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Synthesis and Antifungal Screening of Some Novel Coumarin Linked Imidazole Derivatives

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ABSTRACT

A novel series of coumarin linked imidazole derivatives 4(a-l) were synthesized by reacting 3-(1H-imidazol-1yl-acetyl) coumarin (3) with various substituted phenyl hydrazines. The compound (3) itself was synthesized by reacting 3-(2-bromoacetyl)coumarin (2) with imidazole in 1,4-dioxan.The structures of all the synthesized compounds have been established on the basis of physical, spectral and elemental analyses data. Among the 12 compounds that have been screened for antifungal activity against *C.krusei, C.albicans, A.niger, A.flavus* and *P.notatum,* compounds 4b,4d and 4e possessing o, p-chloro and p-bromo substitutions showed significant activity mainly against *C.krusei and C.albicans,* moderate activity against *A.niger* and *A.flavus* and negligible against *P.notatum* at a concentration of 250µg/mL and 500µg/mL.

Keywords: Zinconazole, Imidazole, Coumarin, Antifungal activity, Substituted phenyl hydrazines

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INTRODUCTION

Inspired by the various biological activities of coumarin [1-5] and imidazole [6-10] derivatives, in the present work, an attempt has been made to synthesize title compounds 4(a-l). The scheme of synthesis is planned in such a way that the synthesized compounds will have the structural similarity with the anti fungal drug zinconazole and the rationale in linking the coumarin heterocycle with imidazole is important as antibacterial antibiotics such as novobiocin contain coumarin moiety as a main heterocycle.

MATERIALS AND METHODS

Experimental

All the melting points were recorded on Cintex melting point apparatus and are uncorrected. IR spectra in KBr were recorded on Schimadzu FTIR spectrophotometer in cm^{-1}. ^{1}HNMR spectra were recorded in $CDCl_3$ or DMSO $-d_6$ on a Bruker DRX-300 MHZ NMR instrument. Chemical shifts were reported in ppm using TMS as internal standard on δ scale. Mass spectra of compounds were recorded on mass spectrometer (Agilant 1100 series). Elemental analyses were carried out on a Heraeus CHN rapid analyzer. Completion of the reactions was monitored from time to time by TLC using E-Merck 0.25 mm silica gel plates.

Preparation of 3-(1H-imidazol-1yl-acetyl)coumarin (3)

A mixture of 3- (bromo acetyl) coumarin (0.01mole) and imidazole (0.01mole) in 1,4 dioxan (30 mL) was stirred for 2 hr on magnetic stirrer. The precipitate obtained was filtered, washed thoroughly with acetone and the crude product was recrystallized from ethanol.

Preparation of 3-[(1Z)-2-(1H-imidazole-1-yl)-1-(2-substituted phenyl hydrazinylidene)ethyl] coumarin, (4 a-l)

A mixture of (1H-imidazol-1yl-acetyl) coumarin (3) (0.002 mole), substituted phenyl hydrazine (0.004 mole) and sodium acetate (0.004 mole) in10-15mL of ethanol was refluxed for 2 hr and cooled. The reaction mixture was poured into ice cold water and the yellow precipitate obtained was filtered and recrystallized from ethanol.

Antifungal activity

Newly prepared compounds, (4a-l) were screened for their antifungal activity against *Candida albicans* (NCIM No.2063), *Candida krusei* (NCIM No.3130), *Aspergillus niger* (NCIM No.620), *Aspergillus flavus* (NCIM No.524) and *Penicillium notatum* (NCIM No.745) in DMSO by cup-plate method[11, 12] at two concentrations 250µg/mL and 500 µg/mL. The standard used for this study is Fluconazole.

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RESULTS AND DISCUSSION

The reaction sequences for synthesis of title compounds are shown in **scheme I**. 3-acetyl coumarin (1) was brominated using bromine in glacial acetic acid to get 3-(2-bromoacetyl)coumarin (2). The structure of this compound was confirmed by its IR and mass spectra. Compound (2), upon reaction with imidazole in 1, 4-dioxan gave 3-(1H-imidazol-1yl-acetyl) coumarin (3). The IR spectrum of compound (3) showed C=O str.of lactone at 1724 cm^{-1}, C=O str of $–COCH_2$ at 1693cm^{-1} and C=N str. of imidazole at 1604$cm^{-1}$and the mass spectrum showed (M+1) peak at m/z 255. ^{1}HNMR spectrum in $CDCl_3$ showed a singlet at 4.80 due to two protons of $–COCH_2$ and multiplet at 6.85-8.0 corresponds to 8 aromatic protons confirmed the structure of the compound (3). This compound was treated with different substituted phenyl hydrazines to get hydrazones 4(a-l). The IR spectrum of 4a showed C=O str. of lactone at 1708cm^{-1}, N-H str. at 3238cm^{-1}, C=N str. at 1600 cm^{-1} and disappearance of band at 1693cm^{-1} indicated the formation of the compound. Mass spectrum of the compound showed (M+1) peak at m/z 345. ^{1}HNMR in DMSO showed a singlet at 4.9 due to two protons of CH_2N, singlet at 10.3 due to N-H proton and a multiplet at 6.9-8.2 which corresponds to 13 aromatic protons. Data from the elemental analyses were found to be in conformity with the assigned structure which has further confirmed the structure of compound 4a. Similarly the structures of other compounds were confirmed by physical, spectral and analytical data.

Compound 2.Pale yellow crystalline solid, Yield 82%, m.p.198^{0}c;
IR(KBr):3035,1724,1693,1604,1238cm^{-1};^{1}HNMR($CDCl_3$):δ4.80(s,2H,-$COCH_2$),6.85-8.0(m,8H,ArH).MS(ESI):m/z 255(M+1).
Anal.Calcd for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02.Found: C, 66.12; H, 3.92;N, 11.00%.

3-[(1*Z*)-2-(1*H*-imidazol-1-yl)-*N*-phenylethanehydrazonoyl]-2*H*-chromen-2-one, 4a
Light yellow crystals, yield 80%, mp142^{0}c; IR KBr):3338, 1708, 1600, 1247 cm^{-1}
^{1}HNMR (DMSO–d_6): δ 4.90(s, 2H, CH_2N), 6.90-8.2(m, 13H, ArH), 10.3(s, 1H, NH).
MS (ESI): m/z 345(M+1).
Anal.Calcd for $C_{20}H_{16}N_4O_2$: C, 69.76; H, 4.68; N, 16.27. Found: C, 69.74; H, 4.65; N, 16.25%.

3-[(1*Z*)-*N*-(2-chlorophenyl)-2-(1*H*-imidazol-1-yl)ethanehydrazonoyl]-2*H*-chromen-2-one,4b
Light yellow crystals, yield 75%, mp148^{0}c; IR (KBr): 3328 1718, 1600, 1240cm^{-1}.
^{1}HNMR (DMSO-d_6): δ 4.79(s, 2H, CH_2N), 7.2-8.0(m, 12H, ArH), 10.0(s, 1H, NH).
MS (ESI): m/z 379(M+1).
Anal.Calcd for $C_{20}H_{15}ClN_4O_2$:C,63.41;H,3.99;N,14.79.Found: C,63.37;H,3.97;N,14.75%.

3-[(1*Z*)-*N*-(4-chlorophenyl)-2-(1*H*-imidazol-1-yl)ethanehydrazonoyl]-2*H*-chromen-2-one,4c
Yellow crystals, yield 79%, mp146^{0}c; IR (KBr): 3378, 1712, 1600, 1246cm^{-1}.
^{1}HNMR (DMSO-d_6): δ 4.76(s, 2H, CH_2N), 7.4-8.3(m, 12H, ArH), 10.1(s, 1H, NH).
MS (ESI): m/z: 379(M+1).
Anal.Calcd for $C_{20}H_{15}ClN_4O_2$:C,63.41;H,3.99;N,14.79.Found:C,63.38;H,3.97;N,14.76%.

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3-[(1*Z*)-*N*-(2,6-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanehydrazonoyl]-2*H*-chromen-2-one,4d
Yellow crystals, yield 78%, mp147^{0}c; IR (KBr): 3372,1722,1600,1242cm^{-1}.
^{1}HNMR (DMSO-d_6) : δ 4.74(s,2H,CH_2N),7.4-8.0(m,11H,ArH),10.1(s,1H,NH).
MS (ESI):m/z 414(M+1).
Anal.Calcd for $C_{20}H_{15}ClN_4O_2$:C,63.41;H,3.99;N,14.79.Found:C,63.38;H,3.97;N,14.76%.

3-[(1*Z*)-1-[2-(4-bromophenyl)hydrazinylidene]-2-(1*H*-imidazol-1-yl)ethyl]-2*H*-chromen-2-one,4e
Light yellow crystals, yield 72%, mp162^{0}c; IR (KBr): 3398, 1714, 1596, 1245, 1072, 1010 cm^{-1}
^{1}HNMR (DMSO-d_6): δ 4.70(s, 2H, CH_2N), 6.70-8.0(m, 12H, ArH), 10.3(s, 1H, NH).
MS (ESI): m/z 424(M+1), 425(M+2).
Anal.Calcd for $C_{20}H_{15}BrN_4O_2$: C,56.75;H,3.57;N,13.27.Found:C,56.74;H,3.55;N,13.25%.
3-[(1*Z*)-2-(1*H*-imidazol-1-yl)-*N*-(2-methoxyphenyl)ethanehydrazonoyl]-2*H*-chromen-2-one,4f
Light yellow crystals, yield 76%, mp152^{0}c; IR (KBr): 3358, 1728, 1610, 1248cm^{-1}.
^{1}HNMR (DMSO-d_6): δ 3.72(s,3H,OCH_3), 4.78(s,2H,CH_2N),7.12-8.20 (m,12H,ArH),10.0(s,1H,NH)
MS (ESI): m/z 375(M+1)
Anal.Calcd for $C_{21}H_{18}N_4O_3$:C,67.37;H,4.85;N,14.96.Found: C,67.35;H,4.81;N,14.92%.

3-[(1*Z*)-2-(1*H*-imidazol-1-yl)-*N*-(4-methoxyphenyl)ethanehydrazonoyl]-2*H*-chromen-2-one,4g
yellow crystals, yield 78%,mp150^{0}c;IR(KBr): 3362,1724,1612,1240cm^{-1}
^{1}HNMR (DMSO-d_6): δ 3.74(s,3H,OCH_3), 4.75(s,2H,CH_2N),7.15-8.12 (m,12H,ArH),10.2(s,1H,NH)
MS(ESI):m/z 375(M+1).
Anal.Calcd for $C_{21}H_{18}N_4O_3$:C,67.37;H,4.85;N,14.96.Found: C,67.34;H,4.82;N,14.92%.

3-[(1*Z*)-2-(1*H*-imidazol-1-yl)-*N*-(2-methylphenyl)ethanehydrazonoyl]-2*H*-chromen-2-one,4h
Light yellow crystals, yield 70%, mp148^{0}c; IR (KBr): 3368, 1712, 1605, 1245cm^{-1}
^{1}HNMR (DMSO-d_6): δ 2.2(s,3H,CH_3),4.76(s,2H,CH_2N),7.10-8.1(m,12H,ArH),10.2(s,1H,NH)
MS (ESI):m/z 359(M+1)
Anal.Calcd for $C_{21}H_{18}N_4O_2$:C,70.38;H,5.06;N,15.63.Found: C,70.36;H,5.04;N,15.62%.

3-[(1*Z*)-2-(1*H*-imidazol-1-yl)-*N*-(4-methylphenyl)ethanehydrazonoyl]-2*H*-chromen-2-one,4i
Yellow crystals, yield 72%, mp152^{0}c; IR (KBr): 3358, 1714, 1606, 1244cm^{-1}
^{1}HNMR (DMSO-d_6): δ 2.3(s,3H,CH_3),4.78(s,2H,CH_2N),6.90-8.1(m,12H,ArH) 10.0(s,1H,NH)
MS (ESI): m/z 359(M+1)
Anal.Calcd for $C_{21}H_{18}N_4O_2$:C,70.38;H,5.06;N,15.63.Found: C,70.35;H,5.04;N,15.61%.

3-[(1*Z*)-2-(1*H*-imidazol-1-yl)-*N*-(2-nitrophenyl)ethanehydrazonoyl]-2*H*-chromen-2-one,4j
Yellow crystals, yield 75%, mp136^{0}c; IR (KBr): 3368, 1726, 1610, 1246cm^{-1}
^{1}HNMR (DMSO-d_6): δ 4.65(s, 2H, CH_2N), 6.82-8.21(m, 12H, ArH), 10.3(s, 1H, NH)
MS (ESI): m/z 390(M+1)
Anal.Calcd for $C_{20}H_{15}N_5O_4$:C,61.69;H,3.88;N,17.99.Found: C,61.64;H,3.85;N,17.98%.

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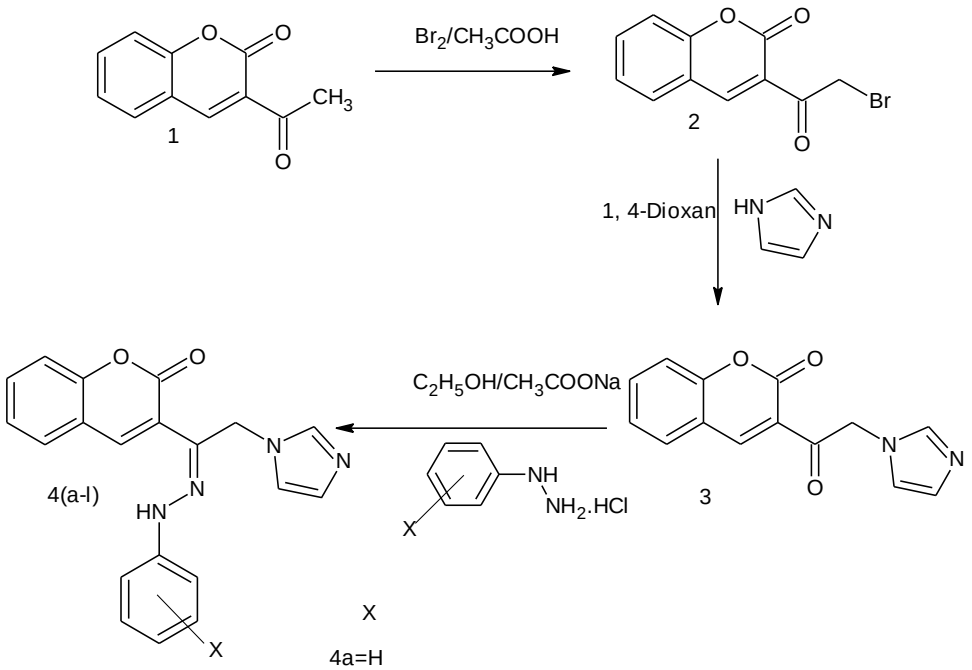
3-[(1*Z*)-2-(1*H*-imidazol-1-yl)-*N*-(4-nitrophenyl)ethanehydrazonoyl]-2*H*-chromen-2-one,4k
Yellow crystals, yield 76%, mp138^{0}c; IR (KBr): 3358, 1718, 1609, 1248cm^{-1}
^{1}HNMR (DMSO-d_6): δ 4.67(s, 2H, CH_2N), 6.80-8.1(m, 12H, ArH), 10.4(s,1H,NH)
MS (ESI):m/z 390(M+1)
Anal.Calcd for $C_{20}H_{15}N_5O_4$:C,61.69;H,3.88;N,17.99.Found: C,61.66;H,3.85;N,17.97%.

3-[(1*Z*)-*N*-(2,4-dinitrophenyl)-2-(1*H*-imidazol-1-yl)ethanehydrazonoyl]-2*H*-chromen-2-one,4l
Yellow crystalline powder, yield 74%, mp144^{0}c; IR (KBr): 3328, 1724, 1604, 1235cm^{-1}
^{1}HNMR (DMSO-d_6): δ 4.85(s, 2H, N), 7.12-8.0(m, 12H, ArH), 10.2(s, 1H, NH)
MS (ESI): m/z 435(M+1)
Anal.Calcd for $C_{20}H_{14}N_6O_6$:C,55.30;H,3.25;N,19.35.Found: C,55.27;H,3.23;N,19.32%.



4a=H
4b= 2-Cl
4c= 4-Cl
4d= 2, 4-Cl
4e= 4-Br
4f= 2-OCH_3
4g= 4-OCH_3
4h= 2-CH_3
4i= 4-CH_3
4j= 2-NO_2
4k= 4-NO_2
4l= 2, 4-NO_2

Scheme I

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Antifungal activity

Table 1– antifungal activity data of synthesized compounds (4a-l)

Compd	Zone of inhibition(Diameter in mm)									
	C. albicans		*C. krusei,*		*A.niger*		*A. flavus*		*P. notatum*	
	250µg /mL	500µg /mL	250µg /mL	500µg /mL	250µg /mL	500µg /mL	250µg /mL	500µg /mL	250µg /mL	500µg /mL
4a	15	19	16	20	11	14	10	14	-	8
4b	25	33	29	37	22	27	21	26	-	13
4c	20	29	22	30	13	17	12	18	-	10
4d	26	34	29	39	23	28	22	27	9	12
4e	26	35	30	40	22	27	21	23	10	13
4f	18	24	20	28	14	18	13	17	-	-
4g	17	23	19	27	12	17	12	18	-	-
4h	19	25	21	30	14	18	13	17	8	10
4i	18	24	19	29	10	14	11	13	9	11
4j	17	22	18	26	12	16	12	16	-	-
4k	16	20	16	24	13	18	11	17	-	-
4l	17	23	17	25	11	17	10	18	-	-
Fluconazole (Std)	28	36	32	41	29	38	28	39	29	40

The zone of inhibition after 24hr of incubation at 28 ± 2^0C was compared with that of standard fluconazole. The screening data shown in table-1 indicated that the compounds 4b, 4d, and 4e showed significant activity against *Candida albicans* and *Candida krusei,* moderate activity against *Aspergillus niger and Aspergillus flavus* but the other tested compounds were inactive against *Penicillium notatum.*

CONCLUSION

In anti fungal, the compounds possessing chloro and bromo substitutions exhibited significant activity at the concentration of 250 µg/mL and 500 µg/mL against fungi *C.albicans* and *C.krusei.*

It is worthwhile to study these compounds further by QSAR and molecular modeling studies in order to establish a lead molecule.

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