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Short communication

Synthesis of vanillin ethers from 4-(bromomethyl) coumarins as anti-inflammatory agents

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Abstract

4-(Bromomethyl) coumarins **1** have been reacted with vanillins, **2** and **2A** to obtain the corresponding ethers **3** and **5**. Ethers **3** have been reacted with ethyl cyanoacetate to obtain the unsaturated esters **4**. Ethers **5** have been converted to the corresponding 4-(2′-benzo[b] furanyl) coumarins **6** by an intramolecular aldol condensation. Eight compounds have been screened for their anti-inflammatory activity. Out of these the 5,6-benzo-4-2′-benzo[b]furanyl) coumarin (**6c**) and the aryloxymethyl coumarin (**4**) with *p*-formyl group were found to be most active.

Keywords: 4-Aryloxymethyl coumarins; 4-(2′-Benzo[b] furanyl) coumarins; Anti-inflammatory activity

1. Introduction

Synthesis of many 4-(aryloxymethyl) coumarins and 3-heteroaryl-coumarins as anti microbial and anti-inflammatory agents has been reported by this laboratory [1–3]. The observed anti-inflammatory activity of coumarins has been because of the generation of a carboxyl group in biological system [4]. Vanillins have been found to exhibit anti-microbial properties and have been accepted as safer flavouring agents [5,6]. In view of this it was thought of interest to synthesise new 4-(aryloxymethyl) coumarins, **3** and **5**, with vanillin moieties. Benzofuranyl coumarins have been found to exhibit anti-inflammatory activity [7]. Hence the ethers **5** with *ortho*-formyl group have been converted to corresponding benzofuranyl cummarins **6**. Ethers **3** have been converted to the corresponding cyanoesters **4** which can act as precursors of carboxylic groups and hence are expected to exhibit anti-inflammatory activity.

2. Chemistry

4-(Bromomethyl) coumarins **1** were synthesised by the Pechmann cyclization of phenols with 4-bromoethylacetoacetate [8]. They were reacted with vanillin **2** to give ethers **3**, which underwent Knovenagel condensation with ethyl cyanoacetate resulting in the formation of unsaturated cyanoesters **4**. Orthovanillin (**2A**) reacted with compounds **1** under similar conditions resulting in the formation of ethers **5**, which contain an active methylene group and the *ortho* carbonyl group. Ethers **5**, when refluxed in alcoholic potassium carbonate underwent an intramolecular aldol reaction leading to the formation of 4-(7′-methoxy 2′-benzo [b] furanyl) coumarins **6**, which is in accordance with our earlier observations [9]. The reactions are outlined in Fig. 1 and the compounds prepared are presented in Table 1 with their physical data.

2.1. Spectral characterisation

The vanillin ethers **3** and **5** exhibited the IR band of lactone carbonyl group around 1710–1720 cm^{-1} whereas the aldehydic carbonyl stretching was observed in the range of 1680–1690 cm^{-1}. In the NMR spectra all the protons resonated at expected fields. The

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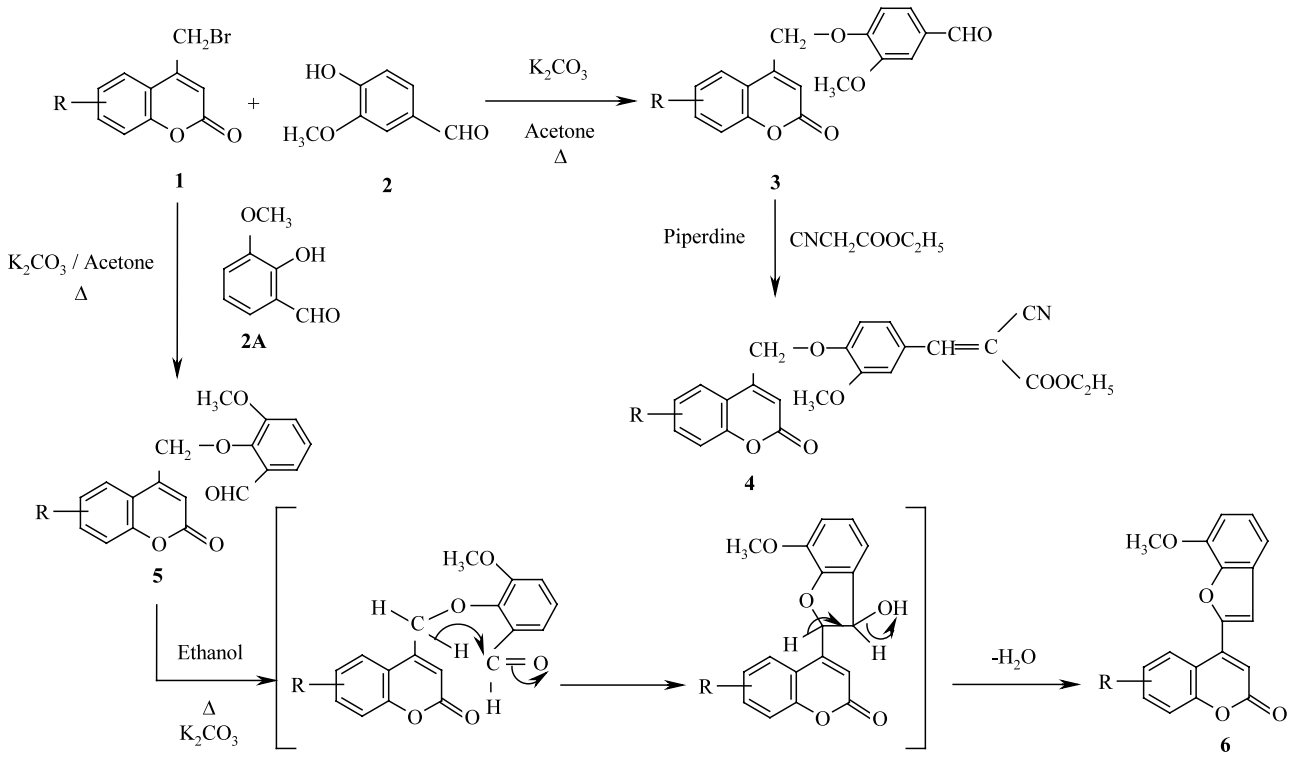

Fig. 1.

aldehydic proton appeared downfield around 9–10 δ, aromatic protons in the range of 7–8 δ and the C_3–H of coumarin around 6.5 δ. The methylene, methoxy and methyl protons resonated around 5, 3.8 and 2.2 δ, respectively.

The cyano esters **4** exhibited a broad band around 1728 cm^{-1}due to carbonyl groups where as the CN stretching band was observed around 2200 cm^{-1}. Compound **4a** showed the absence of aldehydic proton and singlet around 8.15 ppm was assigned to the ethylenic proton located β with respect to the electron withdrawing cyano and ester groups. The benzofuranyl coumarins **6** exhibited the carbonyl-stretching band around 1690 cm^{-1} in the IR spectra. PMR spectra

Table 1
Physical data of compounds **3**–**6**

Compound	R	Molecular formula	Yield (%)	M.p. (°C)	Solvent for crystallisation
3a	6-CH_3	$C_{19}H_{16}O_5$	78	168–169	Chloroform
3b	7-CH_3	$C_{19}H_{16}O_5$	80	198–199	Ethanol
3c	5,6-Benzo	$C_{22}H_{16}O_5$	80	224–225	DMF
3d	7,8-Benzo	$C_{22}H_{16}O_5$	75	232–233	DMF
3e	6-OCH_3	$C_{19}H_{16}O_6$	79	204–205	Ethanol
3f	6-Cl	$C_{18}H_{13}O_5Cl$	60	212–213	Ethanol
4a	6-CH_3	$C_{24}H_{21}NO_6$	80	172–173	Ethanol
4b	7-CH_3	$C_{24}H_{21}NO_6$	82	178–179	Ethanol
5a	6-CH_3	$C_{19}H_{16}O_5$	72	160–161	Ethanol
5b	7-CH_3	$C_{19}H_{16}O_5$	72	178–179	Ethanol
5c	5,6-Benzo	$C_{22}H_{16}O_5$	74	226–227	DMF
5d	7,8-Benzo	$C_{22}H_{16}O_5$	74	182–183	Dioxane
5e	6-OCH_3	$C_{19}H_{16}O_6$	76	204–205	Ethanol
5f	6-Cl	$C_{18}H_{13}O_5Cl$	75	203–204	Ethanol
6a	6-CH_3	$C_{19}H_{14}O_4$	78	164–165	Ethanol
6b	7-CH_3	$C_{19}H_{14}O_4$	74	179–180	Ethanol
6c	5,6-Benzo	$C_{22}H_{14}O_4$	82	220–221	Ethanol
6d	7,8-Benzo	$C_{22}H_{14}O_4$	76	186–187	Dioxane
6e	6-OCH_3	$C_{19}H_{14}O_5$	82	202–203	DMF
6f	6-Cl	$C_{18}H_{11}O_4Cl$	72	196–197	DMF

Table 2
NMR spectral data of compounds **3**–**6**

Compound	R	^{1}H-NMR (δ, ppm)
3a	6-CH_3	2.45 (3H, s, $-CH_3$); 3.96 (3H, s, $-OCH_3$); 5.45 (2H, s, $-OCH_2$); (6.66, 1H, s, C_3-H); 7.2–7.9 (6H, m, Ar–H); 9.2 (1H, s, –CHO)
3b	7-CH_3	2.47 (3H, s, $-CH_3$); 4.0 (3H, s, $-OCH_3$); 5.36 (2H, s,$-CH_2-O$); 6.63 (1H, s, $-C_3-H$); 7–7.5 (6H, m, –Ar–H); 9.86 (1H, s, –CHO)
3c	5,6-Benzo	3.96 (3H, s, $-OCH_3$); 5.3 (2H, s, $-OCH_2$); 6.5 (1H, s, $-C_3-H$); 6.8–7–9 (8H, m, –Ar–H); 10.0 (1H, s, –CHO)
3d	7,8-Benzo	3.98 (3H, s, $-OCH_3$); 5.48 (2H, s, $-OCH_2$); 6.8 (1H, s, $-C_3-H$); 6.81–9.91 (8H, m, –Ar–H); 9.89 (1H, s, –CHO)
3e	6-OCH_3	3.9 (3H, s, $-OCH_3$); 4.0 (3H, s, $-OCH_3$); 5.4(2H, s, $-CH_2-O$); 6.3 (1H, s, $-C_3-H$); 6.5–7.5 (6H, m, –Ar–H); 9.9 (1H, s, –CHO)
4a	6-CH_3	1.41 (3H, t, $-CH_3$, $J = 7.1$ Hz); 4.40 (2H, q, $-CH_2$, $J = 7.1$ Hz); 2.5 (3H, s, $-CH_3$); 3.99 (3H, s, $-OCH_3$); 5.37 (2H, s, $-OCH_2$); 5.25 (1H, s, –CH); 6.64 (1H, s, $-C_3-H$); 6.8–8.0 (6H, m, –Ar–H); 8.15 (1H, s, =CH)
5a	6-CH_3	2.5 (3H, s, $-CH_3$);4.0 (3H, s, $-OCH_3$); 5.45 (2H, s, $-CH_2-O$); 6.63 (1H, s, $-C_3-H$); 6.5–7.5 (6H, m, –Ar–H); 9.9 (1H, s, –CHO)
5b	7-CH_3	2.6 (3H, s, $-CH_3$); 4.0 (3H, s, $-OCH_3$); 5.2 (2H, s, $-CH_2-O$); 6.61 (1H, s, $-C_3-H$); 7.5–8.2 (6H, m, –Ar–H); 10.0 (1H, s, –CHO)
5c	5,6-Benzo	3.94 (3H, s, $-OCH_3$); 5.36 (2H, s, $-CH_2-O$); 6.47 (1H, s, $-C_3-H$); 6.9–7.9 (8H, m, –Ar–H); 9.9(1H, s, –CHO)
6a	6-CH_3	2.29 (3H, s, $-CH_3$); 4.0 (3H, s, $-OCH_3$); 6.62 (1H, s, $-C_3-H$); 6.5–7.9 (7H, m, –Ar–H)
6d	7,8-Benzo	4.0 (3H, s, $-OCH_3$); 6.66 (1H, s, $-C_3-H$); 6.9–8.1 (9H, m, –Ar–H)
6e	6-OCH_3	3.9 (3H, s, $-OCH_3$); 4.0(3H, s, $-OCH_3$); 6.46 (1H, s, $-C_3-H$); 6.9–8.1 (9H, m, –Ar–H)
6f	6-Cl	4.0 (3H, s, $-OCH_3$); 6.63 (1H, s, $-C_3-H$); 7–7.5 (6H, s, –Ar–H)

data for thirteen compounds are given in Table 2. The EI mass spectrum of **3a** showed molecular ion peak at *m*/*z* 324 (41%).

3. Pharmacology

3.1. Acute toxicity studies

For testing the acute toxicity potential of the test compounds, albino rats of either sex weighing 100–200 g were selected, separated into five groups each containing six rats. The dosage was varied from 10 up to 1000 mg kg^{-1} body weight.

The rats were continuously observed for 8 h for any signs of acute toxicity such as increased–decreased motor activity, ataxia, tremors, convulsions, sedation, lacrimation, etc. After 24 h the rats were sacrificed, stomach, intestine and liver were inspected under the magnifying lenses for any ulcer–haemorrhagic spots.

3.2. Analgesic activity

Analgesic activity is measured by rat tail flick method used by D'Amour [10] and Smith. The reaction time was measured at the end of 60, 90 and 120 min after the administration of the compound and the standard employed was Aspirin. The compounds **3c**, **5a**, **6a** are tested for analgesic activity.

3.3. Ulcer index

Mean ulcer index was measured for the compound **3c**, **4a** and **6a**. using phenylbutazone as a standard.

3.4. Anti-inflammatory activity

3.4.1. Acute inflammation

Carragennan induced rat paw oedema inhibition method according to Winter et al. [11]. Ten groups of rats (six in group) received carragennan (0.1 mL of 1% carragennan in 0.5 mL of carboxy methyl cellulose) subplantarly into the left hind paw. One group was kept as control. Other nine groups received standard and test compounds (100 mg kg^{-1} body weight in 0.2 mL of 5% gum acacia) orally 1 h prior to the subplanter injection of carragennan. The paw oedema volume was measured with the help of Plethysmograph by mercury displacement method at 0 h (immediately after injecting carragennan). Then the oedema volume is observed at 1 h interval for 5 h. Control groups received 0.2 mL of 5% gum acacia. Phenylbutazone 100 mg kg^{-1} body weight in 0.2 mL of 5% gum acacia is used as reference compound.

3.4.2. Subacute inflammation

Cotton pellet granuloma method using phenylbutazone as a standard. The rats of 100–200 g of either sex were divided into five groups containing six rats. By using ether anaesthesia the hair in axillary and groin

region were cut and sterile cotton pellets of 15 mg each were implanted in subcutaneous tissue on either sides of axilla and sterile grass pith (25 × 2 mm) in groin region. The wounds were sutured and animals were caged individually after recovery from anaesthesia. The administration of drug was started on the day of implantation and repeated every 24 h regularly for 7 days. On eighth day rats were sacrificed and cotton pellets and grass piths were removed. The pellets free from the tissues were dried over night at 60 °C. The net granuloma formation was calculated by weighing the pellets.

4. Results and discussion

4.1. Acute toxicity studies

All the compounds have shown good safety profile till the highest dose. There was no sedation, convulsions and tremors upon inspection, no ulceration and no haemorrhagic spots were observed. Post mortem examination of the stomach, and intestine did not reveal any ulcer haemorrhagic spots. Liver was also examined and showed no necrosis.

4.2. Analgesic activity

The mean reaction time was around 5.5–6 s, which did not differ significantly from the control group. Hence it was inferred that these compounds did not show good analgesic activity.

4.3. Ulcer index

The mean ulcer index was measured for the compound **3c**, **4a**, and **6a**. The mean ulcer index for the phenylbutazone treated group is 40, which is highly significant. The value for control and the compounds exhibited value 3.33, 1.67, 6.56 and 10.00, respectively. These observations indicate that said compound do not cause any ulceration.

4.4. Anti-inflammatory activity

4.4.1. Carragennan induced rat paw oedema method

The present study reports the anti-inflammatory activity of vanillin ethers, benzo[b]furanyl coumarins and unsaturated cyanoesters of 4-aryloxymethyl coumarins **3**. When compared with the control all the compounds showed reduction in oedema volume. Amongst the ethers **3** with *p*-formyl group the 5,6-benzo substituted compound **3c** was found to exhibit maximum activity. The ethers with ortho formyl group **5a** though showed a quick onset of action initially, the activity showed a decreasing trend at the end of 5 h. However, the corresponding benzofuranyl coumarin **6a** exhibited uniform activity comparable with the standard. The unsaturated cyano ester **4a** obtained from **3a** was found to be the most active in the series along with **3c** and **6a** as the other active compounds. The results indicating oedema volume and percentage inhibition of inflammation at various time intervals have been summarised in Table 3.

4.4.2. Cotton pellet granuloma method

Since compounds **3c**, **4a** and **6a** showed significant activity by carragennan induced rat paw oedema method they were further studied cotton pellet granuloma method. The compounds **3c**, **4a** and **6a** showed considerable inhibition of inflammation which was comparable with standard i.e. phenylbutazone. The compound **4a** is most active among these three compounds. The weight of the pellets and percentage inhibition of inflammation have been summarised in Table 4.

5. Conclusions

It can be seen that though the aldehydic function is important for anti-inflammatory activity as observed in compounds **3**, its conversion to the unsaturated cyanoesters **4** shows on increase in the activity. Similarly the ethers **5** with ortho aldehydic group upon conversion to benzofurans **6** show a slight increase in the activity. None of the compounds tested showed any analgesic activity.

6. Experimental

6.1. Chemistry

The melting points (m.p.) were determined by open capillaries and are uncorrected. The IR spectra were recorded on an IR Nicolet-Impact-410 FTIR spectrometer. ^{1}H-NMR spectra were recorded on C JEOL GSX 400 MHz spectrometer in DMSO-d_6 with TMS as internal standard. The mass spectra were recorded at CDRI Lucknow. The *ortho* and *para* vanillins were commercial samples. All the new compounds have given C, H, N analyses within ±0.4% of the theoretical values.

6.1.1. Preparation of substituted 4-[(4-formyl-2-methoxyphenoxy)methyl] coumarins (3a–f) (general procedure)

Substituted 4-bromomethxyl coumarin (I) (0.004 mol) was refluxed with *p*-vanillin (II) (0.004 mol) and 0.004 mol of anhydrous potassium carbonate in 20 mL of dry acetone for about 6 h on a water bath. The reaction mixture was concentrated and filtered in hot and gradually added to ice. The residue treated with dilute

Table 3
Comparison of oedema volume at different time intervals

Group	Compound	Oedema volume at different time intervals ** ±S.E. and (% inhibition)				
		1 h	2 h	3 h	4 h	5 h
1	Control	0.76±0.15	1.03±0.14	1.08±0.16	1.23±0.20	1.43±0.25
2	Phenylbutazone	0.22±0.07[a] (71)	0.22±0.07[a] (78)	0.29±0.05[a] (75)	0.34±0.03[a] (74)	0.30±0.02[a] (79)
3	**3a**	0.18±0.04[a] (76)	0.34±0.03[a] (67)	0.29±0.05[a] (75)	0.24±0.03[a] (79)	0.27±0.02[a] (81)
4	**3b**	0.14±0.03[a] (81)	0.32±0.04[a] (69)	0.31±0.04[a] (73)	0.37±0.03[a] (70)	0.31±0.04[a] (78)
5	**3c**	0.24±0.04[a] (68)	0.18±0.4[ac] (82)	0.20±0.07[a] (83)	0.24±0.04[a] (80)	0.26±0.04[a] (81)
6	**3d**	0.30±0.05[a] (62)	0.40±0.06[ab] (61)	0.50±0.06[acde] (57)	0.58±0.04[acde] (53)	0.58±0.04[acde] (59)
7	**4a**	0.09±0.05[ab] (88)	0.09±0.07[acdf] (90)	0.16±0.05[ah] (86)	0.22±0.07[af] (82)	0.20±0.07[afh] (87)
8	**5a**	0.13±0.02[a] (83)	0.30±0.05[ae] (72)	0.34±0.03[af] (70)	0.34±0.04[af] (70)	0.41±0.07[a] (72)
9	**6a**	0.12±0.03[ab] (84)	0.19±0.02[ac](81)	0.24±0.02[ah] (79)	0.25±0.03[af] (79)	0.28±0.04[a] (80)
	F value *	51.86	81.69	84.49	88.50	83.86
		$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

** Index for anti-inflammatory activity. Model: acute inflammation; method: carragennan induced oedema; test animal: albino rats; number of animals per group: 6; route of administration: oral; standard: phenylbutazone (100 mg kg^{-1}); test compounds: 100 mg kg^{-1}.

* The superscript a, b, c, d, e, f, g, h indicates significance difference from 1, 2, 3, 4, 5, 6, 7, 8 groups, respectively. The data is analysed by one-way ANOVA (*F*-test) followed by Newman Keul's Studentised range test.

HCl to neutralise the potassium carbonate and added to ice. It was filtered and washed with aqueous ethanol, crystallised from suitable solvent.

*6.1.2. Preparation of substituted 4-[(6-formyl-2-methoxyphenoxy)methyl] coumarins (**5a–f**) (general procedure)*

Substituted 4-(bromomethyl) coumarin (**1**) (0.004 mol) was refluxed with *ortho*-vanillin **2A** (0.004 mol) and 0.004 mol of potassium carbonate in 20 mL of dry acetone for about 6 h on a water bath the reaction mixture was concentrated and filtered in hot and gradually added to ice. The residue treated with dilute HCl to neutralise the potassium carbonate and added to ice. It was filtered and washed with aqueous ethanol, crystallised from suitable solvent.

*6.1.3. Preparation of substituted 4-[(7-methoxy-2-benzo[b]furanyl)] coumarins (**6a–f**) (general procedure)*

The substituted 4-(bromomethyl) coumarin (0.004 mol) (**1**) was refluxed with *ortho*-vanillin **(2A)** (0.004 mol) and anhydrous potassium carbonate (0.004 mol) in 20 mL of super dried ethanol for about 10 h on a water bath. The reaction mixture was concentrated, added to ice water and neutralised with dilute HCl (10%). The separated solid was filtered, washed with water and crystallised from suitable solvent.

*6.1.4. Preparation of 1-cyano-1-carbethoxy-2-[(4-2-methoxyphenoxy-methyl) coumarinyl]ethylenes (**4a–b**) (general procedure)*

Substituted **3a**–**b** 0.005 mol and ethyl cyanoacetate (0.005 mol) and two drops of piperidine were added in 10 mL of rectified spirit. The mixture was stirred for about 4 h at room temperature. The resulting yellow coloured liquid was added to ice water. The separated solid was filtered, washed with water and crystallised form suitable solvent.

6.2. Pharmacology

6.2.1. Animals

Albino rats of Wister strain of either sex weighing between 100 and 200 g were selected. The animals were kept on a standard diet and allowed food and water ad libitum.

6.2.2. Standards

6.2.2.1. Analgesic activity. Aspirin is used as a standard for analgesic activity at a dose of 200 mg kg^{-1} body weight in suspension of 5% gum acacia.

6.2.2.2. Ulcer index. Phenylbutazone is used as a standard for ulcer index administered orally at a dose of 100 mg kg^{-1} body weight in a suspension of 5% gum acacia.

Table 4
Anti inflammatory activity by cotton pellet granuloma method

Compound	Percentage inhibition of inflammation	Dry weight of the pellet (mg)
Control (gum acacia)	–	33.91
Phenylbutazone	64.74	12.04
3c	55.28	15.16
4a	64.60	12.00
6a	63.62	12.33

Index for anti-inflammatory activity: model: subacute inflammation; method: cotton pellet granuloma; test animal: albino rats; number of animals per group: 6; route of administration: oral; standard: phenylbutazone (100 mg kg^{-1}); test compounds: 100 mg kg^{-1}.

6.2.2.3. Anti-inflammatory activity. Phenylbutazone is used as standard for anti-inflammatory activity administered orally at a dose of 100 mg kg^{-1} body weight in a suspension of 5% of gum acacia. Carragennan: 0.1 mL of carragennan in 1% solution suspended in 0.5% carboxy methyl cellulose.

6.3. Statistical analysis

Datas were analysed by one-way ANOVA (F-test) followed by Newman Keul's Studentised range test. Differences below the 0.05 level ($P < 0.05$) were considered as statistically significant.

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