Aflatoxin Analogues as Possible Anticoagulants. III

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The synthesis of a series of derivatives of 4-hydroxycoumarin analogues of aflatoxin and a preliminary testing of their anticoagulant activity is described.

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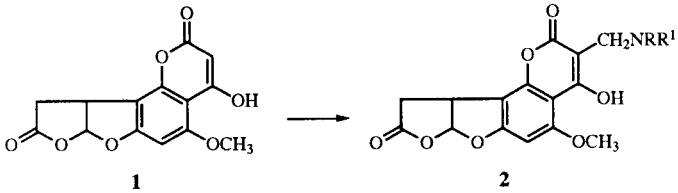
In our previous publications [1,2], we described the synthesis of various linear and angular derivatives of 4-hydroxycoumarin analogues **1**, containing the basic skeleton of aflatoxin-B_1 [3,4]. We now report the synthesis and characterization of warfarin analogues, Mannich bases and 3-acyl derivatives of 4-hydroxy-5-methoxy-7a,9,10,10a-tetrahydro-2*H*-furo[3',2':4,5]-furo[2,3-*h*]-1-benzopyran-2,9-dione **1** [1]. As electrophilic substituents like aminomethyl, acyl and α-acetonylbenzyl groups in the 3-position of a 4-hydroxycoumarin unit enhance the anticoagulant activity [5], similar types of substituents were introduced at the 3-position of compound **1**, in the course of the present work.

When various amines reacted with **1** and formaldehyde in alcoholic medium a series of Mannich bases namely 3-(*N*-substituted amino)methyl-4-hydroxy-5-methoxy-7a,9,10,10a-tetrahydro-2*H*-furo[3',2':4,5]furo[2,3-*h*]-1-benzopyran-2,9-diones, **2a-2f** (Chart 1), were obtained.

Chart 1

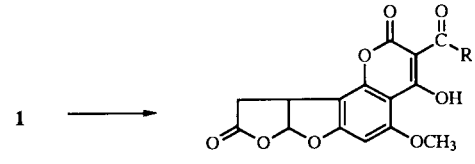


1 **2**

2	R	R^1
a	CH_3	CH_3
b	CH_3	C_2H_5
c	H	Phenyl
d	H	*p*-tolyl
e	H	*p*-chlorophenyl
f	H	2-pyridyl

As the tricarbonylmethane has been found to impart remarkable anticoagulant activity [6], a series of 3-acyl-4-hydroxy-5-methoxy-7a,9,10,10a-tetrahydro-2*H*-furo-[3',2':4,5]furo[2,3-*h*]-1-benzopyran-2,9-diones **3a-f** (Chart 2) were also synthesised by the reaction of **1** with various carboxylic acids in phosphorous oxychloride.

Chart 2

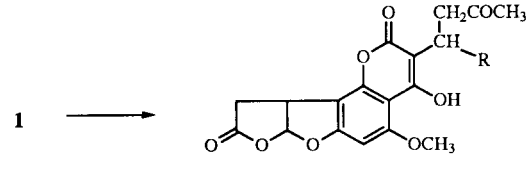


3

3	R
a	CH_3
b	C_2H_5
c	Phenyl
d	*p*-tolyl
e	*p*-chlorophenyl
f	2-pyridyl

Warfarin is a well known anticoagulant on the market [7]. When **1** is treated with α, β-unsaturated ketone like 1-substituted-3-oxobut-1-ene, a Michael addition takes places to produce Warfarin analogues of Aflatoxin, **4a-f** (Chart 3).

Chart 3



4

4	R
a	CH_3
b	C_2H_5
c	Phenyl
d	*p*-tolyl
e	*p*-chlorophenyl
f	2-pyridyl

Anticoagulant activity.

Some of the compounds synthesized were screened for their anticoagulant activity upon albino (*Mus rattus*) rats at the dose level of 5 mg by adopting the method of Quick and coworkers (8,9). The average increase in prothrombin time of plasma (12.5 %) over normal was noted. From this data the relative anticoagulant index was calculated.

Table 1

Compound	Increase in prothrombin time over normal (sec)	Relative anticoagulant index
2a	14.72	7.418
2b	9.3	4.687
2c	Nil	Nil
2d	8.58	4.324
2e	Nil	Nil
2f	Nil	Nil
3a	7.62	3.84
3b	10.97	5.528
3c	Nil	Nil
3d	32.29	16.27
3e	Nil	Nil
3f	Nil	Nil
4a	64.25	32.382
4b	58.24	29.352
4c	41.82	21.077
4d	36.5	19.396
4e	28.21	14.217
4f	24.33	12.262

This index serves as an approximate basis for the comparison of the activity (Table 1).

EXPERIMENTAL

Melting points are uncorrected. The ir spectra were obtained in potassium bromide pellets with a Perkin-Elmer 283 Spectrometer. The ^1H nmr spectra were run on a Varian A-90, at 90 MHz in DMSO-d_6 or deuteriochloroform using TMS as the internal standard, Mass spectra were obtained on JMS-D 300 Mass Spectrograph at 70 ev. Elemental analyses were performed on KARLO-ERBA CHNS-O analyser (Table 2).

3-(*N*-Substituted amino)methyl-4-hydroxy-5-methoxy-7a,9,10,10a-tetrahydro-2*H*-furo[3',2':4,5]furo[2,3-*h*]-1-benzopyran-2,9-diones, **2a-f**.

General Procedure.

A suspension of **1** (0.29 g, 1 mmole) in 6 ml of ethanol, *N*-substituted amine (1.2 mmoles) in 5 ml of ethanol and

Table 2

Compound No.	Yield (%)	Mp (°C)	Molecular Formula	Elemental Analyses (%) Calcd./Found C	H	N	MS (M^+)	^1H NMR (δ ppm)
2a	49	>300	$C_{17}H_{17}O_7N$	58.78 58.63	4.89 4.92	4.03 4.09	347	3.12 (d, 2H, 10-CH_2), 3.26 (s, 2H, 3-CH_2), 3.58 (s, 3H, 5-OCH_3), 3.75 (m, 1H, 10a-H), 3.93 (s, 6H, $N(CH_3)_2$), 6.16 (d, 1H, 6-H), 12.12 (bs, 1H, 4-OH)
2b	53	289-290	$C_{18}H_{19}O_7N$	59.83 59.79	5.26 5.31	3.87 3.81	361	1.90 (t, 3H, -CH_2CH_3), 2.98 (d, 2H, 10-CH_2), 3.12 (s, 3H, -NCH_3), 3.28 (q, 2H, -CH_2CH_3), 3.34 (s, 3H, 5-OCH_3), 3.65 (s, 3H, 5-OCH_3), 3.89 (m, 1H, 10a-H), 6.15 (d, 1H, 7a-H), 6.78 (s, 1H, 6-H), 12.31 (bs, 1H, 4-OH)
2c	23	243-244	$C_{21}H_{17}O_7N$	63.79 63.81	4.30 4.27	3.54 3.56	395	2.91 (d, 2H, 10-CH_2), 3.31 (s, 2H, 3-CH_2), 3.70 (m, 1H, 10a-H), 3.92 (s, 3H, 5-OCH_3), 5.78 (b, 1H, -NH), 6.53 (s, 1H, 6-H), 7.2-7.8 (m, 5H, Ph), 12.1 (bs, 1H, 4-OH)
2d	34	254	$C_{22}H_{19}O_7N$	64.54 64.53	4.64 4.61	3.42 3.48	409	2.71 (s, 3H, -CH_3), 3.02 (d, 2H, 10-CH_2), 3.28 (s, 2H, 3-CH_2), 3.65 (s, 3H, 5-OCH_3), 4.21 (m, 1H, 10a-H), 5.64 (bs, 1H, -NH), 5.92 (d, 1H, 7a-H), 6.73 (s, 1H, 6-H), 7.14 (d, 2H, Ar), 7.75 (d, 2H, Ar), 12.32 (bs, 1H, 4-OH)
2e	27	>300	$C_{21}H_{16}O_7NCl$	58.67 58.65	3.72 3.79	3.25 3.21	429	2.86 (s, 2H, 3-CH_2), 3.28 (d, 2H, 10-CH_2), 3.60 (s, 3H, 5-OCH_3), 4.27 (m, 1H, 10a-H), 5.82 (bs, 1H, -NH), 6.35 (d, 1H, 7a-H), 6.64 (s, 1H, -6H), 7.34 (d, 2H, -Ar), 7.90 (d, 2H, -Ar), 11.93 (bs, 1H, 4-OH)
2f	37	264-265	$C_{20}H_{16}O_7N_2$	60.60 60.63	4.04 4.01	7.07 7.12	396	3.25 (d, 2H, 10-CH_2), 3.80 (d, 2H, 3-CH_2), 4.33 (m, 1H, 10a-H), 5.48 (s, 3H, 5-OCH_3), 5.92 (bs, 1H, -NH), 6.12 (d, 1H, 7a-H), 6.71 (s, 1H, 6-H), 7.21 (dd, 1H, py-5-H), 7.86 (dd, 1H, py-4-H), 8.48 (d, 1H, py-3-H), 8.61 (d, 1H, py-6-H), 12.14 (bs, 1H, 4-OH)
3a	43	251-252	$C_{16}H_{12}O_8$	57.83 57.85	3.61 3.63	— —	332	2.30 (s, 3H, -CH_3), 3.28 (d, 2H, -CH_2), 4.28 (m, 1H, 10a-H), 5.38 (s, 3H, 5-OCH_3), 5.96 (d, 1H, 7a-H), 6.24 (s, 1H, 6-H), 12.16 (bs, 1H, 4-OH)
3b	39	289-290	$C_{17}H_{14}O_8$	58.95 58.91	4.04 4.11	— —	346	1.91 (t, 3H, -CH_2CH_3), 3.37 (d, 2H, 10-CH_2), 3.56 (s, 3H, 5-OCH_3), 3.71 (q, 2H, -CH_2CH_3), 3.98 (m, 1H, 10a-H), 6.14 (d, 1H, 7a-H), 6.37 (s, 1H, 6-H), 12.31 (bs, 1H, 4-OH)

Table 2 (continued)

Compound No.	Yield (%)	Mp (°C)	Molecular Formula	Elemental Analyses (%) Calcd./Found C	H	N	MS (M^+)	^{1}H NMR (δ ppm)
3c	28	>300	$C_{21}H_{14}O_8$	63.95 63.98	3.55 3.50	— —	394	3.19 (d, 2H, 10-CH_2), 3.68 (s, 3H, 5-OCH_3), 4.16 (m, 1H, 10a-H), 5.95 (d, 1H, 7a-H), 6.13 (s, 1H, 6-H), 7.3-8.1 (m, 5H, Ph), 11.87 (bs, 1H, 4-OH)
3d	19	278-279	$C_{22}H_{16}O_8$	64.70 64.75	3.92 3.87	— —	408	2.33 (s, 3H, -CH_3), 2.98 (d, 2H, 10-CH_2), 3.52 (s, 3H, 5-OCH_3), 4.89 (m, 1H, 10a-H), 5.67 (d, 1H, 7a-H), 6.14 (s, 1H, 6-H), 7.24 (d, 2H, Ar), 7.72 (d, 2H, Ar), 12.21 (bs, 1H, 4-OH)
3e	41	293	$C_{21}H_{13}O_8Cl$	58.80 58.73	3.03 3.11	— —	428	2.94 (d, 2H, 10-CH_2), 3.50 (s, 3H, 5-OCH_3), 4.14 (m, 1H, 10a-H), 5.83 (d, 1H, 7a-H), 6.28 (s, 1H, 6-H), 7.14 (d, 2H, -Ar), 7.54 (d, 2H, -Ar), 12.34 (bs, 1H, 4-OH)
3f	35	>300	$C_{20}H_{13}O_8N$	60.76 60.70	3.29 3.22	3.54 3.50	395	3.12 (d, 2H, 10-CH_2), 3.48 (s, 3H, 5-OCH_3), 4.05 (m, 1H, 10-aH), 5.81 (d, 1H, 7-aH), 6.22 (s, 1H, 6-H), 7.41 (t, 1H, py-5-H), 8.20 (d, 1H, ph-4-H), 8.42 (s, 1H, py-2-H), 8.63 (d, 1H, py-6-H), 12.41 (bs, 1H, 4-OH)
4a	32	>300	$C_{19}H_{18}O_8$	60.96 60.99	4.81 4.72	— —	374	1.96 (d, 3H, -CH_3), 2.85 (d, 2H, 10-CH_2), 3.21 (s, 3H, -$COCH_3$), 3.42 (d, 2H, -CH_2CO-), 3.73 (s, 3H, 5-OCH_3), 3.95 (m, 1H, 10a-H), 4.13 (m, 1H,-CH-), 6.18 (d, 1H, 7a-H), 6.52 (s, 1H, 6-H), 11.87 (bs, 1H, 4-OH)
4b	47	>300	$C_{20}H_{20}O_8$	61.85 61.93 66.05	5.15 5.09 4.58	— — —	388	1.82 (t, 3H, -CH_2CH_3), 2.98 (d, 2H, 10-CH_2), 3.19 (d, 2H, -CH_2CO-), 3.31 (s, 3H, -$COCH_3$), 3.43 (m, 2H, -CH_2CH_3), 3.72 (s, 3H, 5-OCH_3), 4.06 (m, 1H, 10a-H), 4.24 (m, 1H, -CH-), 5.95 (d, 1H, 7a-H), 6.29 (s, 1H, 6-H), 12.32 (bs, 1H, 4-OH)
4c	52	264-265	$C_{24}H_{20}O_8$	66.05 66.11	4.58 4.47	— —	436	2.92 (d, 2H, 10-CH_2), 3.15 (s, 3H, -$COCH_3$), 3.37 (d, 2H, -CH_2CO-), 3.76 (s, 3H, 5-OCH_3), 4.09 (m, 1H, 10a-H), 4.21 (t, 1H,-CH-), 5.89 (d, 1H, 7a-H), 6.14 (s, 1H, 6-H), 7.4-8.2 (m, 5H, Ph), 12.18 (bs, 1H, 4-OH)
4d	31	291-292	$C_{25}H_{22}O_8$	66.66 66.69	4.88 4.98	— —	450	2.49 (s, 3H, -CH_3), 2.87 (d, 2H, 10-CH_2), 3.21 (s, 3H, -$COCH_3$), 3.40 (d, 2H, -CH_2CO-), 3.78 (s, 3H, -$COCH_3$), 4.07 (m, 1H, 10a-H), 4.30 (t, 1H, -CH-), 5.80 (d, 1H, 7a-H), 6.59 (s, 1H, 6-H), 7.35 (d, 2H, -Ar), 7.80 (d, 2H, -Ar), 11.93 (bs, 1H, 4-OH)
4e	41	274-275	$C_{24}H_{19}O_8Cl$	61.21 61.17	4.03 3.97	— —	470	2.87 (d, 2H, 10-CH_2), 3.18 (s, 3H, -$COCH_3$), 3.34 (d, 2H, -CH_{2c}O-), 3.58 (s, 3H, 5-OCH_3), 3.98 (t, 1H, -CH-), 4.23 (m, 1H, 10a-H), 5.82 (d, 1H, 7a-H), 6.73 (s, 1H, 6-H), 7.24 (d, 2H, -Ar), 7.56 (d, 2H, -Ar), 12.13 (bs, 1H, 4-OH)
4f	39	>300	$C_{23}H_{19}O_8N$	63.15 63.10	4.34 4.41	3.20 3.13	437	3.15 (d, 2H, 10-CH_2), 3.25 (s, 3H, -$COCH_3$), 3.42 (d, 2H, -CH_2CO-), 3.56 (s, 3H, 5-OCH_3), 4.08 (t, 1H, -CH-), 4.23 (m, 1H,10a-H), 5.92 (d, 1H, py-3-H), 6.71 (s, 1H, 6-H), 7.52 (t, 1H, py-5-H), 8.11 (d, 1H, py-4-H), 8.32 (s, 1H, py-2-H), 8.55 (d, 1H, py-6-H), 11.83 (bs, 1H, 4-OH)

formaldehyde (1.8 ml, 40%) was stirred at room temperature for 2 hours. It was allowed to stand for 4-5 hours at 5°. The precipitates were collected by suction filtration, washed with ethanol and dried in a vacuum desiccator. The crude products were crystallized from aqueous ethanol (Table 2).

3-Acyl-4-hydroxy-5-methoxy-7a,9,10,10a-tetrahydro-2*H*-furo-[3',2':4,5]furo[2,3-*h*]-1-benzopyran-2,9-diones, **3a-f**.

General Procedure.

A mixture of **1** (2.9 g, 1 mmole), carboxylic acid (1.5 mmoles) and phosphorous oxychloride (0.6 ml) was refluxed for 1 hour. The crystalline compound was washed with cold ethanol repeatedly, dried in a vacuum desiccator, and recrystallized from dioxan-water (Table 2).

3-(1-Substituted-3-oxobut-1-enyl) 4-hydroxy-5-methoxy-

7a,9,10,10a-tetrahydro-2*H*-furo[3',2':4,5]furo[2,3-*h*]-1-benzopyran-2,9-diones, **4a-f**.

General Procedure.

A solution of substituted benzalacetone (substituted-3-oxobut-1-ene) (1.4 mmoles) **1** (2.9 g, 1 mmole) in 30 ml of dioxan containing a few drops of piperidine was refluxed for 4-5 hours. Later it was cooled and poured into ice water with vigorous stirring. The crude solid separated was recrystallized from acetone-water (Table 2).

REFERENCES AND NOTES

[1] G. V. P. Chandra Mouli, Y. D. Reddy and V. V. S. Somayajulu, *Tetrahedron*, **39**, 2277 (1983).

[2] G. V. P. Chandra Mouli, R. B. Reddy and Y. D. Reddy, *J. Indian Chem. Soc.*, **67**, 917 (1990).

[3] G. Buchi and E. C. Roberts, *J. Org. Chem.*, **33**, 460 (1968).

[4] R. S. Iyer, M. W. Vochler and T. M. Harris, *J. Am. Chem. Soc.*, **116**, 8863 (1994).

[5] Z. M. Gumminska and M. Eckstein, *J. Med. Pharm. Chem.*, **3**, 583 (1961).

[6] C. Ukita, T. Tamura, R. Matsuda and E. Kashiwabara, *Japan J. Expt. Med.*, **20**, 109 (1949).

[7] K. P. Link and K. B. R. Robertson, *J. Am. Chem. Soc.*, **72**, 5193 (1950).

[8] A. J. Quick, *Am. J. Physiol.*, **118**, 260 (1937).

[9] A. J. Quick, S. Brown and L. Bancroft, *Am. J. Med. Sci.*, **190**, 501 (1935).