Dedicated to the memory of
acad. Ecaterina Cioranescu-Nenitzescu

Synthesis of New Substituted Indan-1,3-diones

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Herein is reported the synthesis and characterization of several new aldol condensation adducts between ninhydrin and carbonyl derivatives and their study with respect to anticoagulant activity. The structure of the compounds was confirmed by a series of analytical methods such as ^{1}H-NMR and ^{13}C-NMR spectroscopy, mass spectroscopy (MS), IR and thin layer chromatography (TLC). Additionally comparative tests were performed in order to study the biological activity of the title compounds.

Keywords: ninhydrin, aldol, anticoagulant, 1,3-indandione

Many studies have demonstrated that 1,3-indandione derivatives act as inhibitors of blood coagulation *in vivo* and as anti-platelet aggregation agents *in vitro* [1]. Some are still commercially available, although their pharmacological profile is less valuable than coumarin derivatives, which are the most potent among the known anticoagulants agents with low-molecular weight. The phenylindandione-type compounds were the first substances used as therapeutic oral anticoagulants. Phenindione, diphenadione or anisindione are examples of such derivatives [2].

Polyheterocyclic structures containing an indandione ring were tested as inhibitors of monoamine oxidase B, an enzyme involved in Alzheimer's disease [3]. These compounds were firstly synthestised starting from nynhidrin [3, 4] and although the final indeno[1,2-c]pyridazines structures were subjected to intense screening [3,5], the biological properties of the aldol condensation adducts between ninhydrin and ketones were less investigated, being regarded mainly as synthetic intermediates.

Therefore, we decided to synthesize several such adducts, in order to obtain structures similar to known anticoagulation agents (some of them new and others already reported in the literature). We describe here the synthesis and characterization of these aldol adducts, as well as the anticoagulant activity of these products.

Experimental part

Chemistry

All commercial reagents were used without additional purification. P.e. stands for petroleum ether (40-60°C) and DCM for dichloromethane. The melting points were determined in open capillaries using an electric melting point STUART SMP3 apparatus and are uncorrected. The IR spectra were recorded on a BIO-RAD FTS-135 apparatus in KBr pellets in the range 4000-400 cm^{-1}. A Varian Gemini 300 BB spectrometer operating at 300 MHz (proton) and respectively 75 MHz (carbon) was used for NMR recordings using d_6-DMSO as solvent and TMS as internal standard. Chemical shifts (δ) are reported in ppm values relative to TMS. Mass spectra were performed on a Finnigan MAT 90 spectrometer using CI technique. Thin Layer Chromatography was achieved on silicagel plates (Merck) and visualisation was made using UV light (λ=254 nm). The studied compounds were prepared according to the literature [5]. Their detailed characterisation is presented bellow:

2-Hydroxy-2-(3,3-dimethyl-2-oxo-butyl)-indan-1,3-dione (1). Colorless fine needles, R_f=0.19 (silicagel, CH_2Cl_2);

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 8.02-7.98 (m, 2H), 7.87-7.84 (m, 2H), 3.95 (bs, 1H, OH), 3.45 (s, 2H, CH_2), 1.06 (s, 9H, *t*-Bu), ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 213.7 (*t*-Bu**CO**), 198.2 (COAr), 140.6, 135.7, 123.7, 72.9 (C-OH), 42.05 (*C*H_2), 25.7 (3C, C(**C**H_3)$_3$);

2-Hydroxy-2-(2-oxo-cyclopentyl)-indan-1,3-dione (2). Tan powder, R_f=0.15 (silicagel, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 8.01-7.98 (m, 2H, CH_{Ar}), 7.92-7.88 (m, 2H, CH_{Ar}), 3.33 (ddd, 1H, CH-CO, *J* 10.1, 8.1, 1.0 Hz), 2.03-1.88 (m, 2H, CH_2), 1.84-1.66 (m, 2H, CH_2), 1.47-1.36 (m, 2H, CH_2); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 220.6 (CH-CO-CH_2), 199.5 (Ar-CO), 196.5 (Ar-CO), 141.0 (C_{ipso}), 136.7 (HC_{Ar}), 136.6 (HC_{Ar}), 124.1 (HC_{Ar}), 123.8 (HC_{Ar}), 76.5 (C-OH), 49.1 (CH-CO), 38.6 (CH_2CO), 24.9 (CH_2), 20.7 (CH_2). IR (KBr, í cm^{-1}): 3434, 2958, 1746, 1707, 1595, 1455, 1279, 740; MS (CI, m/z, %): calc [M+H]$^+$: 273.3; found 273 (100); 255 (30) [M-OH]$^+$

***2*-Hydroxy-2-(5-methyl-2-oxo-cyclohexyl)-indan-1,3-dione (3)**. Tan powder, R_f=0,2 (silica gel, CH_2Cl_2);

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 7.95-7.92 (m, 2H, CH_{Ar}), 7.84-7.81 (m, 2H, CH_{Ar}), 2.84 (ddd, 1H, CH-CO, *J* 13.1, 7.2, 1.0 Hz), 2.36-2.18 (m, 2H, CH_2), 2.12-2.07 (m, 1H), 1.98-1.72 (m, 3H), 1.41-1.28 (m, 1H), 1.01 (d, CH_3, *J* 6.1 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 211.6 (CH-CO-CH_2), 199.4 (Ar-CO), 198.6 (Ar-CO), 141.4 (C_{ipso}), 140.6 (C_{ipso}), 135.9 (HC_{Ar}), 135.8 (HC_{Ar}), 123.8 (HC_{Ar}), 123.7 (HC_{Ar}), 73.8 (C-OH), 54.7 (CH-CO), 40.7 (CH_2CO), 34.7 (CH_2), 34.6 (CH_2), 31.6 (CH), 21.2 (CH_3); IR (KBr, ν cm^{-1}): 3373, 2965, 1727, 1702, 1590, 1398, 1261, 926, 765. MS (CI, m/z, %): Calcd. [M+H]$^+$: 273.3; Found 273 (100); 255 (30) [M-OH]$^+$.

2-Hydroxy-2,2'-biindan-1,1',3,3'-tetrone (4). Colorless solid, R_f=0.3 (silica gel, DCM/MeOH: 95/5).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 8.01-7.98 (m, 2H), 7.95-7.84 (m, 6H), 3.95 (s, 1H, CH). ^{13}C-NMR ($CDCl_3$, 75 MHz) ä: 197.3 (Ar-CO), 195.9 (Ar-CO), 142.1 (C_{ipso}), 141.1 (C_{ipso}), 136.6 (HC_{Ar}), 136.3 (HC_{Ar}), 124.1 (HC_{Ar}), 123.6 (HC_{Ar}), 76.6 (C-OH), 53.3 (CH-CO). IR (KBr, ícm^{-1}): 3430, 3087, 1750,

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1711, 1587, 1353, 1264, 1103, 931, 764, 600. MS (CI, m/z, %): Calcd. $[M+H]^+$: 307.3; Found 307 (38); 291 (17) $[M-OH]^+$; 161 (97); 147 (100).

2-Hydroxy-2-(4,4-dimethyl-2,6-dioxo-cyclohexyl)-indan-1,3-dione (**5**). Colorless prisms, R_f=0.3 (silica gel, DCM/MeOH: 95/5).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 8.00-7.60 (m, 4H), 3.22-2.81 (broad s, 2H, CH and OH), 2.14-2.11 (s, 4H, CO-CH_2), 1.01 (s, 6H, CH_3). IR (KBr, νcm^{-1}): 3390, 3255, 2942, 1711, 1661, 1611, 1392, 1265, 1156, 1006, 950, 882, 779; MS (CI, m/z, %): Calcd. $[M+H]^+$: 301.3; Found 301(100); 283 (17) $[M-OH]^+$; 163 (61); 141 (92).

2-Hydroxy-2-[2-oxo,2-(4-nitro-phenyl)ethyl]-indan-1,3-dione (**6**). Colorless prisms, R_f=0.67 (silica gel, AcOEt/e.p: 1/1).

^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 8.31 (d, 2H, *J* 8.9 Hz, CH-C-NO_2), 8.15 (d, 2H, *J* 8.9 Hz, CH_{Ar}), 8.05 (m, 4H, CH_{Ar}), 6.92 (s, 1H, OH), 4.03 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6, 75 Mz) ä: 200.1 (CH-CO-Ar), 197.2 (Ar-CO), 197.0 (Ar-CO), 150.4 (C-NO_2), 140.6 (HC_{Ar}), 139.3 (CO-C_{Ar}), 136.4 (HC_{Ar}), 129.7 (HC_{Ar}), 123.8 (HC_{Ar}), 123.5 (HC_{Ar}), 72.4 (C-OH), 44.2 (CH_2CO). IR (KBr, νcm^{-1}): 3568, 3106, 2900, 1683, 1596, 1524, 1211, 1153, 1082, 1045,1002, 942, 866, 792. MS (CI, m/z, %): Calcd. $[M+H]^+$: 326.3; Found 325.9 (100), 308 (14) $[M-OH]^+$; 166 (17).

Biological evaluation

All synthesized compounds were evaluated *in vivo* using NMRI white mice with 20-25 g body weights. The animals were maintained with water and food *ad libitum*. The compounds were administered orally using a gastric tube, in the morning.

The LD_{50} was assessed for 4-5 different doses, in the range 1000 – 5000 mg/kg body weight, each on six animals and calculated using Litchfield-Wilcoxon method [6].

The differences between the control and test groups were analysed using the Student's t-test for independent samples. $p \leq 0.05$ was used as the criterion of statistical significance.

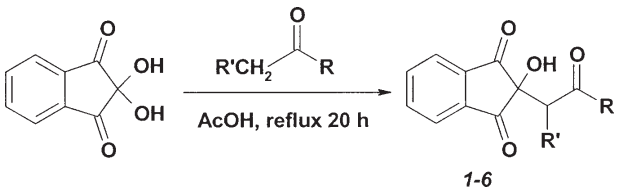
All compounds were suspended in 1% Tween 80 and completely dissolved in saline solution under ultrasounds before administration. For determination of the anticoagulant activity, the compounds were administered in three doses (10, 5 and 2.5%) on three test groups, in order to assess the effect on blood coagulation, according to the method of Moravitz [7].

The anticoagulant activity was determined by measuring the Quick time, using a Coatron (Teco GmbH) coagulometer and Teco GmbH standardised thromboplastin (ISI = 1.1) as standard reagent. The coagulation process was triggered by incubation of plasma with the optimal amount of thromboplastin and calcium. The time to formation of a fibrin clot was then measured.

Results and discussion

Chemistry

The necessary adducts are readily available in one pot by direct aldol condensation between ninhydrin as hydrated carbonyl partner and ketones bearing methyl or methylene groups as depicted in scheme 1.

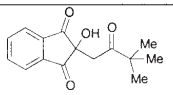
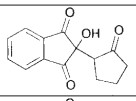
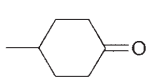
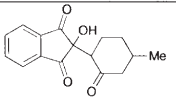
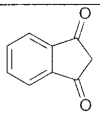
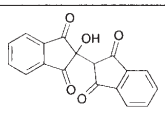
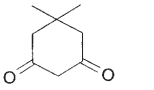
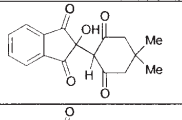
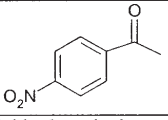
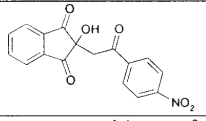


Scheme 1. Aldol condensation of ninhydrin and ketones

The purified products were isolated in very good yields, except from the cyclopentanone and *p*-nitrocetophenone which reacted less satisfactory. Table 1 presents the results and observations regarding the preparation of the compounds **1-5**.

Some of these adducts are already reported in the literature (table 1), either as products of condensation in solution [8], in solid state [9] or by other methods [10-11]. They are all stable colorless solids. The structure of the compounds was confirmed by NMR spectroscopy, IR and thin layer chromatography (TLC). The new compounds were also characterized by mass spectrometry; all spectra

Table 1
ALDOL CONDENSATION ADDUCTS OF NINHYDRIN AND KETONES

Entry	Ketone	Product	M.p.	Yield [a]
1			171-174 °C [b] (AcOEt/p.e.[c])	76
2			134 °C (AcOEt/p.e.) 140 °C [10]	60
3			88-90 °C [b] (AcOEt/p.e.)	86
4			184-185 °C (AcOEt/p.e.) 189-191 °C [8]	85
5			194-196 °C (95% EtOH) 193-195 °C [9]	89
6			122-124 °C [b] (AcOEt/p.e.)	50

[a] Isolated yields; 2 equivalents of ketone were used (except for entry 4);
[b] new compounds (see experimental part) [c] petroleum ether

Table 2
DL_{50} OF COMPOUNDS **1-5** AFTER SINGLE DOSE ORAL ADMINISTRATION

Entry	DL_{50} (mg/kg) Range of values	Statistically significant differences (compared to the compounds in parantheses)
1.	> 5000	$p \leq 0.05$ **(P)**
2.	3095 (1514 - 4676)	$p \leq 0.05$ **(P)**
3.	> 5000	$p \leq 0.05$ **(P)**
4.	2258 (1537 - 2979)	$p \leq 0.05$ **(P)**
5.	> 5000	$p \leq 0.05$ **(P)**
Sintrom®	1470 [12]	$p \leq 0.05$ **(1, 2, 3, 4, 5)**

Table 3
EFFECT OF COMPOUNDS **1-5** ON PROTHROMBIN ACTIVITY (%) AFTER SINGLE DOSE (1000 MG/KG BODY WEIGHT) ORAL ADMINISTRATION

Entry	Prothrombin activity (%)	Statistically significant differences (compared to the compounds in parantheses)
Control (C)	82 ± 9.89	
1.	71.8 ± 8.38	$p \leq 0.05$ **(2, 3, 4)**
2.	45.4 ± 33.64	$p \leq 0.05$ **(1, 5, C)**
3.	34.6 ± 9.40	$p \leq 0.05$ **(1, 5, C)**
4.	39,3 ± 5,8	$p \leq 0.05$ **(1, 5, C)**
5.	67,2± 7,5	$p \leq 0.05$ **(2, 3, 4)**

show, besides the molecular ion-peak, a common behaviour, namely the presence of a $[MH-18]^+$ peak, corresponding to the loss of a water molecule after protonation.

The NMR data of the compounds **1-5** are in good accordance with proposed structures. The ^{1}H-NMR attributions were made on the base of multiplicity and by 2D experiments. The signals are as expected for these types of compounds. The ^{13}C-NMR signals were attributed by HETCOR experiments. The general features of the ^{13}C-NMR are the carbonyl carbons which appear at around 197-199 ppm for the carbonyl groups in the indandione moiety. The carbonyl group in the radical attached in the two positions is more deshielded due to a smaller degree of conjugation and appears at about 201-211 ppm. The C_{sp3}-OH carbon is deshielded by OH radical, as well as the two carbonyl 2-hydroxy-indandione groups and appears at about 72 ppm.

*Biological evaluation of compounds **1-5***

The freshly prepared compounds were first investigated for acute toxicity in mice following oral administration. A preliminary screening in the changes of prothrombin activity was subsequently performed. The results of the preliminary tests prompted us to make an assessment of the compounds effect on blood coagulation at different doses, calculated as percentages of LD_{50}. Finally, we investigated the effect of compounds 1-6 on the decrease of prothrombin activity while applying a daily dose oral administration during nine days. Sintrom® (Acenocumarol) was used as reference compound.

To evaluate the anticoagulant profile of the synthetised compounds, LD_{50} doses were firstly assessed for each compound and the results are shown in table 2. The acute toxicity was determined by administering doses of compounds between 1000 mg/ kg body weight and 5000 mg/ kg body weight. All the compounds present a lower toxicity than the reference compound Sintrom®.

We further evaluated the changes in prothrombin activity for the groups of animals that received the dose equal to 1000 mg/ kg body weight (the lowest dose at which no death was recorded after five days of observation). The results are shown in table 3.

Compound 1 shows a prothrombin activity value that is very close to the control group (table 3) while compound **2** exhibits a higher toxicity for this dose. Consequently, we could also notice a smaller prothrombin activity, significantly changed with respect to the control group. Compund **3** showed the prothrombin acitvity significantly modified with respect to the control group. The modification of prothrombin activity induced by administration of compound **4** is moderate, while in the case of compound **5,** the change is insignificant.

After establishing the LD_{50}, we investigated the effect of the compounds **1-5** on prothrombin activity after a single dose, administered for 9 days. A dose of 100 mg/kg body weight was chose to be administered on a daily base to groups of 6 animals for nine days in order to assess the

Entry	Prothrombin activity (%)	Statistically significant differences (compared to the compounds in parantheses)
Control	82 ± 9.89	
1.	78.4 ± 10.21	$p \leq 0.05$ **(2, 4)**
2.	2.4 ± 0.89	$p \leq 0.05$ **(1, 3, 4, 5, C)**
3.	51 ± 20.7	$p \leq 0.05$ **(2)**
4.	32,1 ± 9,8	$p \leq 0.05$ **(1, 2, 3, C)**
5.	72,8± 8,4	$p \leq 0.05$ **(2, 4)**

Table 4
EFFECTS OF THE COMPOUNDS **1-5** ON THE PROTHROMBIN ACTIVITY AFTER 100 mg/kg BODY WEIGHT DAILY DOSE ORAL ADMINISTRATION

Entry	Dose (mg/kg)	Prothrombin activity (%)
Control		82 ± 14
1.	500	79.5 ± 4
	250	83.5 ± 2
	125	79.5 ± 4
2.	300	86.83 ± 4.62
	150	[b]
	75	79.33 ± 9.02
3.	500	76 ± 2.78
	250	83.82 ± 9.22
	125	23.33 ± 4.51 [a]
4.	225	34.6 ± 9.40 [a]
	112.5	40.5 ± 10.5 [a]
	56.25	79.33 ± 11.59
5.	500	63.5 ± 9.52
	250	79 ± 4.36
	125	78.33 ± 7.77
Sintrom® (Acenocoumarol)	150	[b]
	75	[b]
	38	[b]

[a] Statistically significant different $p \leq 0.05$ compared to the control group
[b] no coagulation

Table 5
EFFECTS OF THE COMPOUNDS **1-5** ON THE PROTHROMBIN ACTIVITY AFTER THREE DAYS ORAL ADMINISTRATION

chronic toxicity of the compounds. The results are shown in table 4.

Since the compounds obtained by us exhibited low toxicity, we further decided to determine the ratio between dose and the anticoagulant response for the compounds **1-5**. Therefore, we proceeded to test the anticoagulant effect of each compound at doses of 10.5 and 2.5% of LD_{50}.

Saline solution (0.9%) was administered to the control group. The anticoagulant activity was determined by measuring the Quick time and the results were analysed according to the prothrombin activity (table 5). None of the substances exhibited as strong anticoagulant activity as acenocoumarol. However, one can notice the similar effects on the prothrombin activity of the compound **2** in the case of the 5% dose (150 mg/kg body weight) with the effects produced at 100 mg/kg body weight daily dose oral administration (table 5).

Conclusions

A series of aldol-condensation adducts of ninhydrin with carbonyl compounds was synthesized and completely characterized regarding their chemical structure. In this respect analytical methods comprising NMR spectroscopy, IR and mass spectroscopy and thin layer chromatography were performed. The biological activity of the compounds was also investigated regarding their anticoagulant properties. The prothrombin activity changes indicate a moderate anticoagulant activity for compounds **2**, **3** and **4**. Moreover the compounds exhibited low-to-moderate acute toxicity.

Acknowledgments: This research was funded by the Romanian Ministry of Education and Research, grants CEEX – VIASAN 43/2005. The authors are indebted to Dr. Sabin Cinca and staff from the "Alex Trestioreanu" Oncological Insitute for enabling the in vivo experimental work.

References

1. AVERY, G.S., Cardiovascular Drugs. **3**: Antithrombotic Drugs, University Park Press, Baltimore, Md., 1978, p.217.
2. NEGWER, M., Organic-Chemical Drugs and Their Synonyms, Berlin, 1978
3. KNEUBUEHLER, S., THULL, U., ALTOMARE, C., CARTA, V., GAILLARD, P., CARRUPT, P.A., CAROTTI, A., TESTA, B., J. Med. Chem. **38**, 1995, p.3874
4. KNEUBUEHLER, S., CARTA, V., ALTOMARE, C., CAROTTI, A., TESTA, B., Helv. Chim. Acta **76**, 1993, p. 1956
5. FREDERICK, R., DUMONT, W., OOMS, F., ASCHENBACH, L., VAN DER SCHYF, C.J., CASTAGNOLI, N., WOUTERS, J., KRIEF, A., J. Med. Chem., **49**, 2006, p.3743
6. LITCHFIELD, J.T., WILCOXON, F., J. Pharmacol. Exp. Ther., **96**, 1949, p.99
7. TIETZ, N.W., Clinical Guide to Laboratory Tests. 3rd ed., WB Saunders Company, Philadelphia, PA, 1995
8. DAS, S., PRAMANIK, A., FROEHLICH, R., PATRA, A., Tetrahedron **60**, 2004, p.10197
9. KAUPP, G., NAIMI-JAMAL, M.R., SCHMEYERS, J., Chem. Eur. J., **8**, 2002, p.594
10. SCHANK, K., LEIDER, R., LICK, C., GLOCK, R., Helv. Chim. Acta, **87**, 2004, p.869, Compd described at page 914
11. STAN D., BACIU, I., NICOLAE, A., NEAGU, L., DUMITRASCU, F., MATACHE, M. Rev. Chim. (Bucharest), **60**, nr. 3, 2009, p. 264
12. WINDHOLZ, M., The Merck Index, 10th ed,, Rahway, USA, 1983, p.5

Manuscript received: 21.08.2008