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SYNTHESIS, CHARACTERIZATION AND ANTIFUNGAL ACTIVITY OF COUMARIN CARBOHYDRAZIDE CONTAINING QUINOLINE DERIVATIVES

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ABSTRACT

Ten novel 2-oxo-N[1]-[(E)-quinolin-3-ylmethylidene]-2H-chromene-3-carbo hydrazones were synthesized by the reaction of substituted the 2-oxo-2H-chromene-3-carbohydrazide in N,N-dimethyl form amide various 2-Chloro,2-hydr oxy,2-thione-3-formyl quinolines and tetrazolo[1,5-a]quinoline-4-carbaldehyde in presence of catalytic amount of glacial aceticacid. Newly synthesized compounds were characterized by spectroscopic and physical methods. All the synthesized com pounds were screened for antifungal activities by standard method. Results of the activities reveal that, some compounds exhibited moderate to good antifungal activities.

KEYWORDS

Quinoline, Vilsmayer Haack reagent, Coumarine, Antibacterial and Antifungal activity.

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INTRODUCTION

Coumarin and its derivatives are biologically active compounds and are widely distributed in nature. The coumarin heterocyclic ring is common feature of various bioactive compounds like calanolides, lipid lowering agent. Coumarin and its derivatives exhibit several biological and pharmacological properties such as anticoagulants, anti-fungal, anti-bacterial, insecticidal, anthelminths, hypnotics, phytoalexins, HIV protease inhibitors, CNS depressants, anti-tumors agent, anti-tubercular, anti-inflammatory, anti-oxidant, anti-viral, analgesic, anti-leshmanial,

and ACE inhibitor. As part of a program of medicinal chemistry under-taken a few years ago, we are pursuing investigations on the synthesis and reactivity of heterocycles containing nitrogen. Recently we were confronted with the preparation of quinoline derivatives [1-4].

Quinoline (Figure No.1) and their derivatives occur in numerous natural products. many quinolines display interesting physiological activities and have found important applications as pharmaceuticals(e.g. norfloxacin and ciprofloxacin).moreover fused quinolines are known to bind DNA with high affinity, inhibit DNA topoisomerase and display cytotoxic and antitumor activities anticonvulsant, antidepressant, antimalarial and antihistaminic activities. The vilsmerier-Haack reagent has been proved to be a versatile reagent capable of executing a large variety of synthetic transformations. It finds application in formylation cyclohaloaddition, cyclisation and ring annulations. Recently, its potentiality was explored in the synthesis of 4-(N,N-dimethylaminomethylene)-2-alkyl/aryl-2-oxazolin-5-ones from N-acylderivatives of alpha-amino acid esters and alpha-aminoacetanilides. To develop novel quinoline based fused hetrocyclic systems quinoline nucleus with different substituents at 2-and 3-positions was required which afforded a versatile synthon for further heteroannulations[5-9].

MATERIAL AND METHODS

All chemicals used were of analytical grade from, SD Fine. Melting points of all the synthesized compounds were determined by open capillary tube method. These are uncorrected. The purity of all compounds was checked by TLC was run on Silica Gel G plates using Chloroform: alcohol (9.5:0.5). Spots were visualized using iodine vapour chamber. IR spectra were recorded on Shimadzu IR spectrophotometer by using KBr pellets technique. ^{1}H-NMR was recorded on Bruker AMX 60 MHz spectrophotometer by using DMSO as solvent.

Synthesis of 2-chloroquinoline-3-carboladehyde(3a-d) General procedure: It is followed as per literature Jayakumar swamy B.H.M et al[10].

Synthesis of 2-Hydroxy-3-Formylquinoline-3-carbaldehyde (4a-d)

General procedure: as prescribed in the literature survey pramod *et al*[11].

Synthesis of 2-thiones- 3-formylquinoline(5a)

As prescribed in the literature survey Pramod N *et al*[12].

Synthesis of Tetrazolo[1,5-a]quinoline-4-carbaldehyde(6a)

In dry round-bottomed flask, to a solution of 2-chloroquinoline-3 carbaldehyde (0.001mol 0.191gm) taken in absolute ethanol (5ml) p-toulenesulphonic acid (0.001mol 0.190gm) and sodiumazide (0.0015mol,0.0975gm) were added and reaction mixture was heated under reflux for 65hrs at 125-135^{0}C.After completion of the reaction (monitored by TLC) the reaction mixture was poured into ice cold water (100ml) and the resulting precipitate was filtered, dried and recrystalized from dimethylformamide as whitish light yellow needle shaped crystals M.P. 240-242^0 C, yield76%.

Synthesis of ethyl-2-oxo-2H-chromene-3-carboxylate

In dry round-bottomed flask, containing solution of Salicyaldehyde (0.01 mol, 1.22 gm) and diethylmalonate (1.6 g, 0.01 mol) were dissolved in ethanol (15 ml) to give clear solution. Piperidine (2 ml) was added and the mixture was refluxed for 10hr's. The content was concentrated to small volume. The product ethyl-2-oxo-2H-chromene-3-carboxylate was poured onto crushed ice, filtered out and recrystallized from ethanol to give white shiny crystals, TLC pure (chloroform : methanol, 9ml:1ml,v/v). M.p.120-122^{0}C; yield: 90%.

Synthesis of 2-oxo-2H-chromene-3-carbohydrazide

In dry round-bottomed flask, containing solution of ethyl-2-oxo-2H-chromene-3-carboxylate (0.01 mol,

2.18 gm) and hydrazine hydrate 99% (0.01mol, 0.5 gm) were dissolved in ethanol (50 ml) to give clear solution and refluxed for 13hr's. The content was concentrated to half of the volume and allowed to cool. The solid mass of 2-oxo-2H-chromene-3-carbohydrazide which separated out on cooling was retained by filtering and washed with small amount of ice cooled ethanol (90%). M.p.136-138^{0}C; yield: 87%.

Synthesis of N'-[(E)-(2-chloroquinolin-3-yl) methylidene]-2-oxo-2H-chromene-3-carbohydrazide (NS-1)

In dry round-bottomed flask, mixture of 2-chloro-3 formylquinoline (0.01mol, 1.91gm) 2-oxo-2H-chromene-3-carbohydrazide (0.01mol, 2.04g,) N,N-Dimethyl formamide (20ml) and catalytic amount of glacial acetic acid(1-2drops) were added and the reaction mixture was refluxed for16- 18hr's. After completion of reaction (monitored by TLC), the reaction mixture was poured onto ice cold water (50ml) and resulting precipitate was filtered, washed with Petroleum ether, dried and recrystalised from aqueous alcohol to give N'-[(E)-(2-chloroquinolin-3-yl)methylidene]-2-oxo-2H-chromene-3-carbohydrazide.

Similarly,*N*'-[(*E*)-(2-chloro-6-methylquinolin-3-yl) methylidene]-2-oxo-2*H*chromene-3-carbohydra zide (NS-2)N'-[(E)-(2-chloro-8-methylquinolin-3-yl) methylidene]-2oxo-2H chromene-3-carbohydrazide (NS-3) and N'-[(E)-(2-chloro-7-methylquinolin-3-yl)methylidene]-2-oxo-2H-chromene-3carbohydra zide (NS-4) (Table No.1).

Synthesis of N'-[(E)-(2-hydroxyquinolin-3-yl) methylidene]-2-oxo-2H-chromene-3-carbohydrazide(NS-5)

In dry round-bottomed flask, mixture of 2-hydroxy-3-formyl quinoline (0.01mol, 1.73gm) 2-oxo-2H-chromene-3-carbohydrazide (0.01mol, 2.04gm) N,N-Dimethyl formamide (20ml) and catalytic amount of glacial acetic acid (1-2drops) were added and the reaction mixture was refluxed for 17-20hr's. After completion of reaction (monitored by TLC), the reaction mixture was poured onto ice cold water (50ml) and resulting precipitate was filtered, washed with Petroleum ether, dried and recrystalised from aqueous alcohol to give N'-[(E)-(2-hydroxyquinolin-3-yl)methylidene]-2-oxo-2H-chromene-3 carbohydrazide. Similarly, *N*'-[(*E*)-(2-hydroxy-6-methylquinolin-3yl) methylidene]-2-oxo-2*H*-chrom ene-3-carbohydrazide(NS-6),N'-[(E)-(2-hydroxy-8-methylquinolin-3-yl)methylidene]-2-oxo-2H-chromene-3-carbohydrazide (NS-7) and N'-[(E)-(2-hy droxy-7 methylquinolin-3-yl)methylidene]-2-oxo-2H-chromene-3-carbohydrazide(NS-8) (Table No.1).

Synthesis of (2-oxo-N'-[(E)-(2-sulfanylquinolin-3-yl)methylidene]-2H-chromene-3-carbohydrazide(NS-9)

In dry round-bottomed flask, mixture of 2-thione-3-formyl quinoline (0.01mol, 1.89gm) 2-oxo-2H-chromene-3-carbohydrazide (0.01mol, 2.04gm) N,N-Dimethyl formamide (20ml) and catalytic amount of glacial acetic acid (1-2drops) were added and the reaction mixture was refluxed for 16hr's. After completion of reaction (monitored by TLC), the reaction mixture was poured onto ice cold water (50ml) and resulting precipitate was filtered, washed with Petroleum ether, dried and recrystalised from aqueous alcohol to give (2-oxo-N'-[(E)-(2-sulfanylquinolin-3-yl)methylidene]-2H-chromene-3-carbohydrazide (Table No.1).

Synthesis of 2 - oxo - N' - [(E) – tetrazolo [1, 5 – a] quinoline -4-ylmethylidene] - 2H – chromenes – 3 – carbohydrazide (NS10)

In dry round-bottomed flask, mixture of tetrazolo [1,5-a]quinoline-4-carbaldehyde (0.01mol, 1.98gm) 2-oxo-2H-chromene-3-carbohydrazide (0.01mol, 2.04gm) N,N-Dimethyl formamide (20ml) and catalytic amount of glacial acetic acid(1-2drops) were added and the reaction mixture was refluxed for 21hr's. After completion of reaction (monitored by TLC), the reaction mixture was poured onto ice cold water (50ml) and resulting precipitate was filtered, washed with Petroleum ether, dried and recrystalised from aqueous alcohol to give 2-oxo-N'-[(E)-tetrazolo[1,5-a]quinolin-4-ylmethylidene]-2H-chromene-3-carbohydrazide (Table No.1) (Scheme-I).

CHARACTERIZATION

All those compounds screened for antibacterial activity were also tested for their antifungal activity by using Agar cup method. The fungi employed for screenings were; *Aspergillus niger* (MTCC 282), *Aspergillus clavatus* (MTCC 1323) and *Candida albicans* (MTCC 227). Potato dextrose agar-agar was used as a medium for the growth of fungi and Griseofulvin as reference standard drug.

Preparation of potato dextrose agar-agar medium

Formula

Potato	250 gms
Dextrose	10 gms
Agar-agar	20 gms
Distilled water	100 ml

The sliced potatoes were taken with 500 ml of distilled water in a pan and boiled for half an hour till a spoon when placed on a slice can pierce in to it. Filter it while hot and broth was again taken in a pan with rest of the distilled water. Dextrose dissolved in distilled water and weight agar-agar was added to the broth and heated it to boil. The medium thus obtained was sterilized in autoclave for 30 minutes.

Preparation of suspension of micro-organism

The test organisms (*Aspergillus Niger*, *Aspergillus clavatus* and *Candida albicans)* were sub-cultured using potato dextrose agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 25°C for 48 hr and they were stored at 4°C in a refrigerator.

Method used for screening of Antifungal activity

The stock solution of the reference standard (Griseofulvin) and test compound were prepared by dissolving in dimethylformamide to obtain various concentrations of 50 µg/ml and 100 µg/ml.

The potato-dextrose-agar medium was sterilized by autoclaving at 121°C (15 lb/sq.inch) for 15 minutes. The Petri-plates, tubes and flasks plugged with cotton plugs were sterilized in hot air-oven at 150°C, for an hour. Into each sterilized Petri-plate (10 cm diameter) about 30 ml each of molten potato dextrose-agar medium inoculated with respective fungus (6 ml of inoculums to 300 ml of potato-dextrose-agar medium) was transferred aseptically. After solidification of the medium at room temperature, five cups of 6mm diameter were made in each plate with a sterile borer. Using sterile graduated syringes, the standard and the test compounds solution of known concentrations were fed into the bored cups. The concentration of solutions were as follows; 1250 µg/ml to 50 µg/ml.

Determination of minimal inhibitory concentration (MIC)

The inoculums were prepared by taking a loopful of stock culture to about 100 ml of nutrient broth, in 250 ml clean and sterilized conical flasks. The flasks were incubated at 27°C for 24 hrs before use. The plates were kept undisturbed for at least two hours at room temperature to allow diffusion of the solution properly, into potato-dextrose-agar medium. Then the plates were incubated at 25°C for 48 hrs. The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculum. The experiments were performed in triplicate in order to minimize the errors.

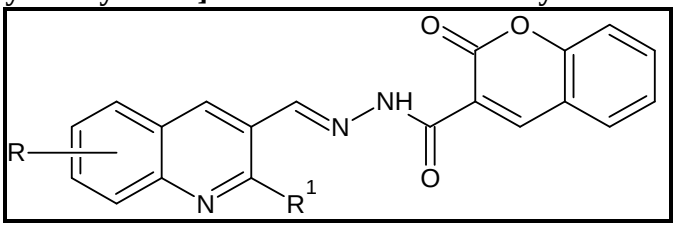
RESULTS AND DISCUSSION

Antifungal activity

The antifungal activity of the synthesized compounds NS 1-10 was determined in-vitro using agar plate method against three strains viz., *Candida albicans, Aspergillus Niger* and *Aspergillus clavatus* at different conc. ranging between 100 µg/ml to 1250 µg/ml.

Out of the tested compounds, NS-7 exhibited a significant activity against *Candida albicans* at conc. 250 µg/ml compared to reference standard Greseofulvin (conc. 500 µg/ml). The compounds NS-4 and NS-8 possess equipotent antifungal activity as compare to that of standard at conc. 500 µg/ml. The compound NS-7 contain 2-hydroxy-8-methyl group. So this indicates presence of electron withdrawing group may favor the potent activity. Whereas, rest of the compounds showed feeble antifungal activity against *C. albicans, A. clavatus* and *Aspergillus Niger* compared to reference standard Griseofulvin (Table No.2).

Table No.1: IR, ^{1}HNMR and Mass spectral characteristic analytical data of 2-oxo-N^1-[(E)-quinolin-3-ylmethylidene]-2H-chromene-3-carbohydrazides



S.No	Compound Code	N-H cm^{-1}	C=O cm^{-1}	C=C cm^{-1}	CH=N cm^{-1}	C=Cl cm^{-1}	C-S cm^{-1}	^{1}HNMR (in δ ppm) and Mass spectral data
1	NS-1	3178	1652	1487	1652	782	-	δ 6.7- 8.0 (m, 9 Ar-H+NH+CH), δ 8.7 (s, 1Ar-H). m/z 377 (M^+), 376(M+2).
2	NS-2	3167	1653	1488	1653	749	-	δ 2.6 (s, 3H, CH_3), δ 6.7- 8.0 (m, 8 Ar-H+NH+CH), δ 8.7 (s, 1Ar-H).
3	NS-3	3179	1650	1488	1650	753	-	δ 2.6 (s, 3H, CH_3), δ 6.7- 7.9 (m, 8 Ar-H+NH+CH), δ 8.8 (s, 1Ar-H).
4	NS-4	3177	1651	1487	1651	752	-	δ 2.4 (s, 3H, CH_3), δ 6.8- 7.8 (m, 8 Ar-H+NH+CH), δ 8.7 (s, 1Ar-H).
5	NS-5	3168	1653	1487	1653	-	-	δ 6.8- 8.1 (m, 9 Ar-H+NH+CH), δ 9.1 (s, 1Ar-H) δ 11.9 (s, 1 Ar-OH). m/z 354.
6	NS-6	3156	1661	1487	1661	-	-	δ 2.4 (s, 3H, CH_3), δ 6.8- 7.9 (m, 8 Ar-H+ NH+CH), δ 8.9 (s, 1Ar-H), δ 11.7 (s, 1 Ar-OH).
7	NS-7	3177	1650	1488	1650	-	-	δ 2.8 (s, 3H, CH_3), δ 6.7- 7.9 (m, 8 Ar-H+ NH+CH), δ 8.7 (s, 1Ar-H), δ 11.3 (s, 1 Ar-OH).
8	NS-8	3176	1651	1488	1651	-	-	δ 2.4 (s, 3H, CH_3), δ 6.7- 7.5 (m, 8 Ar-H+ NH+CH), δ 8.9 (s, 1Ar-H), δ 11.2 (s, 1 Ar-OH). m/z 377.
9	NS-9	3144	1683	1488	-	-	684	δ 6.7- 7.9 (m, 9 Ar-H+NH+CH), δ 8.9 (s, 1Ar-H), δ 13.8 (s, 1 Ar-SH).
10	NS-10	3167	1649	1486	1649	-	-	δ 6.7- 7.9 (m, 9 Ar-H+NH+CH), δ 8.8 (s, 1Ar-H). m/z 380.

Table No.2: Results of Antifungal activity by Mic method for the synthesized compounds (Mic in μg/ml)

S.No	Compound code	*Candida albicans* [MTCC 227]	*Aspergillus niger* [MTCC282]	*Aspergillus clavatus* [MTCC 1323]
1	Greseofulvin (Std)	500	100	100
2	NS-1	1000	1000	1000
3	NS-2	1000	>1000	>1000
4	NS-3	1000	500	500
5	NS-4	500	1000	1000
6	NS-5	250	1000	1000
7	NS-6	250	1000	1000
8	NS-7	200	500	500
9	NS-8	500	500	500
10	NS-9	1000	1000	1000
11	NS-10	1000	1000	1000

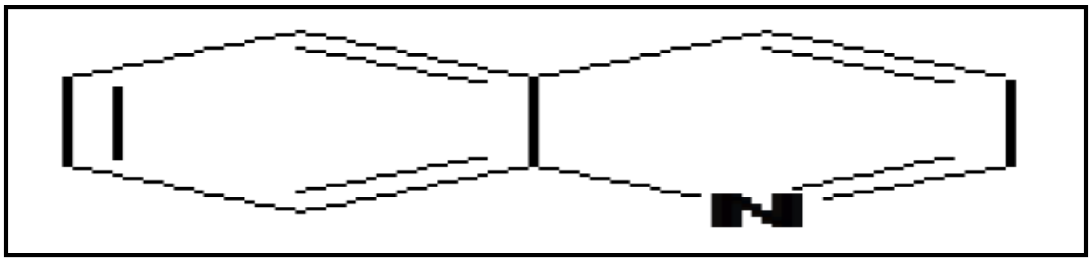
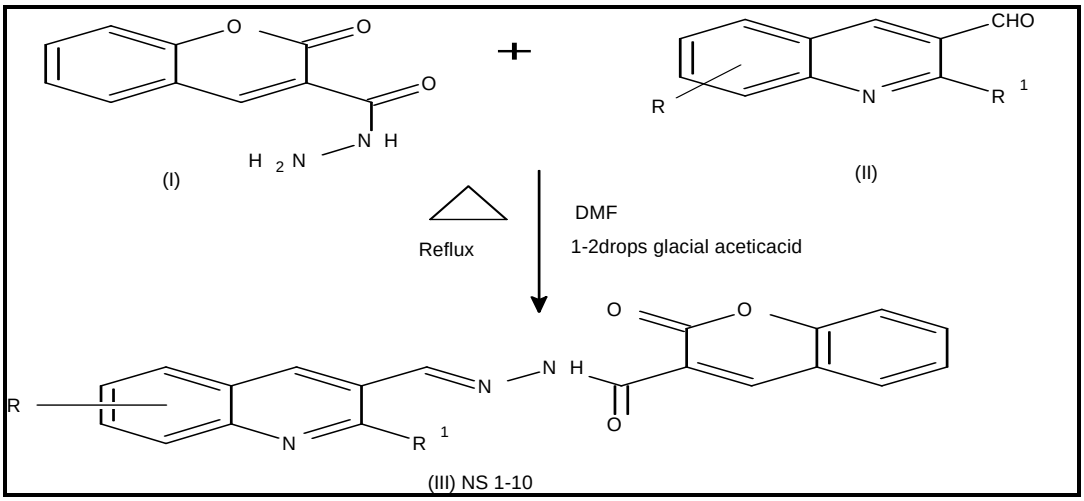


Figure No.1: Structure of Quinoline

Scheme - I



Compounds	R	R	R	R	R^1
NS (1-4)	H	6-CH_3	8-CH_3	7-CH_3	Cl
NS (5-8)	H	6-CH_3	8-CH_3	7-CH_3	OH
NS (9)	H	-	-	-	SH
NS(10)	H	-	-	-	N_3

CONCLUSION

Ten new 2-oxo-N^1-[(E)-quinolin-3-ylmethylidene]-2H-chromene carbohydrazide were synthesized. Analytical and spectral data were used to characterize few synthesized compounds. All synthesized compounds were screened for antibacterial and antifungal activities. Few of the tested compounds exhibited significant and equipotent antifungal activity against *Candida albicans*, but none of the synthesized compounds shown significant antifungal activity against *A. Niger* and *A. clavatus.*

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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