Synthesis, Antiinflammatory, and Cytotoxic Activities of 2-Alkyl and 2-Benzyl-2-Dimethylaminomethyl-5-(E)-Arylidene Cyclopentanone Hydrochlorides

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Purpose. A series of 2-substituted-2-dimethylaminomethyl-5-(E)-arylidene cyclopentanones, **4** were synthesized. The main objective of this investigation was to explore the structural parameters necessary for antiinflammatory activity in this series of compounds, while keeping cytotoxic action to a minimum.

Methods. The target compounds were synthesized in two steps commencing with 2-alkyl-cyclopentanones. Antiinflammatory, analgesic and cytotoxic activities were determined in rats. Cytotoxic results were examined in human cell lines.

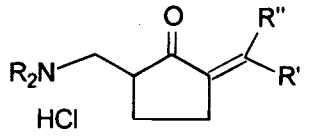
Results. Eight of the eighteen synthetic substances possessed significant antiinflammatory activity and twelve showed appreciable analgesic action. Cytotoxicity was minimal or non-existent for most of the compounds. The stability of one of the compounds, **4b** in both aqueous and non-aqueous media, and an amine exchange reaction with aniline were used to explain the observed antiinflammatory and cytotoxic activities.

Conclusions. Unlike monosubstituted aminomethyl groups (Mannich bases) at the 2-position of 5-arylidene-2-cyclopentanones, a second substituent at the 2-position increases stability of the Mannich base and significantly decreases cytotoxic activity. Antiinflammatory and analgesic action is retained in many of the compounds, thus strongly indicating that these desired pharmacological results can be obtained without untoward damage to cells.

KEY WORDS: 2-aminomethyl-5-(E)-arylidene cyclopentanones; Mannich bases; antiinflammatory; analgesic; cytotoxic.

INTRODUCTION

In previous papers we described the cytotoxic activities (1,2) of structures **1** and **2**. These compounds were designed as progenitors of α-methylene cyclopentanone derivatives which were proposed to inactivate enzyme(s) by an alkylation process. We also found that **2** had potent antiinflammatory activity (1,3) when administered via non-oral routes, but this activity was lost when administered non-orally. Because the antiinflammatory activity of **2** was much greater than that of **1**, the importance of the arylidene moiety relative to the alkylidene group, was demonstrated. In fact, a diversity of antiinflammatory compounds contain the arylidene group (4–9). Our further studies showed that 2-dimethylaminomethylcyclopentanone had little antiinflammatory activity either orally or non-orally whereas 2-(E)-arylidenecyclopentanones were much less potent than **2** upon non-oral administration. These observations strongly suggest that the presence of both an arylidene group and a Mannich base moiety on the cyclopentanone nucleus was necessary for antiinflammatory activity.



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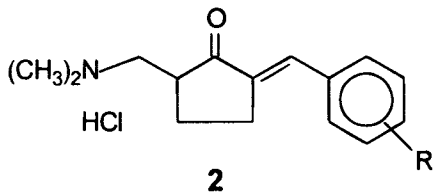

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With the observations that the stability of **2** in aqueous solution decreased as pH increased and its decomposition product, 2-methylene-5-(E)-arylidenecyclopentanone had no activity, we postulated that the oral inactivity was due to non-absorbability of **2** in the stomach, where the pH is 1–3, and instability in the small intestine, at pH 5–6. That 2-methylene-5-(E)-arylidenecyclopentanone had no antiinflammatory activity suggested the Mannich base moiety did not act as an α-methylene precursor for antiinflammatory activity as we had proposed for cytotoxic activity. This reasoning provides the basis for structure modification for eliminating cytotoxic activity while maintaining antiinflammatory properties.

Employing this rationale, we prepared a series of compounds, **4a-r** in which an R group was substituted at the C-2 position of the cyclopentanone. In effect, this substitution would theoretically serve a dual purpose: 1- an increase in oral antiinflammatory action could be gained by increasing stability and 2- by blocking the deamination of the Mannich base or eliminating the formation of the α-methylene function, a decrease or abolishment of cytotoxicity could be achieved. We selected the CH_3-, CH_3CH_2-, $CH_2{=}CHCH_2$-, and $C_6H_5CH_2$-, as the R_1 groups in **4** based on the fact that the lipophilic parameter π (10), electronic parameter σ (10), and steric parameters L and B_4 (11) (Table I), which are more likely to be the main factors affecting biological activity, have sufficient span space.

MATERIALS AND METHODS

Melting points were not corrected. IR spectra were recorded on a IR-27G spectrometer. The ^1H NMR spectra were measured on a BRUKER AC(E)-250 spectrometer with an internal standard of tetramethylsilane. Mass spectra were recorded on a GMS-D300 mass spectrometer. Elemental analyses for **4** were performed on an EA-MOD1106 elemental analyzer and were within ±0.4% of the theoretical values.

General Procedure for Synthesis of 2-Benzyl, or 2-Alkyl-5-(E)-Arylidene Cyclopentanones (3a-r)

To a solution of 32 mmol of 2-benzyl or 2-alkyl cyclopentanone, was added 48 mmol of a benzaldehyde in 20 ml of

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Table I. Lipophilic, Electronic, and Steric Parameters

R_1	π	σ	L	B_4
CH_3-	0.56	0.00	3.00	2.04
CH_3CH_2-	1.02	−1.00	4.11	2.97
$CH_2 = CHCH_2$-	1.10	0.23	5.10	3.78
$C_6H_5CH_2$-	2.10	0.22	3.63	6.02

methanol, followed by 12 ml of 5% NaOH. After stirring for 12h at room temperature, the mixture was filtered to give a solid which was recrystallized from an appropriate solvent. The following compounds were obtained: **3a**, 125-7°C (EtOH), 91%, Lit(12), 130–130.5°C; **3b**, 112–113°C (EtOH), 95%; **3c**, 65–67°C (EtOH), 79%; **3d**, bp 152–155°C/5 mmHg, 52%, Lit(13), bp 140–145°C/1 mmHg; **3g**, 92-4°C (EtOH), 47%; **3h**, 110–112°C (EtOH), 38%; **3i**, 112–114°C (cyclohexane), 55%; **3j**, 115–117°C (EtOH), 63%; **3l**, 71–73°C (EtOH-H_2O), 64%; **3m**, 58–60°C (EtOH-H_2O), 68%; **3n**, 59–60°C (MeOH), 60%; **3o**, 53–55°C (EtOH), 41%; **3p**, 76–78°C (EtOH), 63%; **3q**, 112–114°C (EtOH), 59%; **3r**, 69–71°C (EtOH), 65%. **3e, f, k** were isolated as semi-solids and directly used for the preparation of 4.

General Procedure for Synthesis of 2-Benzyl- or 2-Alkyl-2-Dimethylaminomethyl-5-(E)-Arylidene Cyclopentanone Hydrochlorides (4a-r)

A mixture of 0.02 mol of **3**, 0.038 mol of dimethylamine hydrochloride, 2.4 g of paraformaldehyde and 4 drops of concentrated HCl in 50 ml of ethanol was refluxed with stirring. After 12h, 1.8 g of paraformaldehyde was added and refluxing was continued for another 12h. The resulting solution was concentrated in vacuo to give a residue. The residue was mixed with 100 ml of water and stirred for 30 min at room temperature. The precipitate was removed by filtration and the resulting filtrate was basified with 10% NaOH and then extracted with ether. The combined ether extractions were dried over anhydrous $MgSO_4$. After filtration, the ether solution was treated with ethanolic hydrochloric acid to give a precipitate which was recrystallized from an appropriate solvent. The results are summarized in Table II.

Deaminomethylation (Retro-Mannich Reaction) of 4b to 3b

In ethanol, methanol or acetone: A solution of **4b** was refluxed in the solvent. On cooling, the precipitate was collected by filtration and recrystallized from ethanol to give **3b** as white

Table II. Physical, Antiinflammatory, Analgesic, and Anticancer Data of 2-Alkyl and 2-Benzyl-2-Dimethylaminomethyl-5-(E)-Benzylidene Cyclopentanone Hydrochlorides (4)

				Antiinflammatory[b]		*Analgesic*[c]		*Anticancer*[d] MG-MID		
Compd	Mp (°C)[a]	Yield %	Dose mg/kg (mmol/kg)	No. of rats	Inhibition %	No. of mice	Inhibition %	LogGI50	LogTGI	LogLC50
4a	174–6	53	89 (0, 25)	5	NA	8	84**	−4.42	−4.08	>−4.00
			45 (0.125)			8	47*			
4b	164–6	63	96 (0.25)	5	57**	8	87**	−4.45	−4.11	>−4.00
			48 (0.125)			8	46*			
4c	185–7	65	96 (0.25)	5	52**	8	42*	−5.66	−4.60	−4.08
4d	198–9	43	70 (0.25)	5	NA	8	79**	−4.04	>−4.00	>−4.00
			35 (0.125)			10	47**			
4e	197–9	48	78 (0.25)	5	42**	8	NA	−4.40	−4.09	>−4.00
4f	198–200	36	78 (0.25)	5	30*	8	NA	−4.11	>−4.00	>−4.00
4g	206–8	62	78 (0.25)	5	49**	8	48**	−4.09	>−4.00	>−4.00
4h	194–6	34	98 (0.25)	5	NA	8	NA	−4.72	−4.38	−4.14
4i	182–4	56	81 (0.25)	5	NA	8	62**	−4.09	>−4.00	>−4.00
4j	205–7	50	85 (0.25)	5	NA	8	52*	−4.01	>−4.00	>−4.00
4k	202–4	31	92 (0.25)	5	34**			−4.11	>−4.00	>−4.00
4l	153–5	47	84 (0.25)	5	NA	8	86**	−4.19	>−4.00	>−4.00
			42 (0.125)	5	NA	10	59**			
4m	159–161	32	85 (0.25)	5	34*	8	67*	−4.50	>−4.00	>−4.00
4n	180–2	42	87 (0.25)	5	NA	8	NA	−4.30	>−4.00	>−4.00
4o	173–5	31	99 (0.25)	5	NA			−4.61	−4.25	−4.07
4p	174–5	53	81 (0.25)	5	43**	8	50*	−4.10	>−4.00	>−4.00
4q	195–7	37	88 (0.25)	5	NA	8	NA	−4.21	>−4.00	>−4.00
4r	164–6	43						−4.06	>−4.00	>−4.00

[a] Compounds **4b-h, j, k** were recrystallized from EtOH, **4i, l**, o-r from EtOH-ether, **4a**, n from EtOH-cyclohexane and **4m** from acetone; elemental analyses were within ±0.3% of the theoretical values.

[b] Carrageenin-induced rats paw edema **P < 0.01, *P < 0.05. NA: inhibition% <30%. Ibuprofen gave 52% of inhibition at 52 mg/kg (0.25 mmol/kg) with a group of 5 rats.

[c] Acetic acid-induced mice writhing. **P < 0.01, *P < 0.05. NA: inhibition% <40%. Aspirin gave 64% and 47% of inhibition at 200 mg/kg and 100 mg/kg respectively with a group of 10 mice each.

[d] NCI data. Meangraph-midpoint values. Totally, about 60 human cancer cell lines were used.

platelets, mp 112–113°C. IR (KBr): 1700, 1630, 1605 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.40–1.80 (m, 1H), 2.00–2.30 (m, 1H), 2.34–2.82 (m, 3H), 2.82–3.02 (m, 1H), 3.12–3.48 (m, 1H), 3.84 (s, 3H, OCH_3), 6.92 (d, 2H), 7.20 (d, 2H), 7.30–7.55 (m, 6H).

In water: A solution of **4b** in water was refluxed for 6h or stirred at 37°C for 12h. After cooling, the mixture was extracted with $CHCl_3$ and dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was recrystallized from ethanol to give **3b** as platelets.

In buffer solution: A solution of 4b in pH 7.4 P_i buffer solution was stirred at 37°C for 12h, and treated in a manner similar to the above to give **3b**.

In apolar solvent (benzene or chloroform): No deamino-methylation occurred in these solvents after refluxing for 48h as determined by TLC.

Amine-Exchange Reaction of 4b with p-Chloroaniline

A solution of 380 mg (1.0 mmol) of **4b** and 130 mg (1.0 mmol) of p-chloroaniline in 20 ml of 50% ethanol was left to stand at room temperature for 2h. TLC indicated that no reaction occurred. The solution was then refluxed for 3h. On cooling, the precipitate was collected by filtration and then recrystallized from ethanol to give 20 mg of **3b** (7%). The filtrate was concentrated and the resulting solution was left at room temperature for 2 days to give **5** (280 mg, 70%): mp 98–100°C; ^{1}HNMR ($CDCl_3$) δ 1.7–1.9 (m, 1H), 2.17 (s, 2H), 2.00–2.30 (m, 1H), 2.50–3.30 (m, 4H), 3.85 (s, 3H), 6.55 (d, 2H), 6.91 (d, 2H), 7.00–7.30 (m, 7H), 7.42 (t, 1H), 7.46 (d, 2H); MS (EI) 431 (M^+), 292, 201, 139, 121; IR (KBr) 3400, 1695, 1600 cm^{-1}. Anal. calcd for $C_{27}H_{26}NO_2Cl$: C, 75.08; H, 6.07; N, 3.24. Found: C, 75.21; H, 6.03; N, 3.16.

In a similar process, **4b** and p-chloroaniline were refluxed in 95% ethanol solution for 10h to give 15% of **3b** and 39% of **5** respectively.

Carrageenin-Induced Rats' Paw Edema

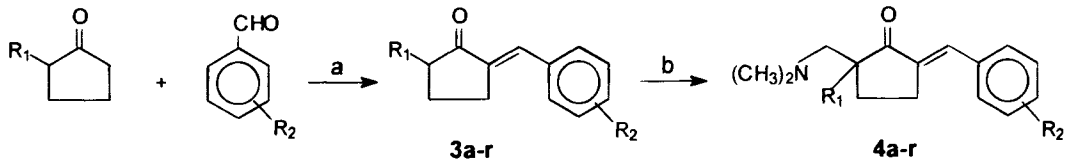
The inhibitory activities of the studied compounds on carrageenin-induced rats' paw edema were determined according to the method of Winter et al. (14) The compounds were orally administered 1h before the injection of 100 μl of a 1% suspension of carrageenin in saline into the subcutaneous tissues of the right hind paw of fasted male Wistar rats (150 ± 30 g). The paw volumes were measured by mercury displacement immediately and again at 3h after the injection of carrageenin. The average foot swelling in a group of drug-treated animals (n = 5) was compared with that of a group of vehicle-treated animals and expressed as percent inhibition. Ibuprofen was employed as a standard against which the test compounds were compared.

Acetic Acid-Induced Mice Writhing

A modified method described by Siegmund et al. (15) was used. One hour after oral administration of the test compounds to fasted female mice (20 ± 2 g), 0.1 ml/10 g of acetic acid was injected intraperitioneally. Five minutes later, the number of writhes of each mouse was counted for a period of 20 min. The average writhes in a group of drug-treated animal was compared with that of a group of vehicle-treated animals and expressed as percent inhibition. The evaluated compounds were compared to aspirin which was used as a standard.

RESULTS AND DISCUSSION

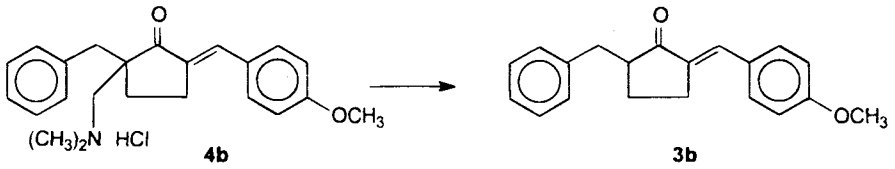
The target compounds **4a-r** were synthesized according to Scheme 1. Thus 2-substituted alkyl cyclopentanones were allowed to react with various benzaldehydes in 5% NaOH to give **3a-r**. The stereochemistry of the **3a-r** products was assigned as the E-form according to ^{1}H NMR and reasons described in our previous papers. (1,2) Compounds **3a-r** were then treated with dimethylamine hydrochloride and paraformal-



(a) 5% NaOH\ MeOH, r.t. (b) $(CH_3)_2NH{\cdot}HCl$\ $(CH_2O)_n$

3 or 4	R_1	R_2
a	$C_6H_5CH_2$	H
b	$C_6H_5CH_2$	4-OCH_3
c	$C_6H_5CH_2$	2-OCH_3
d	CH_3	H
e	CH_3	4-Cl
f	CH_3	3-OCH_3
g	CH_3	4-OCH_3
h	CH_3	3-Br, 4-OCH_3
i	CH_3	3,4-(OCH_2O)
j	CH_3	3,4-$(OCH_3)_2$
k	CH_3	3,4,5-$(OCH_3)_3$
l	CH_2=$CHCH_2$	4-OCH_3
m	CH_2=$CHCH_2$	4-Cl
n	CH_2=$CHCH_2$	3,4-(OCH_2O)
o	CH_2=$CHCH_2$	3,4,5-$(OCH_3)_3$
p	CH_3CH_2	4-OCH_3
q	CH_3CH_2	3,4-$(OCH_3)_2$
r	CH_3CH_2	3,4-(OCH_2O)

Scheme 1.



Conditions	Yield of **3b**
H_2O, reflux, 6h	76%
H_2O, 37°C, 12h	23%
Buffer solution, pH 7.4, 37°C, 12h	9%
MeOH, reflux, 12h	78%
95% EtOH, reflux, 12h	64%
Acetone, reflux, 12h	46%
$CHCl_3$, reflux, 48h	0%
Benzene, reflux, 48h	0%

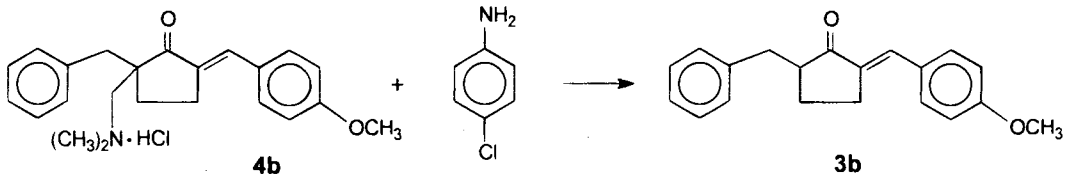
Scheme 2.

dehyde to give Mannich bases **4a-r**. The chemical and biological results pertaining to **4a-r** are summarized in Table II.

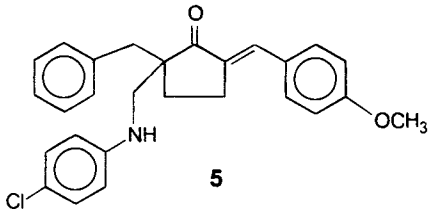
Structures **4a-r** were much more stable in aqueous solution than **2** which gave a deaminated product immediately after it was dissolved in water. Compound **4b** was used as a representative example in some preliminary stability studies. It was shown by TLC that there was no decomposition in water after standing at room temperature for 4 hours. Although there is no reasonable deamination pathway for **4b**, a deaminomethylation, i.e., retro-Mannich reaction is possible and in fact was observed (Scheme 2). When an aqueous solution of **4b** was refluxed for 6h, a 76% yield of **3b** could be obtained. At lower temperature (37°C), there was less decomposition. **4b** was comparatively stable in buffer solution (pH = 7.4) at 37°C. In polar non-aqueous solvents, **4b** was also easily decomposed. But in apolar solvents, no conversion to **3b** could be observed.

Like compound **2** (12), **4** could also react with anilines to give amine-exchanged products. It was shown that **4b** could react with p-chloroaniline in refluxing ethanol or 50% ethanol to give **5** and a small amount of retro-Mannich product **3b** (Scheme 3). However, without refluxing there was no amine-exchange or retro-Mannich reaction. The mechanism of the amine-exchange reaction apparently involves a nucleophilic substitution process. In the case of **2**, the mechanism could be a direct substitution and/or a deamination-Michael addition with the latter more likely (16).

Compounds **4a-q** were evaluated for their oral antiinflammatory and analgesic activities by the carrageenin-induced rats' paw edema and acetic acid-induced mice writhing tests, respectively. In Table II, the results indicate that there are no significant differences in antiinflammatory or analgesic action by varying the R_1 group. However, with regard to changes in R_2 of the



Conditions	Yield	
	3b	**5**
50% EtOH, rt, 2h	0%	0%
50% EtOH, reflux, 3h	7%	70%
EtOH, reflux, 10h	15%	39%



Scheme 3.

Table III. In Vitro Anticancer Activity of **4c**

	Mean LogGI50	Mean LogTGI	Mean LogLC50
Leukemia	−6.36	−5.36	>−4.00
Non-Small Cell Lung Cancer	−5.31	−4.48	−4.14
Small Cell Lung Cancer	−6.24	−5.25	>−4.00
Colon Cancer	−6.37	−4.95	−4.32
CNS Cancer	−5.76	−4.29	−4.05
Melanoma	−5.35	−4.42	>−4.00
Ovarian Cancer	−5.28	−4.33	>−4.00
Renal Cancer	−5.20	−4.35	>−4.00

arylidene portion, all four of the monosubstituted para-methoxy analogs (**4b, g, l, p**) demonstrate significant analgesic action; three of the four para-methoxy derivatives also possessed inhibitory action at greater than 40% in the antiinflammatory screen. Compounds **4b, c, e, f, g, k, m, p** have significant antiinflammatory activity at dose of 0.25 mmol/kg. Compounds **4b, c, g** have a similar inhibitory effect to ibuprofen, which gave 52% inhibition at the same dose. With the exceptions of compounds **4e, f, h, n, q**, all compounds showed appreciable analgesic activity. In particular, **4a, b, d, l** have potent analgesic activity both at 0.25 mmol/kg and at 0.125 mmol/kg. In general, **4b** is a compound that has both potent antiinflammatory activity and potent analgesic activity by oral administration. However, **3b** showed no antiinflammatory or analgesic activity. These results support our hypothesis that the coexistence of a stable Mannich base moiety and arylidene moiety are both necessary for oral antiinflammatory or analgesic activity in the cyclopentanone series. Previous studies (4) of acyclic analogs have described the importance of a Mannich substituted conjugated ketone in antiinflammatory action. Although alkylation of biological nucleophiles appears to be a plausible explanation for cytotoxic activity in the structures **1** and **2** (2), the mechanism of action of **4** for antiinflammatory effects is less clear given a more stable Mannich base present in these structures.

4a-r were also tested for their cytotoxic action on various human cancer cell lines by NCI (17). Results are presented in terms of GI50, TGI and LC50, which represent the concentration required to inhibit cell growth by 50%, 0% and −50% respectively. The results of **4a-r** were summarized in Table II as mean values of log GI50, log TGI and log LC50 of all 60 cell lines. Most of the compounds have no activity at LC50 and TGI levels but for 50% growth inhibition (GI50) some compounds have moderate activity. In general, compounds with an R_1 = benzyl are slightly more active than substances with R_1 = allyl which in turn are slightly more active than compounds either with methyl or ethyl groups. No recognizable differences in activity were observed by variations of R_2. The results indicate that series **4** have little or no cytotoxic and cytostatic effects but have some growth-inhibiting effect against these human cancer cell lines. Surprisingly, compound **4c** had moderate activity at the GI50 level and possessed selectivity to leukemia, small cell lung cancer and colon cancer at GI50 and TGI levels (Table III). Compound **4c** is much more active at the GI50 level especially to leukemia, small cell lung cancer and colon cancer, than any compound of series **1, 2** and the amine-exchanged products of **2** (1,2,16).

Table IV. Stepwise Regression Analysis Results of MG-MID -logGI50

Equation		π	B_4	σ_1	L	R	S	F
(1)	3.95	+0.29				0.72	0.16	15.94
(2)	4.03	+0.47	−0.08			0.77	0.15	10.41
(3)	4.08	+0.46	−0.09	+0.14		0.82	0.13	9.05
(4)	3.90	+0.48	−0.02	+0.15	+0.06	0.85	0.13	7.88

The activity of **4** at the GI50 level may arise from the substitution reaction of biological nucleophiles with the Mannich base moiety. The reaction is presumed to be very slow which may explain the growth inhibition. This hypothesis was supported by the finding that **4** could undergo amine-exchange reaction with anilines. Overall, structures of type **4** were 100–1000 fold less cytotoxic than series **2** compounds. Again, alkylation of nucleophiles by **4** might be less prevalent than that of **2** because of the inability of **4** to provide an α-methylene unit.

We tried to establish a quantitative relationship of the anticancer activity, −log GI50, with physical parameters. The sum π of R_1 and R_2, σ_2 of R_2 and σ_1 (10), L, B_4 (11) of R_1 were used for stepwise regression analysis and the results were summarized in Table IV (the ortho substituted compound 4c was excluded from regression analysis). The fact that σ_2 was not accepted by the equation indicated that the electronic effect of R_2 had little impact on anticancer activity. It is also showed that a lipophilic property played a major role in the 50% inhibition of growth data (Equation 1), and the electronic and steric effect of R_1 had a small effect on anticancer activity (Equations 2, 3 and 4).

In summary, most of the series **4** compounds showed significant antiinflammatory and analgesic activities by oral administration, which may be attributed to their relative stability compared with **2** in aqueous solution. All of the series **4** compounds also had at least some slight anticancer activity at GI50 level which may arise from their substitution interaction with biological nucleophiles.

Appendix

Table V. Elemental Analyses

	Calc.			Found		
	C	H	N	C	H	N
4a	74.42	7.36	3.44	74.26	7.42	3.71
4b	71.38	7.31	3.63	71.53	7.50	3.45
4c	71.58	7.31	3.63	71.60	7.25	3.72
4d	68.70	7.93	5.00	68.75	7.90	5.10
4e	60.97	7.04	4.44	60.81	6.79	4.37
4f	65.90	7.81	4.52	65.94	7.80	4.66
4g	65.90	7.81	4.52	65.74	7.76	4.62
4h	52.53	5.96	3.60	52.23	5.87	3.83
4i	63.06	6.85	4.32	62.90	6.66	4.38
4j	63.61	7.71	4.12	63.78	7.68	4.31
4k	61.70	7.63	3.78	61.48	7.90	3.81
4l	67.97	7.81	4.17	67.75	7.74	4.46
4m	63.54	6.81	4.11	63.50	6.87	4.39
4n	65.23	6.91	4.00	65.42	6.93	4.24
4o	63.71	7.64	3.54	63.74	7.75	3.62
4p	66.76	8.09	4.32	66.30	8.15	4.35
4q	64.48	7.97	3.96	64.32	7.98	4.24
4r	63.99	7.16	4.13	63.95	7.12	4.14

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