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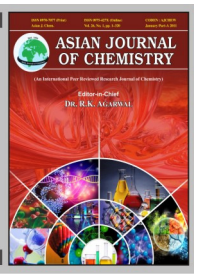

A Novel Route for the Synthesis of Recemic 4-(Coumaryl) Alanines and Their Antimicrobial Activity

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4-Bromomethyl coumarins (**3**) when reacted with diethyl acetamidomalonate (**4**) in presence of sodium hydride afforded the intermediate compounds **5**. Compounds **5** on hydrolysis with 48 % HBr resulted in hydrobromide salt of corresponding alanine derivatives (**6**). Free alanines (**7**) were obtained by neutralizing with ammonia solution. The resulting alanine derivatives (**7**) were characterised by IR, NMR and mass spectroscopic techniques and further screened for their antimicrobial activity.

Keywords: Pechmann cyclization, 4-Bromomethyl coumarin, 4-(Coumaryl) alanine, Antimicrobial activity, Cup plate method.

INTRODUCTION

The α-amino acid fragment when linked to heterocyclic moieties like indole, imidazole and pyrrolidine have given rise to important amino acids like tryptophan, histidine and proline, which play an important role in peptide chemistry in terms of their biological activities. Fluorescent derivatives of biologically active peptides derived from coumarinyl amino acids are useful experimental tools for studying biological structure, function[1] and also for visualizing intracellular process or molecular interactions[2]. The mechanism for antimicrobial activity of α-amino acids and defensins has been proposed, in which the positively charged regions of acids attach or insert in to the negatively charged microbial cell membrane, resulting in the microbial cell death[3]. The thiazole and oxazole containing amino acids and peptides are reported good antimicrobial agents against Gram positive and Gram negative bacteria[4,5]. Coumarin containing amino acids at various positions have been synthesized and studied for their antimicrobial activity[6]. In view of this, present work aims towards fixing the α-amino acid fragment at the allylic position of the pyran ring in the coumarin nucleus and to study the antimicrobial activity.

EXPERIMENTAL

Melting points were recorded using open capillaries and are uncorrected. Analytical TLC was performed on Merck precoated 60 F_{254} silica gel plates. ^{1}H NMR spectra were recorded on Bruker 300 MHz instrument in $CDCl_3$ and DMSO-d_6 with TMS as an internal standard. Chemical shifts (δ) were recorded in ppm, coupling constants (*J*) given in Hz. All the newly synthesized compounds analyzed for C, H, N using Heraus CHN rapid analyzer and the results are in acceptable range. The required 4-bromomethyl coumarins were prepared by Pechman cyclization reported literature methods[7-9].

4-(Diethylacetamidomalonato methyl) coumarins (5a-f): Diethylacetamidomalonate (**4**) (2.75 g, 0.01 mol) was refluxed with sodium hydride (0.36 g, 0.015 mol) in dry benzene (25 mL) for 2 h. The solution of 4-bromomethyl coumarins (**3a-f**) (0.01 mol) in DMF (20 mL) was added. The reaction mixture was refluxed for 18 h. Progress of the reaction was monitored by TLC. The reaction mixture was cooled and diluted with water (50 mL). Neutralized with dilute acetic acid and left overnight at room temperature. The solid formed was filtered and purified by column chromatography (silica gel, 60-120 mesh) using 5-20 % ethyl acetate in hexane.

Diethyl-2-acetamido-2-[(6-methyl-2-oxo-2*H*-chromen-4-yl)methyl]malonate (5a): Colourless solid, column chromatography 5 % ethyl acetate in hexane; yield 60 %; m.p. 60 °C; IR (KBr, ν_{max}, cm^{-1}) 3272, 2976, 1763, 1748, 1725, 1643; ^{1}H NMR ($CDCl_3$), 1.30 (t, 6H, OCH_2CH_3, *J* = 7.1 Hz), 1.93 (s, 3H, CO-CH_3), 2.40 (s, 3H, C_6-CH_3), 3.85 (s, 2H, C_4-CH_2), 4.23 (q, 4H, OCH_2CH_3, *J* = 7.1 Hz), 6.11 (s, 1H, NH, D_2O exchanged), 6.71 (s, 1H, C_3-H), 7.23 (d, 1H, C_7-H, *J* = 8.41 Hz), 7.35 (d, 1H, C_8-H, *J* = 8.39 Hz), 7.43 (s, 1H, C_5-H). Anal. calcd. for $C_{20}H_{23}NO_7$ (%); C, 61.69; H, 5.95; N, 3.60. Found: C, 61.69; H, 5.92; N, 3.59.

Diethyl-2-acetamido-2-[(7-methyl-2-oxo-2*H*-chromen-4-yl)methyl]malonate (5b): Colourless solid, column chromatography 5 % ethyl acetate in hexane; yield 55 %; m.p. 90 °C; IR (KBr, ν_{max}, cm^{-1}) 3290, 2984, 1758, 1745, 1718, 1648;

^{1}H NMR ($CDCl_3$), 1.30 (t, 6H, OCH_2CH_3, J = 7.1Hz), 1.94 (s, 3H, CO-CH_3), 2.45 (s, 3H, C_6-CH_3), 3.84 (s, 2H, C_4-CH_2), 4.25 (q, 4H, OCH_2CH_3, J = 7.1 Hz), 5.79 (s, 1H, NH, D_2O exchanged), 6.07 (s, 1H, C_3-H), 7.05 (d, C_6-H, J = 7.9 Hz), 7.37 (s, 1H, C_8-H), 7.52 (d, 1H, C_5-H, J = 8.0 Hz). Anal. calcd. for $C_{20}H_{23}NO_7$ (%); C, 61.69; H, 5.95; N, 3.60. Found: C, 61.66; H, 5.93; N,3.59.

Diethyl-2-acetamido-2-[(5,6-benzo-2-oxo-2*H*-chromen-4-yl)methyl]malonate (5c): Off white solid, column chromatography 5 % ethyla cetate in hexane; yield 53 %; m.p.178 °C; IR (KBr, ν_{max}, cm^{-1}) 3242, 2980, 1754, 1745, 1731, 1650; ^{1}H NMR (DMSO-d_6) 1.32 (t, 6H, OCH_2CH_3, J = 7.06Hz), 1.95 (s, 3H, CO-CH_3), 3.96 (s, 2H, C_4-CH_2), 4.30 (q, 4H, OCH_2CH_3, J = 7.15 Hz), 6.23 (s, 1H, C_3-H), 6.7 (s, 1H, NH, D_2O exchanged), 7.2-8.5 (m, 6H, Ar-H). Anal. calcd. for $C_{23}H_{23}NO_7$ (%); C, 64.93; H, 5.45; N, 3.29. Found: C, 64.96; H, 5.43; N, 3.28.

Diethyl-2-acetamido-2-[(7,8-benzo-2-oxo-2*H*-chromen-4-yl)methyl]malonate (5d): Off white solid, column chromatography 5 % ethyl acetate in hexane; yield 58 %; m.p. 230 °C; IR (KBr, ν_{max}, cm^{-1}) 3371, 2985, 1754, 1748, 1716, 1670; ^{1}H NMR (DMSO-d_6) 1.04 (t, 6H, OCH_2CH_3, J = 7.05 Hz), 1.61 (s, 3H, CO-CH_3), 4.02 (q, 4H, OCH_2CH_3, J = 6.9 Hz), 4.2 (s, 2H, C_4-CH_2), 5.8 (s, 1H, NH, D_2O exchanged), 7.45 (d, 1H, C_6-H, J = 8.93 Hz), 7.65 (t, 1H, C_{10}-H, J = 7.5 Hz), 7.76 (t, 1H, C_{11}-H, J = 7.6Hz), 8.00 (d, 1H, C_9-H, J = 7.99 Hz), 8.13 (d, 1H, C_5-H, J = 8.93 Hz), 8.22 (d, 1H, C_{12}-H, J = 8.1 Hz). Anal. calcd. for $C_{23}H_{23}NO_7$ (%); C, 64.93; H, 5.45; N, 3.29. Found: C, 64.94; H, 5.43; N, 3.26.

Diethyl-2-acetamido-2-[(6-methoxy-2-oxo-2*H*-chromen-4-yl)methyl]malonate (5e): Pale yellow solid, column chromatography 10 % ethyl acetate in hexane; yield 60 %; m.p. 136 °C; IR (KBr, ν_{max}, cm^{-1}) 3281, 2971, 1756, 1715, 1674; ^{1}H NMR (DMSO-d_6) 1.28 (t, 6H, OCH_2CH_3, J = 7.08 Hz), 1.90 (s, 3H, CO-CH_3), 4.08 (s, 3H, C_6-OCH_3), 4.20 (s, 2H, C_4-CH_2), 4.27 (q, 4H, OCH_2CH_3, J = 7.1 Hz), 6.38 (s, 1H, NH, D_2O exchanged), 7.11 (d, 1H, C_7-H, J = 8.4 Hz), 7.25 (d, 1H, C_8-H, J = 8.2 Hz), 7.36 (s, 1H, C_5-H). Anal. calcd. for $C_{20}H_{23}NO_8$ (%); C, 59.25; H, 5.72; N, 3.46. Found: C, 59.24; H, 5.69; N, 3.45.

Diethyl-2-acetamido-2-[6-chloro-2-oxo-2*H*-chromen-4-yl)methyl]malonate (5f): Pale yellow solid, column chromatography 5 % ethyl acetate in hexane; yield 45 %; m.p. 108 °C; IR (KBr, ν_{max}, cm^{-1}) 3294, 2941, 1758, 1726, 1679; ^{1}H NMR (DMSO-d_6) 1.26 (t, 6H, OCH_2CH_3, J = 7.1 Hz), 1.91 (s, 3H, $COCH_3$), 4.21 (s, 2H, C_4-CH_2), 4.29 (q, 4H, OCH_2CH_3, J = 7.2 Hz), 6.41 (s, 1H, NH, D_2O exchanged) 6.74 (s, 1H, C_3-H), 7.17 (d, 1H, C_7-H, J = 8.41 Hz), 7.28 (d, 1H, C_8-H, J = 8.39 Hz), 7.41 (s, 1H, C_5-H). Anal. calcd. for $C_{19}H_{20}$ Cl NO_7 (%); C, 55.68; H, 4.92; N, 3.42. Found: C, 55.70; H, 4.89; N, 3.42.

4-(Coumaryl) alanins 7(a-f): To the compound **5(a-f)** (500 mg) was added 48 % hydrobromic acid (3 mL) and refluxed for 8 h. The progress of the reaction was monitored by TLC. The reaction mixture concentrated under reduced pressure to remove 75 % of the hydrobromic acid and kept in refrigerator for 10 h. The separated hydrobromide salt of alanine (**6**) was dissolved in warm water. Neutralized with ammonia solution at temperature less than 5 °C. The separated solid was filtered and dried in vacuum to afford compound **7**.

2-Amino-3-(6-methyl-2-oxo-2*H*-chromen-4-yl)propanoic acid (7a): Colourless solid; yield 40 %; m.p. 280 °C; IR (KBr, ν_{max}, cm^{-1}) 3434, 3198, 2923, (DMSO-d_6) 2.45 (s, 3H,CH_3), 3.18 (dd, 1H, H_B, J_{vic} = 9.48 Hz, J_{gem} = 14.29 Hz), 3.47 (dd, 1H, H_A, J_{vic} = 4.65 Hz, J_{gem} = 14.31 Hz), 4.1 (bs, 1H, H_X), 6.42 (s, 1H, C_3-H), 7.0 (d, 1H, C_7-H, J_{ortho} = 8.8 Hz, J_{meta} = 2.1 Hz), 7.18 (d, 1H, C_5-H, J_{meta} = 2.1 Hz), 7.21 (d, 1H, C_8-H, J = 8.8 Hz). Anal. calcd. for $C_{13}H_{13}NO_4$ (%); C, 63.15; H, 5.30; N, 5.67. Found: C, 63.12; H, 5.28; N, 5.65.

2-Amino-3-(7-methyl-2-oxo-2*H*-chromen-4-yl)propanoic acid (7b): Off white solid; yield 45 %; m.p. 295 °C; IR (KBr, ν_{max}, cm^{-1}) 3393, 3993, 2918, 1694, 1677; ^{1}H NMR (DMSO-d_6) 2.48 (s, 3H,CH_3), 3.16 (dd, 1H, H_B, J_{vic} = 9.48 Hz, J_{gem} = 14.30 Hz), 3.48 (dd, 1H, H_A, J_{vic} = 4.64 Hz, J_{gem} = 14.30 Hz), 4.13 (bs, 1H, H_X), 6.44 (s, 1H, C_3-H), 7.1 (d, 1H, C_6-H, J_{ortho} = 8.8 Hz,), 7.18 (d, 1H, C_5-H, J_{ortho} = 8.9 Hz), 7.21 (s, 1H, C_8-H). Anal. calcd. for $C_{13}H_{13}NO_4$ (%); C, 63.15; H, 5.30; N, 5.67. Found: C, 63.14; H, 5.28; N, 5.65.

2-Amino-3-(5,6-benzo-2-oxo-2*H*-chromen-4-yl)-propanoic acid (7c): Off white solid; yield 35 %; m.p. > 300 °C; IR (KBr, ν_{max}, cm^{-1}) 3415, 3060, 2989, 1716, 1694; ^{1}H NMR (DMSO-d_6) 3.27 (dd, 1H, H_B, J_{vic} = 9.24 Hz, J_{gem} = 14.29 Hz), 3.57 (dd, 1H, H_A, J_{vic} = 5.26 Hz, J_{gem} = 14.47 Hz), 4.31 (bs, 1H, H_x), 6.57 (s, 1H, C_3-H), 7.69-8.40 (m, 6H, Ar-H) 8.47 (bs, 3H, NH_3^+). Anal. calcd. for $C_{16}H_{13}NO_4$ (%); C, 67.84; H, 4.63; N, 4.94 Found: C, 67.82; H, 4.65; N, 4.93.

2-Amino-3-(7,8-benzo-2-oxo-2*H*-chromen-4-yl)-propanoic acid (7d): Colourless solid; yield 40 %; m.p. > 300 °C; IR (KBr, ν_{max}, cm^{-1}) 3395, 3123, 2917, 1704, 1681; ^{1}H NMR (DMSO-d_6) 3.32 (dd, 1H, H_B, J_{vic} = 9.66 Hz, J_{gem} = 14.30 Hz), 3.66 (dd, 1H, H_A, J_{vic} = 4.55 Hz, J_{gem} = 14.33 Hz), 4.13 (bs, 1H, H_X), 6.58 (s, 1H, C_3-H), 7.65-7.97 (m, 6H, Ar-H), 8.51 (bs, 3H, NH_3^+). Anal. calcd. for $C_{16}H_{13}NO_4$ (%); C, 67.84; H, 4.63; N, 4.94. Found: C, 67.83; H, 4.62; N, 4.92

2-Amino-3-(6-methoxy-2-oxo-2*H*-chromen-4-yl)-propanoic acid (7e): During reaction with hydrobromic acid 10 % of demethylated product obtained which was removed by washing with water. Off white solid; yield 25 %; m.p. 298 °C; IR (KBr, ν_{max}, cm^{-1}) 3475, 3082, 2918, 1683, 1629; ^{1}H NMR (DMSO-d_6) 3.16 (dd, 1H, H_B, J_{vic} = 9.48 Hz, J_{gem} = 14.01 Hz), 3.48 (dd, 1H, H_A, J_{vic} = 4.66 Hz, J_{gem} = 14.10 Hz), 4.0 (s, 3H, C_6-OCH_3), 4.13 (bs, 1H, H_X), 6.46 (s, 1H, C_3-H), 7.1-7.3 (m, 3H, Ar-H). Anal. calcd. for $C_{13}H_{13}NO_5$ (%); C, 59.31; H, 4.98; N, 5.32. Found: C, 59.26; H, 4.95; N, 5.31.

2-Amino-3-(6-chloro-2-oxo-2*H*-chromen-4-yl)-propanoic acid (7f): Colourless solid; yield 30 %; m.p. 300 °C; IR (KBr, ν_{max}, cm^{-1}) 3391, 3096, 2930, 1698, 1671; ^{1}H NMR (DMSO-d_6) 3.18 (dd, 1H, H_B, J_{vic} = 9.48 Hz, J_{gem} = 14.29 Hz), 3.48 (dd, 1H, H_A, J_{vic} = 4.65 Hz, J_{gem} = 14.31 Hz), 4.08 (bs, 1H, H_X), 6.44 (s, 1H, C_3-H), 7.11 (dd, 1H, C_7-H, J_{ortho} = 8.8 Hz, J_{meta} = 2.1 Hz), 7.18 (d, 1H, C_5-H, J_{meta} = 2.1 Hz), 7.20 (d, 1H, C_8-H, J = 8.9 Hz). 8.43 (bs, 3H, NH_3), Anal. calcd. for $C_{12}H_{10}ClNO_4$ (%); C,53.85; H, 3.77; N, 5.23. Found: C, 53.83; H, 3.82; N, 5.20.

RESULTS AND DISCUSSION

The required 4-bromomethyl coumarins (**3**) were prepared according to the literature methods[7-9]. 4-Bromomethyl coumarins (**3**) were made to react with diethyl acetamido-

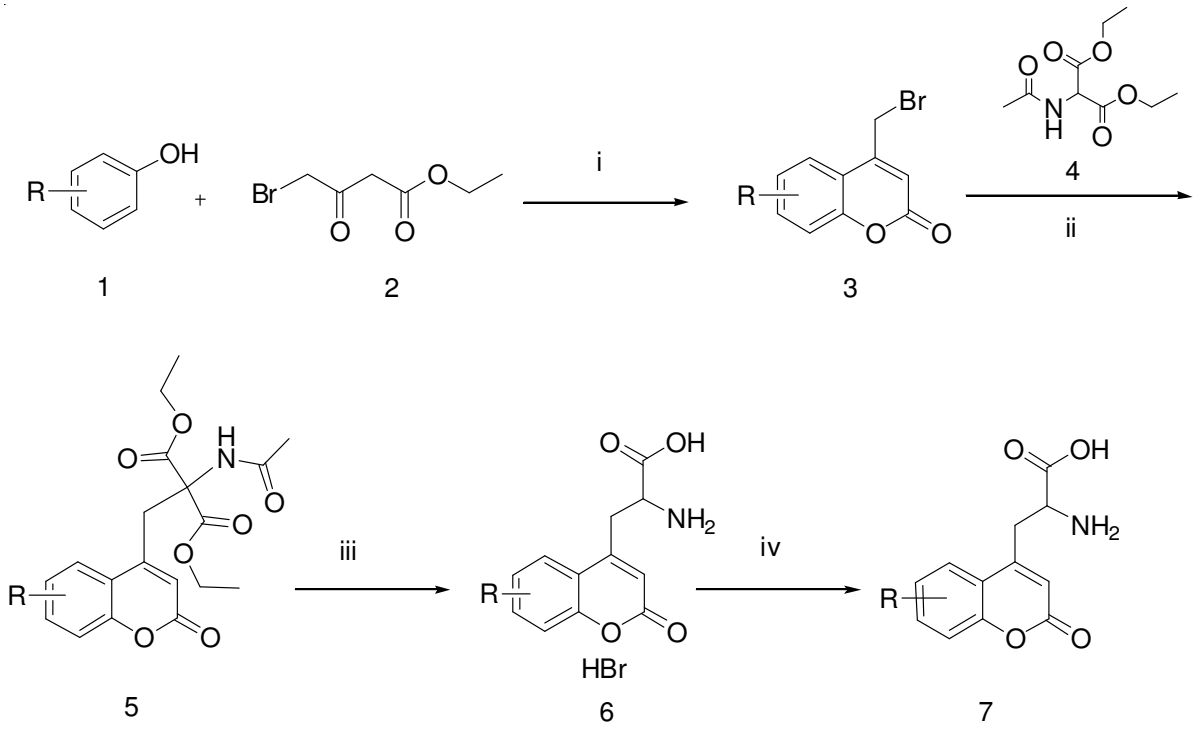
malonate (**4**) in presence of sodium hydride in freshly distilled DMF and dry benzene to afford compounds **5**. The reaction involves the formation of carbanion at the active methylene carbon of diethylacetamidomalonate which attacks the C4-methylene carbon of coumarin leading to a facile C-C bond formation. Compounds **5** were found to be crystalline in nature and showed the melting points in the range of 100-230 °C. The ^{1}H NMR spectrum of compound **5a** (R = 6-CH_3) showed a singlet at δ 2.40 due to C_6-methyl protons and a singlet at δ 1.93 due to methyl protons of amide group. The protons of ethyl ester were observed as triplet and quartet at δ 1.3 (J = 7.1 Hz) and δ 4.23 (J = 7.1 Hz), respectively. The C_3-H of coumarin was observed at δ 6.71 as singlet and the NH proton resonated as singlet at δ 6.11 which was further confirmed by D_2O exchange. The C_5-H of coumarin was observed as singlet at δ 7.43, where as C_7-H and C_8-H were observed as doublets at δ 7.23 (J = 8.41 Hz) and δ 7.35 (J = 8.39 Hz) respectively. The EI mass spectrum of compound **5d** (R = 7,8-benzo) showed base peak at *m/z* 264 (100 %) where as molecular ion peak was not observed.

Reaction of compounds **5** with 48 % HBr led to the hydrolysis of amide and both ester groups. One of the carboxylic acid underwent decarboxylation leading to the formation of hydrobromides of corresponding amino acids (**6**). Free amino acids were obtained by neutralizing the hydrobromides with ammonia solution to afford alanine derivatives of coumarin (**7**). The amino acids (**7**) were insoluble in most of the organic solvents and possessed high melting points.

The IR spectrum of compound **7a** (R = 6-CH_3) showed lactone carbonyl stretching at 1696 cm^{-1}. The acid carbonyl was observed at 1689 cm^{-1}. The NH stretching frequency appeared as broad band at 3198 cm^{-1} and -OH stretching observed at 3434 cm^{-1}. Another characteristic less intensity band observed around 2100 cm^{-1} (combination band) shown by all amino acids and their salts. In fact this band along with the band around 2500 cm^{-1} (overtones) is the two bands which are very common in amino acids and their salts[10]. The ^{1}H NMR spectrum of compound **7a** (R=6-CH_3) showed a singlet at δ 2.45 due to methyl protons and a broad singlet at δ 4.1 due to chiral proton (H_x). The two diastereopic methylene protons H_A and H_B resonated as two separate doublet of doublets. H_A resonated at δ 3.47 with *vicinal* coupling constant J_{vic} = 4.65 Hz and *geminal* coupling constant J_{gem} = 14.31 Hz. H_B resonated at δ 3.18 with *vicinal* coupling constant J_{vic} = 9.48 Hz and with *geminal* coupling constant J_{gem} = 14.29 Hz. Geminal coupling constants are same in both cases, but *vicinal* coupling constants are different. This is because with respect to chiral proton H_x the dihedral angle differs for H_A and H_B. C_3-H of coumarin observed at δ 6.42 and C_5-H observed as doublet at δ 7.18 due to *meta* coupling J_m = 2.1 Hz. Similarly C_7-H of coumarin observed as doublet at δ 7.0 (J = 8.8 Hz) and C_8-H observed as doublet at δ 7.21 (J = 8.8 Hz). Synthetic route is outlined in **Scheme-I**

Antimicrobial activity: All the compounds were screened for their antibacterial activity against *B. subtillis* and *E. coli* using the standard ciprofloxacine and antifungal activity against *A. niger* using grisiofulvin by cup-plate method[11] at the concentration of 1000 µg/mL.

Compound **5f** with chloro substitution at 6th position of coumarin showed 72.2 % inhibition against *B. subtillis* and



R= 6-CH_3, 7-CH_3, 5,6-Benzo, 7,8-Benzo, 6-OCH_3, 6-Cl

i. Conc H_2SO_4, 0 °C/12 h, ii. NaH/Benzene/DMF, Reflux/18 h, iii. 48% HBr, Reflux/8 h, iv. NH_3 solution

Scheme-I

TABLE-1
ANTIMICROBIAL ACTIVITY OF COMPOUNDS **5**(**a-f**) AND **7**(**a-f**)

Compd.	R	*B. subtillis*		*E. coli*		*A. niger*	
		Zone of inhibition (mm)	Relative inhibition (%)	Zone of inhibition (mm)	Relative inhibition (%)	Zone of inhibition (mm)	Relative inhibition (%)
5a	6-CH_3	15	50.0	16	55.5	15	50.0
5b	7-CH_3	16	55.5	16	55.5	17	61.1
5c	5,6-Benzo	14	44.4	14	44.4	15	50.0
5d	7,8-Benzo	15	50.0	15	50.0	14	44.4
5e	6-OCH_3	18	66.6	18	66.6	17	61.1
5f	6-Cl	19	72.2	19	72.2	20	77.7
7a	6-CH_3	20	77.2	20	77.2	21	83.3
7b	7-CH_3	19	72.2	20	77.2	21	83.3
7c	5,6-Benzo	18	66.6	18	66.6	19	72.2
7d	7,8-Benzo	20	77.2	19	72.2	20	77.2
7e	6-OCH_3	21	83.3	21	83.3	22	88.8
7f	6-Cl	23	94.4	22	88.8	23	94.4
DMF		6	-	6	-	6	-
Ciprofloxacin		24	100	24	100	-	-
Grisiofulvin		-	-	-	-	24	100

E. coli. The same compound **5f** showed 77.7 % of inhibiting activity against A. *niger.* Compound **7f** with chloro substitution showed 94.4 % inhibition against *B. subtillis* and *A. niger* and 88.8 % against *E. coli*. All other compounds exhibited moderate to good activity. Antimicrobial activity of all newly synthesised compounds **5**(**a-f**) and **7**(**a-f**) is shown in Table-1.

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