An Efficient One Pot Three Component Synthesis of *N*-(Methylene-4-oxo coumarinyl)anilines; Their *Z,E*-Isomeric Product Distribution Determination From ^{1}HNMR and their Antibacterial Studies

Abdul Rauf*[,1], Hamish McNab[2] and Ashfaq Mahmood Qureshi*[,3]

[1]*Department of Chemistry, The Islamia University, Bahawalpur, 60000-Pakistan*

[2]*School of Chemistry, The University of Edinburgh, Joseph Black Building, The King's Buildings, west Mains Road Edinburgh, EH9 3JJ-UK*

[3]*Department of Chemistry, Bahauddin Zakariya University, Multan, 60800-Pakistan*

Received December 09, 2011: Revised January 30, 2012: Accepted March 04, 2012

Abstract: The new series of *N*-(methylene-4-oxo coumarinyl)anilines **1-13** was synthesised and screening of different solvents led to the discovery of high boiling alcohols as the suitable candidate for one pot efficient three component reaction. Particularly in 2-butanol the product was precipitated out in excellent yields without the hassle of purification. The product distribution of *Z* and *E*-isomers was established by ^{1}HNMR. These compounds were screened for their *in vitro* antibacterial activity against *Escherichia Coli, Bacillus Subtilis, Shigella flexenari, Staphylococous aureus, Pseudomonas aeruginosa* and *Salamonella typhi.* Standard drug impenium was used as control.

Keywords: N-(methylene-4-oxo coumarinyl)anilines, Antibacterial, *Escherichia Coli, Bacillus Subtilis, Shigella flexenari, Staphylococous aureus, Pseudomonas aeruginosa* and *Salamonella typhi.*

INTRODUCTION

Synthetic and naturally occurring coumarins (1,2-benzopyrone) **1a** and its derivatives are well known cytotoxic agents [1]. Auraptene, a coumarin derivative, which was isolated from citrus plant, has reportedly exhibited anti-tumour and anti-carcinogenic biological activities in rats and mice [2]. The synthesis of 3-ureidomethylene coumarins **1b** [3], *N*-(methylene-4-oxo coumarinyl)carbamates **1c** [4] and *N*-(methylene-4-oxo coumarinyl)amino acids **1d** [5] has been reported *via* three component reaction from low to moderate yields (Fig. **1**).

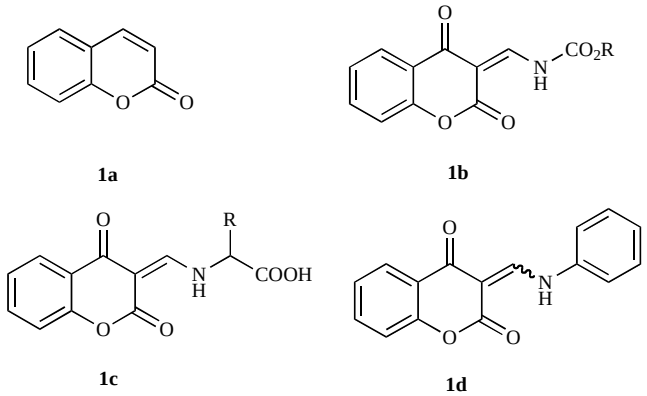


Fig. (1). Some important coumarins derivatives.

Previously, we have synthesised *N*-(methylene-4-oxo coumarinyl)sulfonamides and reported their antibacterial, antifungal and cytotoxic properties [6]. There biological activity is resultant of the coumarin structural motif, which is extensively employed as antimicrobial and anticoagulant [7, 8]. The reported proton NMR data of **1a-1c** are remarkably intriguing [2, 3] which prompted our interest in the synthesis of a new series of *N*-(methylene-4-oxo coumarinyl)anilines **4**. The chemical shifts and splitting pattern of ethylenic proton and NH are revisited. The synthesis of new series of *N*-(methylene-4-oxo coumarinyl)anilines were afforded and their antibacterial activities were also studied.

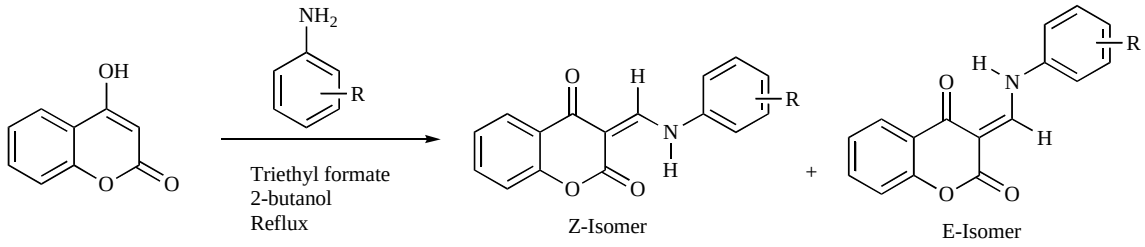
MATERIALS AND METHODS

General Procedure-Chemistry

All reactions were carried out under a nitrogen atmosphere unless otherwise stated. The reaction glassware was flame dried as standard. TLC was performed using Merck plastic/aluminium coated plates covered with a 0.2 mm of layer of silica gel 60 F_{254}. Product spots were visualised either by UV irradiation at 254 nm or by staining with suitable stains followed by heat drying. Melting points were determined on a Gallenkamp apparatus and were uncorrected. ^{1}HNMR spectra were recorded as solution in the d_6-DMSO, using tetramethylsilane as internal standard. The spectra were recorded at 500 MHz (Burker AM 500) and 400 MHz (Burker AM 400) spectrometers. The chemical shift values were measured in part per million (ppm), coupling constants *J*, were measured in Hz. Mass spectra were recorded on a micromass analytical machine, using either chemical ionisation (CI) or electron ionisation (EI). The mass of the fragment is given followed by the relative % in brackets.

*Address correspondence to this author at the Department of Chemistry, The Islamia University, Bahawalpur, 60000-Pakistan; Tel: 0092629250473; E-mail: lecorganic@yahoo.com, shamashfaq@yahoo.com

1875-628X/12 $58.00+.00



Scheme 1.

RESULTS AND DISCUSSION

An equimolar ratio of 4-Hydroxy coumarin and substituted anilines in the presence of triethylorthoformate (1.5eq) gave *Z* and *E*-isomers of *N*-(methylene-4-oxo coumarinyl)anilines in excellent yields (Scheme **1**).

The *Z* and *E*-isomeric ratio was determined by intrinsic analysis of the ^{1}HNMR data; NH proton splits into two non-isochronous doublets, the one low field doublet is assigned to the *Z*-isomer NH which is deshielded as compared to *E*-isomer's NH. Indeed this effect is due to the +M (Mesomeric) effect of lactone which is favouring stronger hydrogen bonding in case of *Z*-isomer. The ethylenic proton generally showed a second order splitting but in case of **7** and 1**2** it is first order, splitting into two characteristic doublets. At these instances the doublet due to ethylenic proton of *E*-isomer is found to be lowfield revealing the same *Z* to *E*-isomeric product distribution as NH proton suggested. On the basis of these grounds the *Z* and *E*-isomeric product distribution was determined and presented in Table **1**.

Table 1. *Z* and *E* Isomeric Distribution

Compounds	Z-Isomer %	E-Isomer %
1	73	27
2	71	29
3	62	38
4	65	35
5	66	34
6	57	43
7	64	36
8	73	27
9	43	57
10	50	50
11	61	39
12	62	38
13	68	32

The product distribution of the *Z* and *E*-isomer clearly depicted that *Z*-isomer is more favoured. This is quite reasonable as in *Z*-isomeric substrates the *N*-substituted phenyl ring is away having less steric interactions. In case of **9** and **12**, the presence of carboxylic acid group at *para* position tends to favour the formation of *E*-isomer.

Screening of the Solvents

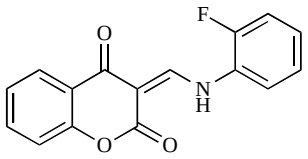
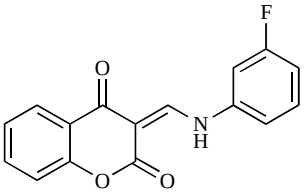
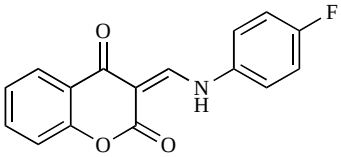
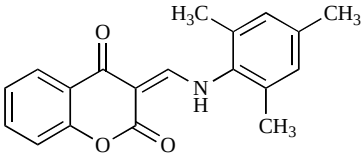
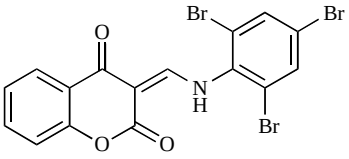
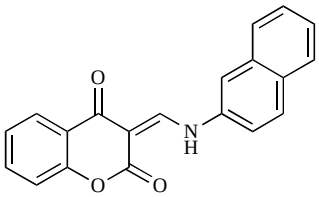
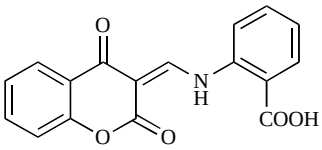
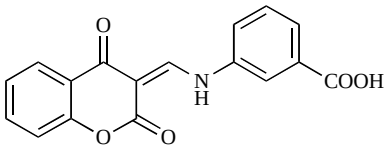
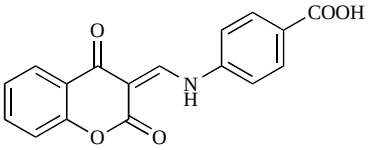
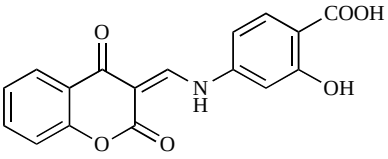
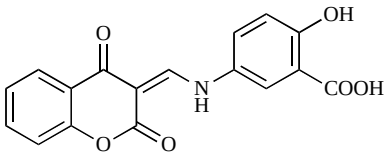
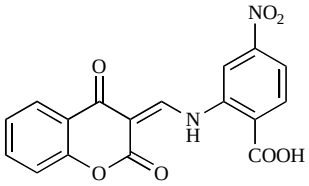
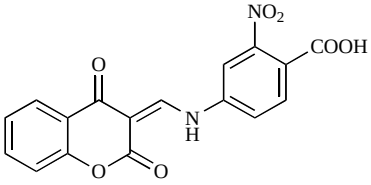
The commercially available aromatic amines were condensed with 4-hydroxycoumarin in the presence of triethylorthoformate and *N*-(methylene-4-oxo coumarinyl) aromatics *via* three component reaction under reflux. Reported methods afforded low yields after tedious separation, therefore screening of the solvents was done, which could address these two issues i.e. low yield and tedious separation. The results are tabulated in Table **2**.

Table 2. Solvent Screening

Compounds	MeOH % yields	EtOH % yields	2-Propanol % yields	2-Butanol % yields
1	7	24	37	92
2	5-7	21	39	88
3	0	16	32	92
4	0	16.5	43	90
5	11	22	45	87
16	8	17	43	83
17	10	24	46	82
8	0	16	53	88
9	12	19	52	90
10	8	21	38	88
11	7	24	31	92
12	5	11	38	85
13	0	13	41	80

The reactions carried out in methanol, ethanol and 2-propanol and products collected were impure and yields were low. The introduction of 2-butanol resulted in high yields and purity. These results can be explained on the basis of the comparison of the boiling points of these solvents; 2-butanol is high boiling solvent in comparison to methanol, ethanol and 2-propanol. The activation energy required for the completion of this three component reaction is sufficiently available if the reaction is performed in the high boiling solvent, whereas in the low boiling solvents small number of molecules can acquire this energy and low yields and unreacted reactants are observed. Although, increase in the reaction time in low boiling solvents can enhance the yields to some extent but still the purification issues prevail. The high boiling solvent and 2-butanol in comparison to methanol, ethanol and 2-propanol are efficiently taking the reaction to completion and above all the desired product is

Table 3. Compounds Synthesised 1-23

 **1**	 **2**	 **3**
 **4**	 **5**	 **6**
 **7**	 **8**	 **9**
 **10**	 **11**	 **12**
 **13**		

highly pure as it is completely insoluble in 2-butanol. These two factors i.e. high boiling point and very low solubility for the targeted compounds make 2-butanol a stand out choice for this multicomponent reaction.

General Procedure for the Preparation of Compounds 1-13

To a stirred solution of 4-hydroxycoumarin (1.62 g, 0.01mole) and triethylorthoformate (2.25 g, 0.015mole) in n-butanol (30 mL) was added the respective amines (0.01mole). The mixture was refluxed for 3 h. The precipitates formed during refluxing were collected by suction filtration. Washing with hot ethanol, TLC afforded pure products in excellent yield (Table **3**).

3-[(2-Fluoro-phenylamino)-methylene]-chroman-2,4-dione 1

δ_H(500 MHz; $CDCl_3$) 13.80 (0.77 H, d, *J* 13.1, N***H***, *Z*-isomer), 11.96 (0.23 H, d, *J* 14.2, N***H***, *E*-isomer), 8.99 (1H, dd, *J* 13.1 and 15.7, =C***H***NH), 7.99 (1H, m, Ar***H***), 7.91 (1H, m, Ar***H***), 7.73 (1H, m, Ar***H***), 7.44 (2H, m, Ar***H***), 7.35 (3H, m, Ar***H***); *m/z* (CI) 283(MH^+, 2%), 282(15), 172(42), 166(2), 148(8), 121(16), 91(5), 84(62), 66(100), 57(21), 55(17).

3-[(3-Fluoro-phenylamino)-methylene]-chroman-2,4-dione 2

δ_H(500 MHz; $CDCl_3$) 13.40 (0.71H, d, *J* 13.6, N***H***, *Z*-isomer), 11.82 (0.29H, d, *J* 14.6, N***H***, *E*-isomer), 8.90 (1H, dd, *J* 13.6 and 13.2, =C***H***NH), 7.98 (1H, dd, *J* 7.6 and 1.3 Ar**H**), 7.69 (2H, m, Ar***H***), 7.51 (2H, m, Ar***H***), 7.35 (2H, m, Ar***H***), 7.15 (1H, m, Ar***H***); m/z (EI) 284.2(M+, 16), 283.1(76), 254.1(5), 221.1(4), 173.0(100), 162.1(10), 149.1(27) , 135.1(18), 121(25), 108(7), 95.1(6), 83(10), 71.1(14), 57.1(22), 46.1(100)45.1(13.5), 40.3(2).

3-[(4-Fluoro-phenylamino)-methylene]-chroman-2,4-dione 3

δ_H(400 MHz; $CDCl_3$) 13.42 (0.62 H, d, *J* 13.8, N***H***, *Z*-isomer), 11.85 (0.38 H, d, *J* 14.8, N***H***, *E*-isomer), 8.82 (1H,

dd, *J* 14.6 and 13.8, =C***H***NH), 7.96 (1H, d, *J* 7.8, Ar***H***),7.71 (3H, m, Ar***H***) 7.33(4H, m, Ar***H***); m/z (EI) 284 (M+, 13), 283(72), 254.1(5), 207(3), 173(100), 162(10), 149(9), 139(28), 135(22), 121(30), 111(38), 95(16), 84(22), 71(13), 57(21), 46(80).

3-[(2,4,6-Trimethyl-phenylamino)-methylene]-chroman-2,4-dione 4

δ_H(400 MHz; $CDCl_3$) 12.51 (0.65H, d, *J* 14.4, N***H***, *Z*-isomer), 11.12 (0.35H, d, *J*, N***H***, *E*-isomer), 8.32 (1H, d, *J* 14.3, =C***H***NH), 7.96 (1H, dd, *J* 7.7 and 7.6, Ar***H***), 7.69 (1H, t, Ar***H***), 7.33 (2H, m, Ar***H***), 7.01 (2H, s,) 2.26 (3H, s, CH_3), 2.24 (3H, s, CH_3), 2.20 (3H, s, CH_3); m/z (EI+) 430(M+, 1), 307(60), 306(100), 305(96), 292(30), 278(9), 213(55), 187(44), 173(32), 158(560, 145(65), 131(24), 121(60), 105(16), 83(45), 46(100).

3-[(2,4,6-Tribromo-phenylamino)-methylene]-chroman-2,4-dione 5

δ_H(400 MHz; $CDCl_3$) 12.79 (0.66H, d, *J* 13.4, N***H***, *Z*-isomer), 11.43 (0.34H, d, *J* 14.5, N***H***, *E*-isomer), 8.57 (1H, dd, *J* 14.6 and 12.1, =C***H***NH), 8.11 (2H, s, Ar***H***), 7.96 (1H, dd, *J* 8.8 and 1.1, Ar***H***), 7.71 (1H, m, Ar***H***), 7.36 (2H, m, Ar***H***); m/z (EI) 500(M+, 11), 423(93), 421(100), 420(94), 354(7), 301(12), 273(7), 231(6), 188(5), 173(49), 134(3), 121(45), 92(11), 65(8).

3-(Naphthalen-2-ylaminomethylene)-chroman-2,4-dione 6

δ_H(400 MHz; $CDCl_3$) 14.46 (0.57H, d, *J* 12.6, N***H***, *Z*-isomer), 12.54 (0.43H, d, *J* 14.3, N***H***, *E*-isomer), 9.03 (1H, dd, *J* 14.3 and 12.5, =C***H***NH), 7.98 (2H, s, Ar***H***), 7.96 (1H, dd, *J* 8.8 and 1.1, Ar***H***), 7.71 (1H, m, Ar***H***), 7.36 (2H, m, Ar***H***), 7.28 (4H, m, Ar***H***), 7.11(1H, s, Ar***H***) ; m/z (EI) 314.9(M+, 100), 313.9(89), 298(6), 286(5), 270.1(5), 195(12), 194(35), 173(62), 167.1(39), 154.1(22), 139(14), 127(20), 121(40), 115(16), 94(17), 78(99), 63(99), 46.1(86)

2-[(2, 4-Dioxo-chroman-3-ylidenemethyl)-amino]-benzoic acid 7

δ_H(400 MHz; $CDCl_3$) 14.44 (0.64H, d, *J* 13.7, N***H***, *Z*-isomer), 13.57 (0.36H, d, *J* 14.5, N***H***, *E*-isomer), 8.97 (1H, dd, *J* 14.5 and 13.7, =C***H***NH), 8.03 (1H, d, *J* 7.7, Ar***H***), 7.95 (2H, m, Ar***H***), 7.71 (2H, m, Ar***H***), 7.40 (1H, m, Ar***H***) 7.33 (2H, m, Ar***H***); m/z (EI) 581(M+, 1), 308(7), 265(21), 264(100), 247(18), 221(8), 187(16), 171(23), 172(99), 162(18), 144(23), 121(81), 120(65), 92(43), 77.1(25), 65(23), 63(16), 46(100).

3-[(2,4-Dioxo-chroman-3-ylidenemethyl)-amino]-benzoic acid 8

δ_H(400 MHz; $CDCl_3$) 13.40 (0.73H, d, *J* 13.7, N***H***, *Z*-isomer), 13.2 (1H, broad s, COO***H***), 11.89 (0.27H, d, *J* 14.7, N***H***, *E*-isomer), 8.89 (1H, m, =C***H***NH), 8.10 (1H, d, *J* 9.32, Ar***H***), 7.98 (1H, d, *J* 7.7, Ar***H***), 7.91 (1H, m, Ar***H***), 7.86 (1H, d, *J* 7.6, Ar***H***) 7.70(1H, m, Ar***H***) 7.95(1H, m, Ar***H***) 7.35(2H, m, Ar***H***) ;m/z (CI) 308.9(MH+,61) 309.9(10), 279.9(2), 263.8(3), 188(15), 173.9(11)., 172.9(100), 161(15), 148.9(8), 120.9(40), 91.9(16), 65(24).

4-[(2,4-Dioxo-chroman-3-ylidenemethyl)-amino]-benzoic acid 9

δ_H(400 MHz; $CDCl_3$) 13.42 (0.43H, d, *J* 13.6, N***H***, *Z*-isomer), 13.07(1H, broad s, COO***H***)11.87 (0.57H, d, *J* 14.5, N***H***, *E*-isomer), 8.95 (1H, dd, *J* 14.2 and 13.6, =C***H***NH), 7.99 (2H, dd, *J* 8.4 and 1.7, Ar***H***), 7.76 (2H, dd, *J* 8.7 and 1.9, Ar***H***), 7.69 (1H, m, Ar***H***), 7.36 (3H, m, Ar***H***); m/z (CI) 309(MH+, 10), 308(58), 188(11), 172(100), 161(14), 143(8), 120(40), 91(14), 89(7), 65(20).

4-[(2, 4-Dioxo-chroman-3-ylidenemethyl)-amino]-2-hydroxy-benzoic acid 10

δ_H(400 MHz; $CDCl_3$) 13.27 (0.50H, d, *J* 13.64, N***H***, *Z*-isomer), 11.74 (0.50H, d, *J* 14.5, N***H***, *E*-isomer), 8.89 (1H, dd, *J* 13.5 and 13.3, =C***H***NH), 7.97 (1H, m, Ar***H***), 7.84 (1H, d, *J* 8.5, Ar***H***), 7.70 (1H, m, Ar***H***), 7.36 (1H, d, *J* 7.24, Ar***H***) 7.32 (1H, d, *J* 8.2, Ar***H***) 7.20 (3H, m, Ar***H***) 3.35 (1H, broad s, O***H***); m/z (EI) 589(M+, 4) 325(53), 280(20), 281(92), 264(14), 204(11), 187(16), 173(100), 160(21), 121(84), 120(38), 94(27), 65(16), 46(70).

5-[(2, 4-Dioxo-chroman-3-ylidenemethyl)-amino]-2-hydroxy-benzoic acid 11

δ_H(400 MHz; $CDCl_3$) 13.38 (0.61H, d, *J* 13.9, N***H***, *Z*-isomer), 11.28 (0.39H, d, *J* 14.9, N***H***, *E*-isomer), 8.7 (1H, dd, *J* 14.1 and 13.8, =C***H***NH), 7.96 (2H, m, Ar***H***), 7.82 (1H, ddd, *J* 8.9, 8.5 and 2.7, Ar***H***), 7.67 (1H, m, Ar***H***), 7.34 (1H, d, *J* 7.4, Ar***H***) 7.30 (1H, d, *J* 8.3 Ar***H***) 7.04 (1H d, *J* 8.9, Ar***H***) 3.40 (1H, broad s, O***H***); m/z (EI) 324(M+, 12), 325(64), 307(90), 281(63), 187(13), 173(100), 153(9), 121(6), 120(22), 93(18), 65(13), 46(32).

2-[(2,4-Dioxo-chroman-3-ylidenemethyl)-amino]-4-nitro-benzoic acid 12

δ_H(400 MHz; $CDCl_3$) 14.40 (0.62H, d, *J* 13.2, N***H***, *Z*-isomer), 13.53 (0.38H, d, *J* 14.1, N***H***, *E*-isomer), 9.02 (1H, dd, *J* 14.1 and 12.9, =C***H***NH), 8.68 (1H, m, Ar***H***), 8.23 (1H, dd, *J* 8.6 and 8.6, Ar***H***), 8.11 (1H, m, Ar***H***), 7.96 (1H, m, Ar***H***) 7.71 (1H, m, Ar***H***) 7.34 (2H, m, Ar***H***) 3.31 (1H, broad s, COO***H***); m/z (EI) 332(M+, 5), 310(40), 281(9), 182(18), 173(99), 164(31), 121(48), 120(31), 92(19), 61(11), 63(12), 46(100).

4-[(2,4-Dioxo-chroman-3-ylidenemethyl)-amino]-2-nitro-benzoic acid 13

δ_H(400 MHz; $CDCl_3$) 13.32 (0.68H, d, *J* 13.5, N***H***, *Z*-isomer), 11.90 (0.32H, d, *J* 14.4, N***H***, *E*-isomer), 8.96 (1H, dd, *J* 14.3 and 13.5, =C***H***NH), 8.11 (2H, m, Ar***H***), 7.98 (2H, m, Ar***H***), 7.72 (1H, m, Ar***H***), 7.34 (2H, m, Ar***H***) 3.4 (1H, broad s, COO***H***); m/z (EI) 438(M+, 4), 310(82), 309(100), 263(16), 189(26), 173(74), 172(99), 162(38), 120(99), 119(91), 92(48), 64(31), 53(29), 46(99).

In-vitro antibacterial activities were analysed at HEJ Research Institute of Chemistry, International Centre for Chemical Sciences, University of Karachi, Pakistan.

Antibacterial Bioassay

The compounds **1-13** were studied *in vitro* for *Escherichia Coli, Bacillius Subtilis, Shigella flexenari, Staphylococous aureus, Pseudomonas aeruginosa* and *Salamonella typhi* and standard drug impenium was used as control. The method employed was agar well diffusion method [9, 10]. Using a sterile borer wells (6 mm diameter) were dug in bacterial inoculation was done on the nutrient agar (~ 10^4-10^6 CFU/ml). The concentration of sample

Table 4. Antibacterial Activity of the Synthesised Compounds Against Various Micro-Organisms

Compd No	*Escherichia Coli*	*Bacillius Subtilis*	*Shigella flexenari*	*Staphylococus aureus*	*Pseudomonasa eruginosa*	*Salamonella typhi*
1	-	-	-	-	14	-
2	14	-	-	-	10	10
3	-	-	-	-	10	-
4	-	-	-	-	10	-
5	-	-	-	-	10	-
6	-	-	-	-	-	10
7	-	17	-	-	-	-
8	-	-	-	-	-	08
9	-	12	-	-	-	-
10-13	-	-	-	-	-	-
Impenium Std Drug	30	32	26	32	24	25

employed to the wells is 1mg/mL of DMSO. The control wells were supplemented with DMSO (1 mL) and standard drug 'Impenium' (10 µg/well). These samples were incubated at 37°C for 24-48hrs. The zone inhibition in mm was recorded, DMSO did not show any activity against any organism included in this study. The biological activities are presented in Table **4**.

The compounds **3-5** showed biological activity against *Pseudomonas aeruginosa*, which can be co-related with the presence of halogen substituents on the aromatic ring of these anilines. In **7** the increased activity against *Bacillius subtilis* showed that the presence of -COOH group at ortho position made the substrate biologically potent. The sample **10-13** did not show any activity which emphasizes that future work should consider the SAR (structure activity relationship) of the substituents on these substrates.

CONCLUSION

Various novel *N*-(methylene-4-oxo coumarinyl)anilines were synthesized in excellent yield using an efficient one pot multicomponent reaction. The methodology was scrutinised for the best solvent to increase yield and avail hassle free separation and 2-butanol was the appropriate solvent for this reaction in comparison to low boiling solvent.

N-(methylene-4-oxo coumarinyl)anilines derivatives exhibited intriguing ^{1}HNMR; which was employed to determine *E/Z* product distribution among the synthesised compounds. All the synthesised compounds, when screened against 6 different microorganisms, exhibited moderate to no inhibition; which enlightens that halogens and carboxylic acids substituents are potent pharmacophore against these microorganisms.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

One of the authors (A. Rauf) wishes to thank the Higher Education Commission of Pakistan for Postdoctoral fellowship award that made this research possible.

REFERENCES

[1] Kostova, I. Synthetic and natural coumarins as cytotoxic agents. *Curr. Med. Chem..*, **2005**, *5*, 29-46.

[2] Murakami, A.; Nakamura, Y.; Tanaka, T.; Kawabata, K.; Takahashi, D.; Koshimizu, K.; Ohigashi, H. Suppression by citrus auraptene of phorbol ester-and endotoxin-induced inflammatory responses: role of attenuation of leukocyte activation. *Carcinogenesis.*, **2000**, *21*, 1843-50.

[3] Sakellariou, R.; Speziale V.; Hamdi, M. Preparation of a new series, the 3-ureidomethylenecoumarins by condensation of 4-hydroxycoumarin with substituted ureas. *Synth. Commun.*, **1990**, *20*, 3443-3451.

[4] Speziale, V.; Sakellariou, R.; Hamdi, M. New method of synthesis of *N*-substituted carbamates from 4-hydroxycoumarin., *Synthesis*, **1992**, *10*, 921-922.

[5] Hamdi, M.; Sakellariou R.; Speziale, V. Synthesis of *N*-substituted α-aminoacids from 4-hydroxycoumarin., *J. Heterocyclic. Chem.*, **1992**, *29*, 1817-1819.

[6] Chohan, Z.H.; Shaikh, A.U.; Rauf, A.; Supuran, C.T. Antibacterial, antifungal and cytotoxic properties of novel *N*-substituted sulfonamides from 4-hydroxycoumarins, *J. Enzy. Inhibit. Med. Chem.*, **2006**, *21*, 741-748.

[7] Manolov, I.; Danchev, N.D. Synthesis toxicological and pharmacological assessment of some 4-hydroxy coumarin., *Eur .J .Med. Chem. Chim. Ther.*, **1995**, *30*, 531-536.

[8] Abd-Allah, O. A. Synthesis and biological studies of some benzopyrano[2,3-*c*]pyrazole derivatives., *Farmaco*, **2000**,*55*, 641-649.

[9] Atta-ur-Rahman; Choudhary, M.I.; Thomsen, W.J. Bioassay Techniques for Drug Development., **2001**, The Netherlands: Harwood Academic Publishers, 16.

[10] Qureshi, A.M., Qadir, M., Rauf, A., Idrees, M., Mumtaz, S., Najam-ul-Haq, M., Aziz-ur-rehman, Ismail, M., Athar, M., Khushal, R., Riaz, S., Bokhari, H. Antimicrobial efficacy of metal-barbiturate conjugates against pathogenic strains of Escherichia coli and Staphylococcus aureus, *Lett. Drug. Dis & Des*, **2011,** *8*, 10, 980-987.