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Synthesis and biological evaluation of novel angularly fused polycyclic coumarins

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Abstract—In a three-step sequence, an array of angularly fused polycyclic heterocycles with coumarin, benzofuran and pyridine rings were synthesized from 4-bromomethylcoumarins and salicylonitrile. All the final compounds were fully characterized and screened for anti-microbial, anti-inflammatory and analgesic activities. Several compounds exhibited promising inflammation inhibiting and anti-microbial properties.

Coumarins have been found to exhibit a wide range of biological and controlled therapeutic activities in view of their extensive occurrence in nature and low toxicity.[1] Their potential for anti-inflammatory activity and related metabolic processes has been recently reviewed.[2–4] A variety of coumarin derivatives with diverse substituents at C4 have been found to exhibit anti-coagulant,[5] cytochrome P450 inhibiting,[6] anti-microbial[7] and anti-tumor[8] activities. Introduction of an azomethine linkage at the allylic position with respect to the biogenetic C3–C4 double bond in the form of oximes, amidines, oxadiazoles, isoxazolines, etc., has resulted in compounds with promising anti-proteolytic, anti-oxidant and anti-inflammatory properties.[9–11] To this end, we have found that bi-heterocycles, such as 4-(2-benzo[*b*]furanyl)coumarins and 4-aryloxymethylcoumarins from vanillin, exhibit good inflammation inhibiting properties and high levels of molecular tolerance in animal models.[12,13] Studies on the mode of the anti-bacterial action of benzopyranopyridine esters[14] have revealed that they act via the DNA gyrase inhibition pathway. The other properties of this skeleton, such as DNA adduct formation[15] and energy transfer in photophysical processes[16], make them promising molecular skeletons for further biological explorations. The benzofurano[3,2-*b*]pyridines have been reported as potential anti-allergic agents[17] and endothelin receptor antagonists[18] and the tetrahydropyridines have been identified as potential anti-depressants[19] (Fig. 1).

It was envisaged that the two tricyclic templates could be modified into a pentacyclic system with a common pyridine ring, which might have interesting biological functions in animal model studies (Fig. 2). The present paper reports the synthesis and preliminary biological evaluation of a series of pyridine-fused benzofuranocoumarins from various 4-bromomethylcoumarins.

The present synthetic sequence was initiated by an allylic substitution of various 4-bromomethylcoumarins **1** with salicylonitrile **2** giving rise to the *o*-cyano-4-phenoxymethylcoumarins **3** at ambient temperature (Scheme 1). An intramolecular carbanion addition across the nitrile was brought about by refluxing ethers **3** in ethanol in the presence of anhydrous potassium carbonate, resulting in the formation of 3-aminobenzofuranylcoumarins **4**. Aromatisation is the driving force for such an intramolecular condensation. The last step in the sequence was the construction of the fused pyridine ring by a C–C bond formation. An attempt was first made to achieve this ring closure via an intramolecular approach using the *N*-acetyl and the *N*-benzoyl derivatives **4a**. Conversion to **4a** was achieved by the reaction of the amines **4** with acetyl or benzoyl

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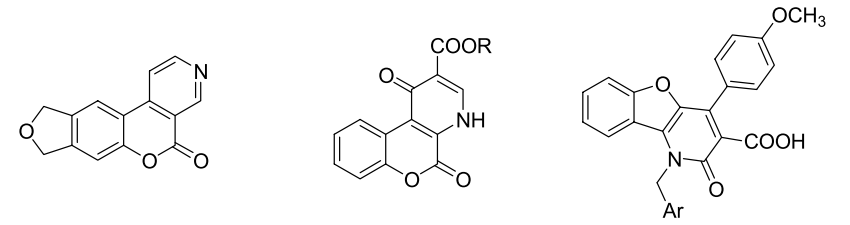


Figure 1. Structures of biologically active pyridine-fused coumarins and benzofurans.

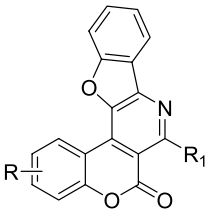
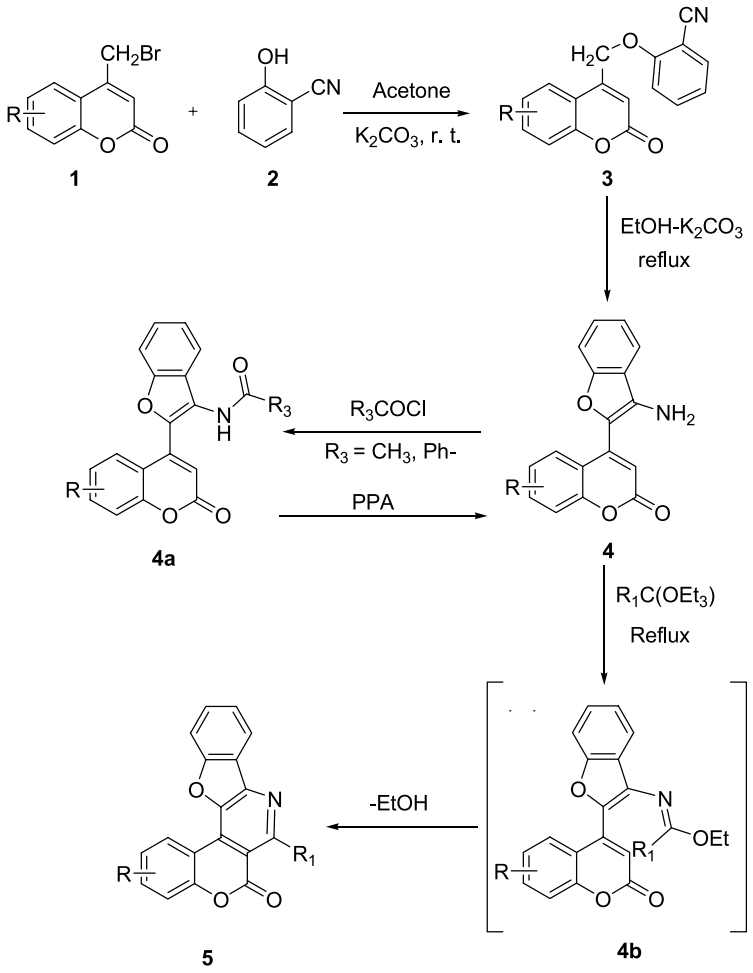


Figure 2. Novel angularly fused pentacyclic heterocycles.

chlorides. The high temperature (120–150 °C) reactions of anilides **4a** in polyphosphoric acid (PPA) led to the hydrolysis of anilides and no cyclised product was detected. Alternatively, the [5 + 1] approach was then attempted with orthoesters as one-carbon electrophiles. The amines **4** were refluxed with excess orthoesters, which resulted in a high yielding ring closure at the C3 position of coumarin. The driving force for such a facile cyclisation is due, in part, to the built-in leaving group abilities of the incipient alkoxy imines **4b**. Another plausible reason for the ring closure is the aromatic stability of the resulting pentacyclic ring system. The formation of the intermediates in the synthetic sequence and the spectral properties of all the fused coumarins **5** were consistent with their structures.[20] The isolated yields of the intermediates **3** and **4** were in the range of 70–80% after purification by crystallization. For the ring



Scheme 1. Synthesis of new angularly fused polycyclic coumarins.

Table 1. Biological activity of benzofuropyridinocoumarins **5**

Compound	R	R_1	Dose (mg/kg)	% Inhibition of inflammation (3 h)	% MPE
5a	7-CH_3	–H	200	24.6	1.3
			300	31.4	22.9
			400	18.3	10.8
5b	7-CH_3	–CH_3	200	80.7	22.9
			300	88.7	24.9
			400	97	18.3
5c	7-CH_3	–C_2H_5	200	35.4	2.4
			300	41.7	4.9
			400	46.5	7.1
5d	7-Cl	–H	200	19.2	1.8
			300	26.3	3.4
			400	29.4	6.8
5e	7-Cl	–CH_3	200	21.6	4.7
			300	25.9	7.3
			400	27.4	9.2
5f	7-OCH_3	–C_2H_5	200	35.7	24.3
			300	78.1	24.4
			400	67.2	39.5
5g	6-CH_3	–H	200	89.0	20.9
			300	96.3	32.9
			400	67.2	20.9
Dicyclofenac sodium			100	94.5	NA
Acetyl salicylic acid			100	NA	74.9

closure triethyl orthoformate, acetate and propionate were employed to generate a library of 14 compounds. The isolated yield in the last step was around 70–80% after recrystallization from dioxane. Out of these, seven compounds (**5a**–**5g**) containing the representative groups R and R_1 have been selected for the biological activity screening.

These were tested for their anti-inflammatory, analgesic and anti-microbial activities. In the in vivo acute toxicity experiments, all the compounds showed LD_{50} values >800 mg/kg body weight. No tremors and convulsions were observed upon inspection and a post mortem examination revealed no haemorrhagic spots. To evaluate the in vivo anti-inflammatory activity of the polycyclic coumarins, the carrageenan induced rat paw oedema method described by Winter et al.[21] was used employing dicyclofenacsodium as the standard drug. The compounds were administered as suspensions in 2% Tween 80. Albino rats (Wister strain) obtained from NIMH-ANS-Bangalore were used in groups of six animals. The paw volumes were measured using a plethysmometer. The percentage of inhibition for inflammation was calculated according to literature methods.[22] The analgesic activity was evaluated by acetic acid induced abdominal constriction method using Swiss albino mice of either sex (20–30 gm) and a group of six animals was used. The analgesic response was assessed by counting the number of abdominal constrictions for 20 min starting 3 min after the injection of the acetic acid solution. Analgesic activity was calculated[23] as the percentage maximum possible effect (% MPE) from the ratio of the mean number of constrictions in control group and the mean number of constrictions in the treated group.

Among the compounds tested, we found that compound **5b** showed significant inhibition of inflammation at 200 and 300 mg/kg doses, respectively. In the analgesic activity results, four compounds **5a**, **5b**, **5f** and **5g** were found to give about one-third of the protection compared to that of acetylsalicylic acid. The most promising compound in the series was the 7-methoxy derivative **5f**, which showed 78% inhibition of inflammation at 300 mg/kg dose level and provided considerable protection against the acetic acid induced abdominal constrictions (Table 1). In the anti-microbial screening by the cup-plate method,[24] all the compounds had a MIC of 25 μg/ml against *Pseudomonas chinchori*, whereas at the same concentration they were inactive against *Micrococcus aureus*. In the anti-fungal screening, all the compounds inhibited the growth of *Aspergillus fumigatus* at 25 μg/ml, whereas against *Penicillium wortmanni* they were active at a concentration of 100 μg/ml.

In summary, we have described a three-step synthetic sequence for the synthesis of polycyclic-fused coumarins by N–C–C3 annealation, a method which has a potential for wider applicability to create new fused heterocyclic systems for their preliminary pharmacological evaluation.

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20. Synthesis of 6-methyl benzofurano[3,2-*b*]pyridino[4,3-*d*]benzpyran-3-2(*H*)-ones **5g**: 0.01 mole of 6-methyl-[3′-amino-2′-benzo (*b*) furanyl]coumarin **4** in 2 mL triethyl orthoformate was refluxed for 16 h, the reaction mixture was cooled, triturated with ethyl alcohol, filtered, dried and the compound **5g** was crystallized from dioxane in 82% isolated yield. mp 246 °C; IR (cm^{-1}) 1734; ^1H NMR (300 MHz, $CDCl_3$) δ 2.58 (s, 3H); 7.31 (d, J = 8.4 Hz, 1H); 7.45 (dd, J = 8.4, 1.8 Hz, 1H); 7.56 (dd, J = 8.4, 1.8 Hz, 1H); 7.74 (t, J = 7.8 Hz, 1H); 7.80 (d, J = 8.4 Hz, 1H); 8.32 (d, J = 7.8 Hz, 1H); 8.60 (s, 1H); 9.5 (s, 1H); MS [M+H] 302 *m*/*z*.
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