pradesh, India) were washed with normal saline to remove all the fecal matter and waste surrounding their body. The earth worms (*Pheritima posthuma*) 5-8 cm in length and 0.2-0.3 cm width weighing 0.8–3.04g were used for all experiment protocols. The earthworms resemble the intestinal roundworm parasites of human beings both anatomically and physiologically and hence were used to study the antihelmintic activity. Gum acacia solution (1%) prepared in normal saline, 50 mg/ml, 100mg/ml of test and 50mg/ml of standard was prepared by using this solution. Sample were taken in petriplates and adult healthy earth worms (n=6) were introduced into them. Observations were made for the time taken to paralyze and time taken for death of the organism. Paralysis was said to occur when the worms do not survive even in normal saline. Death was concluded when worms lost their motility followed by fading away of their body color.

RESULTS AND DICUSSION

Chemistry

A series of N^1-benzylidene-2-cyano-3-(2-naphthyl) acrylohydrazides (1a-10a) were synthesized by two step procedure. In the first step various N^1-substituted benzylidene-2-cyanoacetohydrazides were synthesized by taking various substituted aromatic/heterocyclic aldehydes and cyanoacetohydrazide in few ml of ethanol by adding few drops of glacial acetic acid. The free amino group of cyanoacetohydrazide was condensed with carbonyl group of

aldehyde to form imine linkage. In the second step the various N^1- substituted benzylidene-2-cyanoacetohydrazides were condensed with the 2-naphthaldehyde at the electrophilic carbon of cyanoacetohydrazide . The products are obtained in good yields with high purity. All the synthesized hydrazone structures were characterized by their TLC, IR, ^{1}H-NMR and mass spectra. The IR spectra of compounds (1a-10a) showed absorption bands at 1628 - 1693 cm^{-1} due to the presence of C=O functional group, while the bands observed at 1573 - 1653 cm–1 corresponded with C=N linkage and 3307 - 3436 cm–1 observed due to -NH group. From the ^{1}H NMR spectra, the structures of the synthesized compounds (1a-10a) were confirmed on the basis that the CONH δ protons appearing as singlets resonated at δ values between 9.6 and 10.4 and a singlet due to the azomethine (N=CH) group appeared at *δ* values between 8.0 - 9.1 in all the compounds. The aromatic protons showed δ values between 6.7-8.9 ppm.

Biological evaluation antimicrobial activity

The synthesized compounds were evaluated for antibacterial, antifungal activity and the diameter of zones of inhibition was measured in millimeters and results were compared with that of standard oxytetracycline and fluconazole respectively. Among the series of compounds the 4-cinnamyl (7a) and the heterocyclic moiety 4-pyridyl (8a) showed good antibacterial and antifungal activities .The compounds unsubstituted (1a), 3,4,5- trimethoxy (3a) and 4-methoxy (4a) were found to be inactive to all species at 50 µg/ml and 100 µg/ml and shown less to moderate activity at 200 µg/ml. The other derivatives 2a,5a,6a ,9a and 10a showed moderate to good antibacterial and antifungal activity. The results were represented in table 1.

Table 1: Antimicrobial activity

\Compound	R	*Escherichia coli*			*Staphylococcus aureus*			*Pencillinium notatum*			*Pencillinium crysogenum*			*Aspergillus niger*		
		Concentration in micrograms per milliliter (µg/ml)														
		50	**100**	**200**	**50**	**100**	**200**	**50**	**100**	**200**	**50**	**100**	**200**	**50**	**100**	**200**
1a	Phenyl-	0	0	11	0	0	11	0	0	10	0	0	11	0	0	10
2a	(4-hydroxy,3- methoxy) phenyl-	8	10	16	8	10	15	0	8	12	8	11	14	8	10	18
3a	(3,4,5-tri methoxy) phenyl-	0	0	10	0	0	10	0	0	11	0	0	12	0	0	16
4a	(4-methoxy) phenyl-	0	0	11	0	0	11	0	0	12	0	0	14	0	0	14
5a	(3-nitro) phenyl-	8	11	22	8	12	20	8	13	22	8	11	23	8	11	15
6a	(4-cyano) phenyl-	12	16	23	11	14	22	10	18	23	10	19	24	10	20	23
7a	Cinnamyl-	18	20	24	17	20	24	18	20	25	18	20	25	17	20	25
8a	4-pyridyl	20	21	25	20	22	25	18	19	24	16	20	25	17	19	25
9a	2-Thiophenyl	12	17	18	10	11	17	12	14	18	11	12	18	11	12	16
10a	3-Indolyl	14	16	22	16	17	22	18	20	25	20	24	26	20	22	25
standard	oxytetracycli ne	17	19	24	14	16	24									
standard	fluconazole							16	18	24	15	18	24	14	17	24

Table 2: Anthelmintic activity

Compound	R	Concentration in mg/ml	Time taken for paralysis in minutes	Time taken for death in minutes
1a	Phenyl-	50	25	19
		100	20	14
2a	(4-hydroxy,3-methoxy) phenyl-	50	30	20
		100	20	20
3a	(3,4,5-tri methoxy) phenyl-	50	25	20
		100	19	17
4a	(4-methoxy) phenyl-	50	25	20
		100	20	18
5a	(3-nitro) phenyl-	50	4	10
		100	2	5
6a	(4-cyano) phenyl-	50	4	5
		100	2	2
7a	Cinnamyl-	50	8	5
		100	3	5
8a	4-pyridyl	50	6	6
		100	2	2
9a	2-Thiophenyl	50	15	14
		100	10	10
10a	3-Indolyl	50	5	10
		100	2	5
Standard	Albendazole	50	20	16
		100	18	12

Anthelmintic activity

Among the test compounds 5a, 6a, 7a, 8a, 9a and 10a showed good anthelmintic activity when compared with the standard albendazole. The unsubstituted (1a), vanillyl (2a), 3,4,5- trimethoxy (3a) and 4-methoxy (4a) derivatives were found to be less active than the standard albendazole. (Table 2)

CONCLUSION

In the present study we have described the synthesis by simple and effiecient techniques, under mild conditions. The antimicrobial and antihelmintic activity of various N^1-(substituted benzylidene)-2-cyano-3-(2-naphthyl)acrylohydrazides were found to be moderate to good. The acyl hydrazone derivatives containing cinnamyl, (4-cyano) phenyl and heterocyclic moieties4-pyridyl, 3-indolyl on the imine carbon showed good antibacterial, antifungal and anthelmintic activities. It is hopeful to study toxicities and QSAR in order to improve their biological and pharmacological properties.

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