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Short communication

One pot synthesis of oxygenated tri-heterocycles as anti-microbial agents

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Abstract

A one pot synthesis of an array of angularly linked tri-heterocycles with coumarin, benzofuran and furan rings is described. This high yielding synthesis is achieved by the reaction of various 4-bromomethylcoumarins with furyl *o*-hydroxyphenyl ketones involving benzylic nucleophilic displacement and intramolecular aldolization. All the compounds have been tested in vitro for their anti-microbial activity against *Micrococcus aureus*, *Pseudomonas chinchori*, *Asperigillus fumigatus* and *Penicillium wortmanni* at 100, 50, and 25 μg ml^{-1} concentrations. Chloro groups in the benzofuran ring enhanced the activity.

Keywords: Coumarins; Tri-heterocycles; Antibacterial activity

1. Introduction

Biheterocycles containing coumarin and benzofuran ring systems have been of structural and pharmacological interest (Fig. 1). 2-Heterocyclic benzofurans with pyridine [1], thiazole [2], and chromone [3] templates have been found to exhibit anti-microbial, psychotropic and anti-inflammatory activities. For examples, Efaroxan (2-imidazolinyl-2,3-dihydrobenzofuran), is a well-known antagonist ofα-2-adrenoreceptor [4]. 4-Isoxazolyl and oxadiazolyl coumarins are reported as lipo-oxygenase and glucouronidase inhibitors [5], whereas 4-pyridyl and piperidyl coumarins showed CNS depressant properties in animal models [6]. With a view to combine these two naturally occurring nuclei, our efforts were focused on the synthesis of 4-2′-benzofuranyl coumarins which exhibited moderate anti-inflammatory activity in the carrageenan induced rat-paw edema method [7]. The orientation of the benzofuran ring in these compounds was studied by difference NOE experiments using sterically hindered benzofuranyl coumarins [8]. Recently we have reported the biological evaluation of 4-vanillyl ethers and benzofurans from bromomethyl coumarins [9]. Computer aided structural and statistical analysis of natural products and synthetic drugs has clearly emphasized the need to develop libraries of multifunctional oxygen compounds [10], which is evidenced by a recent report on the potent antibacterial and hepatoprotective properties of new 2-substituted benzofurans isolated from *Propolis* [11]. Tri-heterocyclic coumarins with a nitrogen heterocyclic system like oxadiazole, isoxazole, dioxadiazine etc. have been found to be anti-inflammatory agents [12]. In continuation of our work on benzofuranyl coumarins [7–9] the present paper outlines a logical sequence of reactions leading to the construction of an oxygenated tri-heterocyclic library using the positions in the fused benzene rings as the center of molecular diversity.

2. Chemistry

The two synthons required for the construction of tri-heterocyclic library have been generated by utilizing the reactivity of the *ortho* position of phenols (Scheme 1). In pathway **A** phenols **1** are treated with 4-bromoethylacetoacetate **2**, a β-ketoester acting as a double electrophile, in presence of sulfuric acid to yield various 4-bromomethyl coumarins **3** [13]. Pathway **B** utilizes the easy cleavage of the acyl-oxygen bond in phenyl esters **4** which were formed by the reaction of various phenols with furoyl chloride in presence of magnesium turnings in refluxing benzene for 1 h. These esters **4** undergo migration of the furoyl group to the *ortho*

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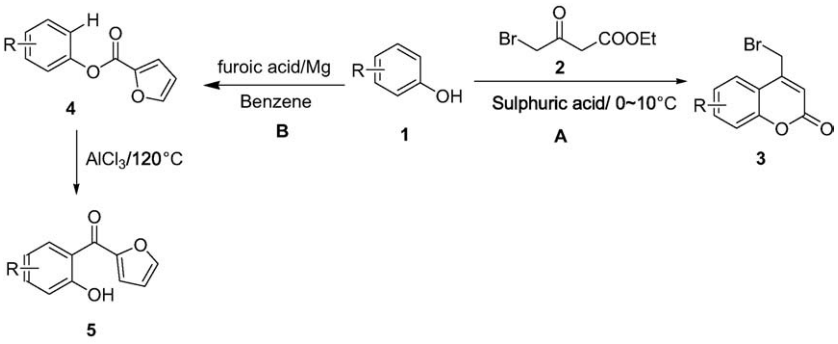


Scheme 1. Generation of synthons **3** and **5**.

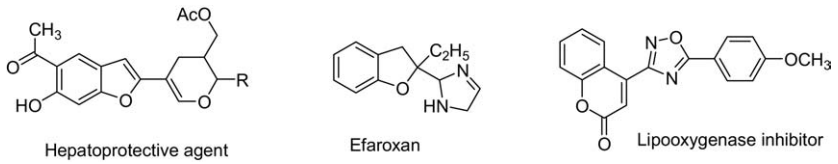


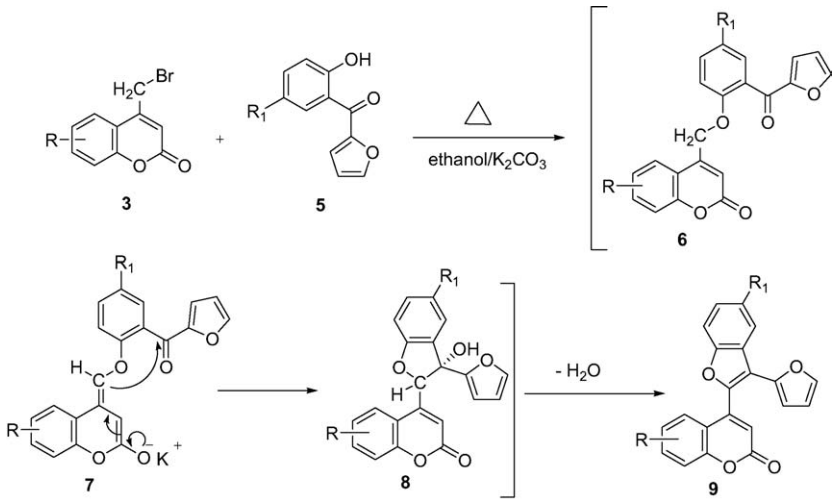
Fig. 1. Biologically active coumarin and benzofuran bi-heterocycles.

position of phenols by anhydrous aluminum chloride. The reaction occurs in 2 h at high temperatures (120 °C) by an intra molecular acylation according to Fries rearrangement resulting in *o*-hydroxyphenyl-furyl ketones **5** [15]. The two synthons **3** and **5** are combined to generate the tri-heterocycles **9** in a single step, which is brought about when equimolar quantities of 4-bromomethylcoumarins **3** and the furyl ketones **5** are refluxed in ethanol in presence of potassium carbonate for 14–16 h.

The reaction mechanism (Scheme 2) is *via* the intermediacy of an *o*-carbonylated-4-aryloxymethylcoumarin **6** resulting from the benzylic S_N reaction leading to O–C bond formation. This intermediate has an active methylene group and a carbonyl group meeting the stereo electronic requirements for a carbanion **7** (in the form of the conjugated enolate), addition across the carbonyl group to form an incipient dihydrobenzofuranol **8** which, in an irreversible fast step undergoes β-elimination to form a C–C double bond. Aromatization is the driving force for such an intramolecular aldol condensation. The proposed intermediacy of the 4-aryloxymethylcoumarin **6** is based on our earlier work involving the isolation and characterization of *o*-carbonylated-4-aryloxymethylcoumarins in the formation of benzofurans [7]. All the final compounds were colorless crystalline solids and are presented in Table 1 with their physical data.

2.1. Spectral characterization

The formation of tri-heterocycles **9** was confirmed by the IR spectra which showed the lactone C=O stretching band in the region of 1709–1740 cm^{-1}. The C=C and C–O–C stretching vibrations were observed in the region of 1610–1550 and 1025–1250 cm^{-1}, respectively. The proton NMR was a useful probe to check the formation of the products **9**. In the NMR spectrum of the reactants **3**, the methylene protons were observed as a singlet around 5.2 ppm. The products **9** showed no signal in the region of 4–6 ppm clearly indicating the absence of methylene protons and formation of the products.



Scheme 2. Mechanism of formation of tri-heterocycles.

Table 1
Physical data of compounds **9a–9l**

Compound	R	R_1	Molecular formula	Yield (%)	M.p. (°C)
9a	6-CH_3	CH_3	$C_{23}H_{16}O_4$	75	168
9b	7-CH_3	CH_3	$C_{23}H_{16}O_4$	70	164
9c	6-Cl	CH_3	$C_{22}H_{13}O_4Cl$	72	187
9d	6-OCH_3	CH_3	$C_{23}H_{16}O_5$	80	164
9e	6-CH_3	Cl	$C_{22}H_{13}O_4Cl$	75	149
9f	7-CH_3	Cl	$C_{22}H_{13}O_4Cl$	72	207
9g	6-Cl	Cl	$C_{21}H_{10}O_4Cl_2$	74	218
9h	6-OCH_3	Cl	$C_{22}H_{13}O_5Cl$	73	192
9i	6-CH_3	Br	$C_{22}H_{13}O_4Br$	76	174
9j	6-CH_3	5′ 7′,Cl	$C_{22}H_{12}O_4Cl_2$	80	172
9k	7-CH_3	5′ 7′,Cl	$C_{22}H_{12}O_4Cl_2$	82	166
9l	6-OCH_3	5′ 7′,Cl	$C_{22}H_{12}O_5Cl_2$	79	205

The most downfield signal was observed around 7.7–8.1 ppm which has been assigned to the C_5–H of coumarin, the deshielding being due to the ring oxygen of benzofuran which is in agreement with the literature reports [14]. The C_3–H of coumarin appeared as a singlet around 6.7 ppm. The protons of the furan ring appeared upfield to C_3–H as multiplets. The other protons due to methyl, methoxy groups resonated at expected fields. The NMR spectral data for all the compounds are given in Table 2. The FAB-MS of compound **9b** showed a peak at *m/z* 357 (M + H) confirming its molecular weight.

3. Biological evaluation and results

In vitro anti-microbial evaluation of all the tri-heterocycles was done against two bacterial and two fungal strains viz. *Micrococcus aureus*, *Pseudomonas chinchori*, *Asperigillus fumigatus* and *Penicillium wortmanni*. The results indicated that compounds **9e–9l** showed total inhibition of bacterial growth at 100 μg ml^{-1} concentration. None of them were active against *M. aureus* at 25 μg ml^{-1}. However against *P. chinchori* **9e–9l** showed a moderate inhibition, at 25 mg ml^{-1} concentration. Their fungicidal activity revealed that **6e–6l** showed a moderate inhibition of *A. fumigatus* at 25 μg ml^{-1} concentration. Against *P. wortmannii*, **9a–9c**, **9j** and **9l** showed moderate inhibition at 25 μg ml^{-1} concentration (Table 3).

4. Experimental

4.1. Chemical synthesis

Melting points were determined in open capillaries and are uncorrected. IR spectra (KBr) were recorded on a Nicollet FT-IR spectrometer. NMR spectra were recorded on a Bruker-300 MHz instrument in $CDCl_3$ using TMS as the internal standard. TLC was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapor or by irradiation with UV light (254 nm). All the new compounds gave satisfactory elemental analyses.

4.1.1. Tri-heterocycles *9a–l*

To a solution of 4-bromomethylcoumarin **3** (0.005 m), and *o*-hydroxyphenyl furyl ketone **5** (0.005 m), in absolute ethanol (50 ml) taken in a 100 ml round bottom flask, anhydrous potassium carbonate 1.38 g (0.01 mol) was added. The mixture was refluxed on a water bath for 14–16 h. The reaction mixture was then filtered hot, the filtrate concentrated, cooled to room temperature and poured on to crushed ice (100 g). The separated solid was washed with water and crystallized from ethanol. The physical and spectral data for the tri-heterocycles are given in Tables 1 and 2.

5. In vitro anti-microbial activity assays

Various coumarin derivatives synthesized during present investigation have been subjected to a preliminary anti-

Table 2
NMR spectral data of compounds **9a–9l**

Compound	R	R_1	^{1}H-NMR (δ, ppm)
9a	6-CH_3	CH_3	2.28 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 6.50–6.63 (m, 2H, Fur-H), 6.73 (s,1H, C_3–H), 7.32–7.56 (m, 6H,Ar-H), 7.78 (s,1H, C_5–H)
9b	7-CH_3	CH_3	2.46 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 6.48–6.60 (m, 2H, Fur-H), 6.67 (s,1H, C_3–H) 7.30–7.60 (m, 6H, Ar-H), 7.76 (d, J = 8.7 Hz, 1H, C_5–H)
9c	6-Cl	CH_3	2.55 (s, 3H, CH_3), 6.54–6.70, (m, 2H, Fur-H), 6.78 (s, 1H, C_3–H), 7.30–7.57 (m, 6H, Ar-H), 7.74 (s, 1H, C_5–H)
9d	6-OCH_3	CH_3	2.54 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 6.52–6.64 (m, 2H, Fur-H), 6.77 (s, 1H, C_3–H), 6.95–7.50 (m, 6H, Ar-H), 7.76 (s, 1H, C_5–H)
9e	6-CH_3	Cl	2.29 (s, 3H, CH_3), 6.50–6.60, (m, 2H, Fur-H), 6.73 (s, 1H, C_3–H), 7.25–7.57 (m, 6H, Ar-H), 8.00, (s, 1H, C_5–H)
9f	7-CH_3	Cl	2.47 (s, 3H, CH_3), 6.48–6.58 (m, 2H, Fur-H), 6.69 (s, 1H, C_3–H), 7.00–7.55 (m, 6H, Ar-H), 8.00 (d, J = 8.4 Hz. 1H, C_5–H)
9g	6-Cl	Cl	6.54–6.68 (m, 2H, Fur-H), 6.80 (s,1H, C_3–H), 7.27–7.58 (m, 6H, Ar-H), 7.97 (s,1H, C_5–H).
9h	6-OCH_3	Cl	3.62 (s, 3H, OCH_3), 6.50–6.60, (m, 2H, Fur-H), 6.78 (s, 1H, C_3–H), 6.95–7.45 (m, 6H, Ar-H), 7.98 (s,1H, C_5–H)
9i	6-CH_3	Br	2.29 (s, 3H, CH_3), 6.50–6.60 (m, 2H, Fur-H), 6.77 (s, 1H, C_3–H,), 7.24–7.61 (m, 6H, Ar-H), 8.16 (s, 1H, C_5–H)
9j	6-CH_3	5′7′, Cl	2.29 (s, 3H, CH_3), 6.50–6.58 (m, 2H, Fur-H), 6.77 (s, 1H, C_3–H), 7.25–7.50 (m, 5H, Ar-H), 7.92 (s, 1H, C_5–H)
9k	7-CH_3	5′7′, Cl	2.47 (s, 3H, CH_3), 6.50–6.57 (m, 2H, Fur-H), 6.72 (s, 1H, C_3–H), 7.04–7.50 (m, 5H, Ar-H), 7.92 (d, J = 8.4 Hz, 1H, C_5–H)
9l	6-OCH_3	5′7′, Cl	3.66 (s, 3H, OCH_3), 6.52–6.62 (m, 2H, Fur-H), 6.82 (s, 1H, C_3–H), 6.96–7.50 (m, 5H, Ar-H), 7.91 (s, 1H, C_5–H)

Table 3
In vitro anti-microbial spectrum of tri-heterocycles

Compound	Concentration (μg ml^{-1})	Inhibition (%)			
		M. aureus	*P. chinchori*	*A. fumigatus*	*P. wortmanni*
9a	100	45.83	41.65	42.85	96.42
	50	Nil[a]	Nil	Nil	64.28
	25	Nil	Nil	Nil	39.28
9b	100	41.65	45.83	39.28	78.57
	50	Nil	Nil	Nil	60.17
	25	Nil	Nil	Nil	42.85
9c	100	50.00	41.65	75.00	89.28
	50	Nil	Nil	53.57	64.28
	25	Nil	Nil	Nil	46.42
9d	100	45.83	50.00	42.85	60.17
	50	Nil	Nil	Nil	39.28
	25	Nil	Nil	Nil	Nil
9e	100	91.66	54.16	96.42	71.42
	50	66.66	Nil	67.85	42.85
	25	Nil	Nil	42.85	Nil
9f	100	87.50	91.66	89.28	67.85
	50	62.50	66.66	60.17	46.42
	25	Nil	41.65	39.28	Nil
9g	100	95.83	91.66	96.42	82.14
	50	66.66	62.50	64.28	57.14
	25	Nil	45.83	42.85	Nil
9h	100	91.66	95.83	89.28	100
	50	62.50	70.83	64.28	60.17
	25	Nil	50.00	39.28	Nil
9i	100	87.50	100	96.42	64.28
	50	62.50	75.00	67.85	39.28
	25	Nil	45.83	39.28	Nil
9j	100	100	100	92.85	67.25
	50	62.50	70.83	78.57	42.85
	25	Nil	58.33	46.42	Nil
9k	100	95.83	100	100	100
	50	62.50	66.66	71.42	71.42
	25	Nil	45.83	50.85	46.42
9l	100	100	95.83	100	96.42
	50	66.66	62.50	75	64.28
	25	Nil	50.00	53.57	46.42
Norfloxacin	100	100	100	----[b]	-----
	50	100	100	-----	-----
	25	62.50	58.33	----	----
Griesofulvin	100	----	----	100	100
	50	-----	-----	100	100
	25	-----	----	3.57	50.85

[a] In the above table, Nil refers to inhibitions less than 20%.
[b] ----- Indicates not applicable.

microbial screening by the cup plate method [16], against two bacterial and two fungal species at three concentrations using DMF as solvent. The methodology adopted for the evaluation is described follows. Antibacterial activity of test compound was evaluated against Gram positive bacteria *M. aureus* and Gram negative bacteria *P. chinchori* using norfloxicin as standard by cup plate method. Dimethyl formamide was used as solvent control. The bacteria were subcultured in a medium containing peptone (0.5%), yeast extract (0.15%), sodium chloride (0.35%), potassium dihydrogen phosphate (0.13%) and potassium monohydrogen phosphate (0.13%). Nutrient agar which served as the basal medium was prepared by dissolving bacteriological peptone (0.6%), yeast (0.3%), beef extract (0.13%) and agar (2.1%) in distilled water. The solution was sterilized for 20 min at 15 lbs. pressure in an autoclave. The basal medium (25–30 ml) (with glucose solution to hasten the bacterial growth) with bacterial culture was poured in sterile petri dishes. After the solidification medium holes of 9 mm diameter were bored to form cups with the help of a sterile cork borer. To this cup 0.02 ml of the solution of the test compound was added by sterilized pipettes. The petridishes were kept in a cold room to facilitate the diffusion of the solvent for about 2 h. The plates were then incubated at 37 °C for 24 h. The extent of inhibition was measured by the width of the inhibition zone in mm. Minimum inhibitory concentration (MIC) of the test solution was determined by diluting the test solution of required concentration.

Fungicidal activity of test compounds was evaluated against *A. fumigatus* and *P. wortmannii* by cup plate method [16]. The fungi were sub-cultured in the following medium containing peptone (1%), yeast extract (0.6%), sodium chloride (0.5%), potassium dihydrogen phosphate (0.3%) and glucose (1%) in distilled water. The pH of the medium was adjusted to 6.0 and sterilized at 15 lbs, for 20 min. The method of testing for anti-fungal activity is the same as that adopted for evaluating antibacterial activity. Griseofulvin was used as the standard and DMF was used as a solvent control.

6. Results and discussion

All the tri-heterocycles were tested against two bacterial and two fungal species by the standard cup plate method [16]. The dimethyl compounds **9a** and **9b** did not show any inhibitory effect at 25 μg ml^{-1} concentration except a moderate inhibition of *P. wortmannii*, 39% and 42%, respectively. Introduction of the chloro substitution in the coumarin ring as in the case of **9c** did not change the observed trend. It is interesting to note that chloro group located in benzofuran ring showed considerable inhibition of *P. chinchori*, *A. fumigatus*, and *P. wortmanni*, as observed in the case of **9e**, **9f** and **9h** (Table 3). The dichloro compound **9g** showed a moderate inhibition of *P. chinchori* (45%) and *A. fumigatus* (42%) at 25 μg ml^{-1} concentration. Introduction of the dichloro group in benzofuran resulted in the enhancement of the antimicrobial potency and the two compounds **9j**, and **9k** exhibited considerable inhibition of the microbial growth of all the species at 50 μg ml^{-1} (Table 3). Further at a lower concentration of 25 μg ml^{-1} also, they were active against *P. chinchori* and *A. fumigatus* (58–46%). These results are consistent with the observation that introduction of chloro group in heterocycles [17] increases the anti-microbial activity. Comparison of the activities of **9e** and **9i** showed that the bulky bromo group made the compound more potent even at 25 μg ml^{-1} especially against *P. chinchori* (45%). The methoxy cou-

marin derivative **9d** was inactive against all the species at 25 μg ml^{-1}. The corresponding benzofuranyl monochloro compound **9h** was active against *P. chinchori* (50%) and *A. fumigatus* (39%). The dichloro derivative **9l** showed the inhibition of *P. chinchori* (50%), *A. fumigatus* (53%) and *P. wortmanni* (46%) proving to be the most promising compound in the present series. Thus the preliminary structure activity relationship (SAR) studies indicate that chlorination in the benzofuran ring enhances both the antibacterial and anti-fungal potencies of the triheterocycles. The zones of inhibition of Norfloxacin and Griseofulvin were taken as 100% and the observed zones of inhibition of the newly synthesized compounds have been expressed as related to the standards.

7. Conclusion

It can be seen that without the introduction of any nitrogen functionality, the present synthetic compounds have the potential to exhibit anti-microbial properties. In particular, they have shown promising anti-fungal activity.

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