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SYNTHESIS CHARACTERISATION AND ANTIMICROBIAL SCREENING OF HYDRAZONES OF COUMARIN HYDRAZIDES AND ISATIN.

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ABSTRACT
Substituted 2-oxo-2H-chromen-4-ylmethyl Sulphanyl/Sulphonyl)-acetic acid hydrazides or coumarin carbahydrazides treated with isatin in ethanol reflux in presence of catalytic amount of acetic acid to yield desired corresponding hydrazones, thus newly synthesised compounds were established by IR, ^{1}HNMR and mass spectral studies. All the title compounds were subjected to in vitro antibacterial testing against two pathogenic strains and antifungal testing against two fungi. Among the tested compounds 4c ,4d and 4f displayed significant antibacterial and antifungal activities and rest of them are the moderately active.

KEYWORDS: Coumarin Sulphanyl/Sulphonyl)-acetic acidhydrazides or coumarin carbahydrazides, isatin, *amido-iminol* tautomerism, antibacterial and antifungal.

INTRODUCTION

Nitrogen, Oxygen, Sulphur containing heterocyclic compounds are widespread in nature by showing compatibility and applicability in their biological activity, pharmacology and in agrochemicals. Thus, needfullness for synthetic organic chemist to develop N,O and S containing heterocyclic hybrids has been fulfilled.

It is evident from literature that the hydrazones constitute an important class of biologically active molecules, which have attracted attention of medicinal chemists due to their wide ranging pharmacological activity. Isatin derivatives are known to be associated with broad spectrum of biological activity like antibacterial[1] , anti-inflammatory[2] , analgesic[3], anti-viral[4], antifungal[5] , anti-tubercular[6], anti-HIV[7], antiprotozoal[8], antihelminthic.[9] Isatin hydrazones have been reported to possess anticonvulsant[10] activity also. Isatin molecule bear two carbonyl groups of which ∝-carbonyl exhibits amide carbonyl character whereas β-carbonyl exhibits ketocarbonyl character, essentially both the carbonyl carbon atoms differ in their electrophilic nature.Since ketocarbonyl of isatin has more electrophilic nature than amide carbonlyl, the NH_2 of carbahydrazide attacks the carbon of ketocarbonyl to form corresponding hydrazone derivatives.The one of the interesting feature of hydrazones is their tautomeric ability. They can exhibit *amido-iminol* tautomerism[11,12,13] **Figure-1**. In solid state *amido*-form predominates while in solution state *iminol*-form, this property offer the possibility for the formation of different types of complexes with metals.

In view of these facts and as a continuation of our work in the laboratory, prompted us to synthesize some new substituted[(2-oxo-2H-chromen-4-ylmethyl Sulphanyl/Sulphonyl)-acetic acid hydrazino]-2-indolinones **4(a-f)**. Reaction of substituted 2-oxo-2H-chromen-4-ylmethyl Sulphanyl/Sulphonyl)-acetic acid hydrazides **3(a-f)** reacts with isatin in ethanol reflux in presence of catalytic amount of acetic acid to yield desired corresponding hydrazones, the esters required for the synthesis of hydrazides **3(a-f)** have been synthesised starting from 4-bromomethyl coumarins[14]. All newly synthesized compounds **4(a-f), Scheme 1** were characterised by IR, ^{1}HNMR and mass spectra and have been screened for their in vitro anti-bacterial and anti-fungal activity (antimicrobial activity).

The ^{1}HNMR spectrum of [(Substutited-2-oxo-2H-chromen-4- sulphanyl/sulphonyl)-acetic acid hydrazino]-2-indolinones **4(a-f)** have exhibited some interesting secondary peaks for every primary signals, this indicates the presence of tautomeric equilibrium [15, 16]. The two possible structures A and B which are expected to show changes in chemical shifts. The proportion of the signals in the ratio of 3:1 which indicates the proportion of tautomer as approximately 75:25%.

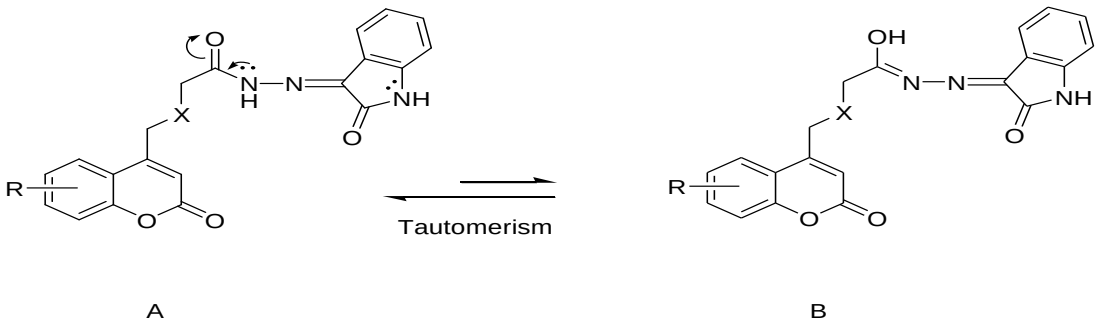
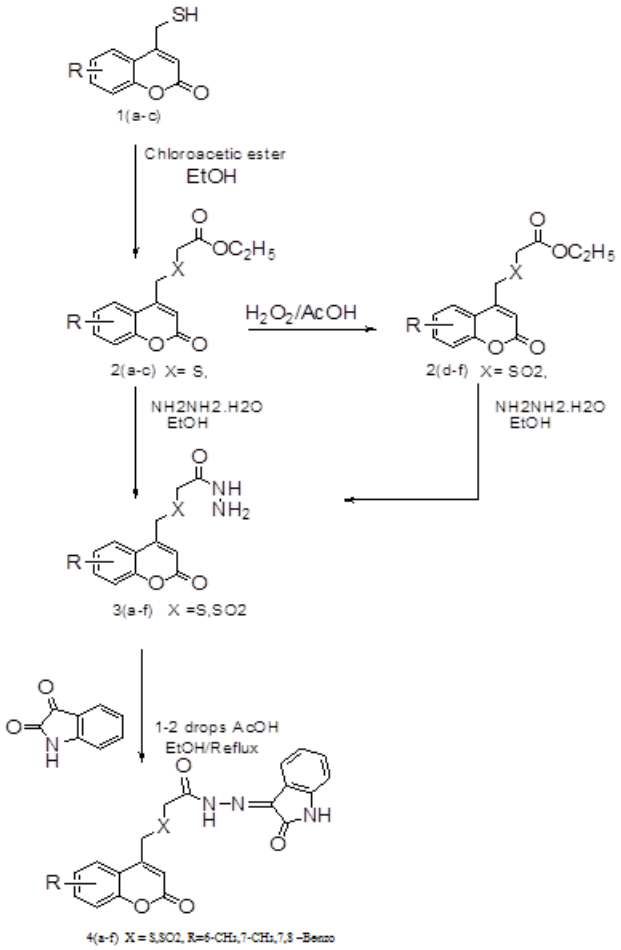


Figure-1.Amido-Iminol Tautomerism

Scheme



Scheme 1. Synthesis of[(2-oxo-2H-chromen-4-ylmethyl-sulphanyl/sulphonyl)-acetic acid hydrazono]-2-indolinones 4(a-f)

RESULTS AND DISCUSSION

The IR spectrum of [7,8-benzo-(2-oxo-2H-chromen-4-ylmethyl-sulphanyl)-acetic acid hydrazono)]-2-indolinone **(4c)**, exhibited 3-carbonyl stretching vibrations at 1716 cm^{-1}, 1700 cm^{-1} and 1672 cm^{-1} due to characteristic lactone carbonyl of coumarin and amide carbonyls respectively, whereas NH, =C-H, C=N, C=C and C-O-C were observed at 3224 cm^{-1}, 2923 cm^{-1}, 1629 cm^{-1}, 1552 cm^{-1} and 1159 cm^{-1} respectively. The IR spectral data of some of the sulphanyl/sulphonylhydrazono derivatives have been tabulated in **Table-1**.

Table-1. IR Spectral data of some of the [(2-oxo-2H-chromen-4-ylmethyl-sulphanyl/sulphonyl)-acetic acid hydrazono]-2-indolinones (4a-f)

Sl. No.	R	ν_{-NH} cm^{-1}	$\nu_{=C-H}$ cm^{-1}	$\nu_{C=O}$ cm^{-1} Lactone	$\nu_{C=O}$ cm^{-1} Amide	$\nu_{C=N}$ cm^{-1}	$\nu_{C=C}$ cm^{-1}	ν_{SO_2} cm^{-1}	ν_{C-O-C} cm^{-1}
4a	6-CH_3	3387 and 3245	2923	1726	1705 and 1683	1617	1590	-	1175
4b	7-CH_3	3262	2923	1710	1683	1612	1524	-	1136
4c	7,8-Benzo	3224	2923	1716	1700 and 1672	1629	1552	-	1159
4d	6-CH_3	3246	2923	1694	1690	1612	1568	1333	1142
4e	7-CH_3	3300	2912	1727	1700	1607	1525	1339	1142
4f	7,8-Benzo	3271	2924	1719	1693	1620	1556	1323	1148

^{1}H NMR spectrum of [(7,8-benzo-2-oxo-2H-chromen-4-ylmethyl sulphanyl)-acetic acid hydrazino]-2-indolinone **(4c)** has displayed some interesting secondary peaks for every primary signals due to a possible tautomeric equilibrium. The ^{1}H NMR values which are inside the parenthesis are associated with one of the possible tautomeric mixture structure-B.

The ^{1}H NMR spectrum of [(7,8-benzo-2-oxo-2H-chromen-4-ylmethyl sulphanyl)-acetic acid hydrazino]-2-indolinone **(4c)** displayed two singlets at 3.79 δ ppm (3.49 δ ppm) and 4.12 δ ppm (3.67 δ ppm) due to S-CH_2 and C_4-CH_2 of coumarin respectively. The C_3-H of coumarin found to resonate at 6.63 δ ppm (6.58 δ ppm) and two singlets at 10.83 δ ppm (10.74 δ ppm) and 12.72 δ ppm (13.55 δ ppm) due to two amide –NH and are D_2O-exchanged , whereas primary and secondary signals of aromatic protons found to resonate in the range of 6.83-8.52 δ ppm. The proton NMR data is briefly described in spectral data. All the sulphanyl/sulphonylhydrazono derivatives **4(a-f)** were screened for their antimicrobial activity **Table - 2**.

EXPERIMENTAL

Instrumentation

Spectral Data

[6-methyl-(2-oxo-2H-chromen-4-ylmethyl-sulphanyl)-acetic acid hydrazono)]-2-indolinone (4a).
DarkYellow solid; yield 73%; m.p 205 ºC; IR (KBr) cm^{-1} 3387,3245 (NH),1726 (C=O) lactone, 1705, 1683 (C=O) amide, 1617(C=N); ^{1}H NMR (DMSO, 400 MHz, TMS): *δ* 2.41(2.37), (s,3H 6-CH_3 of Coum.), *δ* 3.68 (3.45) (s,2H S-CH_2), *δ* 4.02(4.00)(s, 2H C_4-CH_2 of Coum.), *δ* 6.53 (6.49) (s,1H C_3-H of Coum.), *δ* 6.89-7.98 (m 7H, Ar-H), *δ* 10.80 (10.87) (s, 1H, -NH) and *δ* 12.72 (13.64) (s,1H, -NH). NH are D_2O-exchanged. GC/MS *m/z*: 407 observed. Anal.Calcd. for $C_{21}H_{17}N_3O_4S$; C,61.88; H,4.15 ; N,10.30; Found: C,61.91; H,4.17; N,10.31.

[7-methyl-(2-oxo-2H-chromen-4-ylmethyl-sulphanyl)-acetic acid hydrazono)]-2-indolinone (4b).
DarkYellow solid; yield 72%; m.p 218ºC; IR (KBr) cm^{-1} 3262 (NH),1710 (C=O) lactone, 1683 (C=O) amide, 1612(C=N); ^{1}H NMR (DMSO, 400 MHz, TMS): *δ* 2.44 (2.37)(s, 3H 7-CH_3 of Coum.), *δ* 3.76(3.43) (s, 2H S-CH_2), *δ* 4.00(3.99)(s, 2H C_4-CH_2 of Coum.), *δ* 6.48(6.44) (s, 1H, C_3-H of Coum.), *δ* 6.89-7.70(m, 7H, Ar-H), *δ* 10.75 (10.82)(s, 1H-NH) and *δ* 12.71(13.62)(s, 1H,-NH). NH are D_2O-exchanged.GC/MS *m/z*: 407 observed. Anal.Calcd. for $C_{21}H_{17}N_3O_4S$; C,61.89; H,4.13 ; N,10.28; Found: C,61.91; H,4.17; N,10.31.

[7,8-benzo-(2-oxo-2H-chromen-4-ylmethyl-sulphanyl)-acetic acid hydrazono)]-2-indolinone (4c)
DarkYellow solid; yield 74%; m.p 245ºC; IR (KBr) cm^{-1} 3224 (NH),1716 (C=O) lactone, 1700,1672 (C=O) amide, 1629 (C=N); ^{1}H NMR (DMSO, 400 MHz, TMS): *δ* 2.44 (2.37)(s, 3H 7-CH_3 of Coum.), *δ* 3.79(3.49)(s, 2H, S-CH_2), *δ* 4.12(3.67)(s,2H C_4-CH_2 of Coum.), *δ* 6.63(6.58)(s, 1H, C_3-H of coum), *δ* 6.83-8.52 (m, 10H, Ar-H), *δ* 10.83(10.74)(s,1H-NH) and *δ* 12.72 (13.55) (s,1H-NH). NH are D_2O exchanged. GC/MS *m/z*: 443 observed. Anal.Calcd. for $C_{24}H_{17}N_3O_4S$; C,64.98; H,3.80; N,9.45; Found: C,65.01; H,3.83; N,9.48.

[6-methyl-(2-oxo-2H-chromen-4-ylmethyl-sulphonyl)-acetic acid hydrazono)]-2-indolinone (4d)
DarkYellow solid; yield 79%; m.p 287 ºC; IR (KBr) cm^{-1} 3246 (NH),1694 (C=O) lactone, 1612 (C=N); ^{1}H NMR (DMSO, 400 MHz, TMS): *δ* 2.44(2.41)(s, 3H, 6-CH_3 of Coum.), *δ* 4.92(4.62)(s,2H, SO_2-CH_2), *δ* 5.02(4.98)(s, 2H, C_4-CH_2 of Coum.), *δ* 6.68 (s, 1H C_3-H of Coum.), *δ*

Melting points were determined with open capillary method on a Buchi apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5700 FT-IR instrument (Nicolet, Madison, WI, USA) as KBr discs. 1H NMR spectra were recorded on Bruker 400 MHz Spectrometer using $CDCl_3$ and DMSO-d6 as solvents and TMS as internal standard. All chemical shifts were reported as δ values (ppm). Mass spectra were recorded using Shimadzu GCMSQP2010S. The elemental analysis were carried out using Hereaus CHN rapid analyser. Reaction progress was checked by TLC and purity of the compounds were checked by other analytical methods .

General Procedure for the Synthesis of substituted-2-oxo-2H-chromen-4-yl-methyl sulphanyl/sulphonyl)-acetic acid hydrazono)-2-indolinones 4(a-f).
Mixture of (substituted-2-oxo-2H-chromen-4-yl-methyl sulphanyl)-acetic acid hydrazide **3(a-f)** (0.01 mol) and isatin (0.01 mol) was refluxed on a water bath for 2 hrs in ethanol in the presence of catalytic amount of acetic acid. The separated solid was filtered and washed with excess of ethanol, dried and recrystallised from ethanol dioxane mixture**.**

6.91-7.71(m, 7H, Ar-H), *δ* 11.01 (10.90) (s, 1H-NH), *δ* 13.07(13.50) (s, 1H-NH). Compound was precipitated on adding D_2O. GC/MS *m/z*: 439 observed. Anal.Calcd. for $C_{21}H_{17}N_3O_6S$; C,57.38; H,3.85; N,9.52; Found: C,57.40; H,3.87; N,9.56.

[7-methyl-(2-oxo-2H-chromen-4-ylmethyl-sulphonyl)-acetic acid hydrazono)]-2-indolinone (4e)

DarkYellow solid; yield 78%; m.p 245ºC; IR (KBr) cm^{-1} 3300 (NH),1727 (C=O) lactone, 1607 (C=N); ^1H NMR (DMSO, 400 MHz, TMS): *δ* 2.46(2.43)(s, 3H, 6-CH_3 of Coum.), *δ* 4.92(4.73)(s, 2H SO_2-CH_2), *δ* 5.02(4.98)(s, 3H, C_4-CH_2 of Coum.), *δ* 6.63 (6.62)(s, 1H, C_3-H of Coum.), *δ* 6.90-8.15(m, 7H, Ar-H), *δ* 11.03(10.99)(s, 1H, -NH) and *δ* 13.06(13.44)(s, 1H-NH) compound was precipitated on adding D_2O.

GC/MS *m/z*: 439 observed. Anal.Calcd. for $C_{21}H_{17}N_3O_6S$; C,57.36; H,3.86; N,9.54; Found: C,57.40; H,3.87; N,9.56.

[7,8-benzo-(2-oxo-2H-chromen-4-ylmethyl-sulphonyl)-acetic acid hydrazono)]-2-indolinone (4f)

DarkYellow solid; yield 80%; m.p 267ºC; IR (KBr) cm^{-1} 3271 (NH),1719 (C=O) lactone, 1693 (C=O) amide, 1620 (C=N); ^1H NMR (DMSO, 400 MHz, TMS): *δ* 4.95(4.93)(s, 2H SO_2-CH_2), *δ* 5.02(4.99)(s, 2H C_4-CH_2 of Coum.), *δ* 6.68(6.64)(s, 1H, C_3-H of coumn.), *δ* 6.81-8.72 (m, 10H, Ar-H), *δ* 11.05(10.97)(s, 1H, -NH) and *δ* 13.93 (13.01)(s, 1H, -NH) compound was precipitated on adding D_2O. GC/MS *m/z*: 475 observed. Anal.Calcd. for $C_{24}H_{17}N_3O_6S$; C,60.61; H,3.54; N,8.80; Found: C,60.63; H,3.57; N,8.84.

Antimicrobial activity

The agar disc-diffusion method [17] was used for the screening of *in vitro* antimicrobial activity. The anti-microbial activity of the synthesized compounds **4(a-f)** were screened against *Staphylococcus aureus* and *Escherichia Coli* using nutrient agar medium. The antifungal activity of the compounds was tested against *Candia albicans* and *Aspergillus niger* using Sabouraded dextrose agar medium. The minimum inhibitory concentration (MIC) as carried out using micro dilution susceptibility method[18]. Ciprofloxacin was used as a standard antibacterial drug and Flucanazole was used as a standard antifungal drug. The observed data on the antimicrobial activity of compounds and control drugs are given in **Table-2.** The investigation of antibacterial screening **Table-2** revealed that some of the newly synthesized compounds showed moderate to good inhibition at 12.5-100 µg/mL in DMSO. Amongst all the compounds, compounds **4c,4d,4f** showed excellent antibacterial activity against *E-Coli* (MIC: 12.5µg/mL) and *S .aureus* (MIC: 12.5 µg/mL). Compounds **4a** and **4e** exhibited moderate activity against *E. coli.* The investigation of antifungal screening **Table-2** revealed that some of the newly synthesized compounds showed moderate to good inhibition at 12.5-100 µg/mL in DMSO. Amongst the tested compounds, compounds **4c** and **4d** shows excellent inhibitory growth against *C. albicans* (MIC: 12.5 µg/mL) and *A niger* (MIC: 12.5µg/mL) respectively. Compound **4f** exhibited good activity against *A. niger* (MIC: 25µg/m). Remaining compounds showed moderate to least activity against both bacteria and fungi.

Table-2: Antimicrobial activity of compounds 4(a-f).

	Bacterial strains (+ Ve and –Ve) in µg/mL		Fungal strains In µg/mL	
Compound	***S. aureus***	***E. coli***	***C. albicans***	***A. niger***
4a	100	50	75	75
4b	13	13	100	50
4c	12.5	12.5	12.5	12.5
4d	12.5	12.5	12.5	12.5
4e	100	50	50	25
4f	12.5	12.5	50	25
Ciprofloxacin	3.25	3.25	-	-
Flucanazole	-	-	3.25	3.25

Table-3. Physical and analytical data of some of [(2-oxo-2H-chromen-4-ylmethyl sulphanyl/sulphonyl)-acetic acid hydrazino]-2-indolinones 4(a-f).

Sl. No.	R	m. p. (°C)	Yield (%)	Mol. Formula	Analysis Found(Calcd.) (%)		
					C	H	N
4a	6-CH_3	205	73	$C_{21}H_{17}N_3O_4S$	61.88 (61.91)	4.15 (4.17)	10.30 (10.31)
4b	7-CH_3	218	72	$C_{21}H_{17}N_3O_4S$	61.89 (61.91)	4.13 (4.17)	10.28 (10.31)
4c	7, 8-Benzo	245	74	$C_{24}H_{17}N_3O_4S$	64.98 (65.01)	3.80 (3.83)	9. 45 (9.48)
4d	6-CH_3	287	79	$C_{21}H_{17}N_3O_6S$	57.38 (57.40)	3.85 (3.87)	9.52 (9.56)
4e	7-CH_3	245	78	$C_{21}H_{17}N_3O_6S$	57.36 (57.40)	3.86 (3.87)	9.54 (9.56)
4 f	7, 8-Benzo	267	80	$C_{24}H_{17}N_3O_6S$	60.61 (60.63)	3.54 (3.57)	8. 80 (8.84)

CONCLUSION

In conclusion, we have described method for the synthesis of hydrazono coumarin scaffolds by using isatin. The bromomethyl coumarins are converted into mercaptans, thus condensed with chloroacetic ester. This scheme also involves sulphonation by using hydrogen peroxide in acetic acid. All the title compounds were subjected to *in vitro* antibacterial testing against two pathogenic strains and antifungal screening against two fungi. Among the tested compounds, **4c,4d** and **4f** showed significant antibacterial and antifungal activities with MIC 12.5µg/mL. Also the compound **4c** and **4d** showed significant antifungal activity against *A. niger* with MIC 12.5µg/mL. Needless to say, further understanding the mechanism of biological action are still required in order to fully develop of these compounds as potent antimicrobial drugs.

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