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Original Scientific Paper

Synthesis of Novel Indane-1,3-dione Derivatives and Their Biological Evaluation as Anticoagulant Agents

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Abstract. 2-Substituted derivatives of indane-1,3-dione **3a**–**f** , **5a**–**g**, **6a**–**g** were synthesized and investigated as anticoagulant agents. 2-Arylindane-1,3-diones (**3**) were obtained in the reaction of phthalide with appropriate arylaldehydes. 2-Arylmethyleneindane-1,3-diones (**5**) were prepared by condensation of indane-1,3-dione with the corresponding arylaldehydes. The compounds **5** were converted into their methyl analogues **6** by reduction with sodium tetrahydroborate. All of the compounds studied were screened for the anticoagulant activity. The highest prothrombin time was established for 2-[4-(methylsulfanyl)phenyl]indane-1,3-dione (**3c**) (PT = 33.71 (± 26.01) s), which was very close to PT of the drug anisindione (PT = 36.0 (± 26.42) s).

Keywords: indane-1,3-dione, 2-arylindane-1,3-dione, 2-arylmethyleneindane-1,3-dione, 2-arylmethylindane-1,3-dione, anticoagulant activity

INTRODUCTION

Anticoagulants are pivotal agents for prevention and treatment of thromboembolic disorders.[1] Recently, anticoagulant activities were found for sulfated polysaccharides that are either of natural, semisynthetic, or synthetic origin, such as dextran sulfate, chitin and chitin sulfate, polysaccharide sulfate, and others.[2] Various anticoagulant-active fractions from marine algae have been isolated and characterized, especially from red and brown algae.[3]

The most commonly used anticoagulants, discovered in the early 1940s, are heparin, and coumarins.[4] A number of coumarin compounds possessing anticoagulant activity have been synthesized as potential drugs for treatment of myocardial infarction.[5] However, using both heparins and coumarins have well-known limitations. They are not selective, acting on a broad range of substrates in the coagulation cascade.[6] Drug and food interactions are frequently cited as causes of adverse events when using warfarin.[7]

Warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin] acts slower than heparin, though it has a number of advantages. Warfarin acts as an inhibitor of vitamin K epoxide reductase and vitamin K reductase, preventing generation of the reduced form of vitamin K, which is a necessary cofactor for the hepatic synthesis of vitamin K-dependent clotting factors.[8, 9] The onset and duration of warfarin action are similar to those of 2-arylindane-1,3-diones (anisindione, fluindione, and phenindione).[10] Like coumarins, the indane-1,3-diones are competitive inhibitors of vitamin K in the biosynthesis of prothrombin and follow the same biochemical mechanism of action.[11]

In our previous papers, we described the synthesis and the results of our preliminary pharmacological investigations of indane-1,3-diones (**3**), structurally related to anisindione, fluindione, or clorindione.[12, 13] All of the explored compounds exhibit weak toxicity, and given at the doses equivalent to 0.1 LD_{50} they have no neurotoxic activity as they did not affect the motor coordination in the rota-rod and chimney tests. Due to the fact, that various 2-arylindane-1,3-diones, besides their hypolipemic, antiinflammatory and antiallergic activity, act mainly as anticoagulant agents, the aim of the present study was to investigate *in vivo* the anticoagulant activity of **3** and the novel indane-1,3-diones **5** and **6**.

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CHEMISTRY

Experimental

Structures of all synthesized compounds were elucidated by analytical and spectroscopic data. Melting points were determined on a Boëtius apparatus and are uncorrected. IR spectra were measured in KBr pellets on a Bio-Rad FTS-175C spectrophotometer. 1D ^{1}H NMR and 2D heteronuclear ^{1}H-^{13}C correlation spectra were taken on a Varian 300 MHz Mercury-VX apparatus, using $CDCl_3$ as the solvent. The chemical shifts are expressed as δ values in ppm against TMS as an internal standard; the coupling pattern and the coupling constants were taken from expanded spectra. EI mass spectra were recorded with a Varian – MAT 112 spectrometer at 70 eV, and ESI+ were obtained using Finnigan MAT 95S instrument. Elemental analyses (C, H) were performed on a Perkin-Elmer 2400 analyzer located at the Regional Laboratory of Jagiellonian University, and the results are within ± 0.4 % of the calculated values. The reactions and the product purification were monitored by TLC on silica-gel plates (Merck $60F_{254}$) using ethyl acetate/*n*-hexane (vol. ratio 1:1) mixture as eluent. For column chromatography, silica gel (Merck) was used. Starting materials, solvents, and reagents were purchased from commercial sources (Aldrich and Merck) and were used without further purification.

The synthesis of the compounds **3a**–**f** have been described previously.[12, 13]

Synthesis of 2-Arylmethyleneindane-1,3-diones (***5a***–***g***)

General Procedure. To a solution of indane-1,3-dione (**4**) (0.01 mol) in glacial acetic acid (10 mL) appropriate arylaldehydes **2a**–**g** (0.01 mol) and catalytic amounts (2–3 drops) of concentrated sulfuric acid were added. The reaction mixture was let to stand for 72 h at room temperature. The solid formed was collected by filtration and recrystallized from *n*-octane.

2-Benzylideneindane-1,3-dione (***5a***) was obtained from **2a** as brown, glitter needles, 1.38 g (58 %); m.p. 152–153 °C; IR(KBr) ν_{max}/cm^{-1}: 3066 (CH), 1728, 1685 (CO), 1613, 1588, 1568, 1486 (C=C); ^{1}H NMR ($CDCl_3$) δ/ppm: 7.48–7.56 (m, 3H, Ar-H), 7.78–7.84 (m, 2H, Ar-H), 7.90 (s, 1H, CH=), 7.98–8.04 (m, 2H, Ar-H), 8.45 (d, 2H, J = 7.6 Hz, Ar-H); MS (EI) *m/z*: 234 (M^+, 75 %), 233 ([M-H]$^+$, 100 %), 206 ([M-CO]$^+$, 7 %), 205 ([M-H-CO]$^+$, 11 %), 177 ([M-H-2CO]$^+$, 8 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{16}H_{10}O_2$ (M_r = 234.25): C 82.04, H 4.30; found: C 81.86, H 4.23.

2-(4-Methoxybenzylidene)indane-1,3-dione (***5b***) was obtained from **2b** as yellow needles, 1.54 g (58 %); m.p. 156–157 °C; IR(KBr) ν_{max}/cm^{-1}: 3072, 2919 (CH), 1718, 1680 (CO), 1581, 1558, 1509 (C=C), 1268, 1250 (O-CH_3); ^{1}H NMR ($CDCl_3$) δ/ppm: 3.91 (s, 3H, CH_3), 7.00 (d, 2H, J = 8.7 Hz, Ar-H), 7.78 (d, 2H, J = 8.7 Hz, Ar-H), 7.83 (s, 1H, CH=), 7.95–7.99 (m, 2H, Ar-H), 8.54 (d, 2H, J = 7.1 Hz, Ar-H); MS (ESI+) *m/z*: 265 ([M+H]$^+$, 100 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{17}H_{12}O_3$ (M_r = 264.28): C 77.26, H 4.58; found: C 76.82, H 4.49.

2-(4-Methylsulfanylbenzylidene)indane-1,3-dione (***5c***) was obtained from **2c** as yellow-orange needles, 1.51 g (54 %); m.p. 142–143.5 °C; IR(KBr) ν_{max}/cm^{-1}: 3068, 2917 (CH), 1723, 1686 (CO), 1605, 1574, 1540 (C=C), 1331 (S-CH_3); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.54 (s, 3H, CH_3), 7.30 (d, 2H, J = 6.3 Hz, Ar-H), 7.77–7.81 (m, 3H, Ar-H + CH=), 7.96–8.00 (m, 2H, Ar-H), 8.42 (d, 2H, J = 6.8 Hz, Ar-H); MS (EI) *m/z*: 280 (M^+, 100 %), 279 ([M-H]$^+$, 64 %), 265 ([M-CH_3]$^+$, 14 %), 233 ([M-SCH_3]$^+$, 73 %), 205 ([M-SCH_3-CO]$^+$, 8 %), 177 ([M-SCH_3-2CO]$^+$, 7 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{17}H_{12}O_2S$ (M_r = 280.34): C 72.84, H 4.31; found: C 72.89, H 4.23 .

2-(1-Naphthylmethylene)indane-1,3-dione (***5d***) was obtained from **2d** as orange needles, 1.83 g (64 %); m.p. 174–176 °C; IR(KBr) ν_{max}/cm^{-1}: 3090, 3057 (CH), 1720, 1680 (CO), 1604, 1592, 1511 (C=C); ^{1}H NMR ($CDCl_3$) δ/ppm: 7.54–7.67 (m, 3H, Ar-H), 7.82–7.85 (m, 2H, Ar-H), 7.93 (d, 1H, J = 7.5 Hz, Ar-H), 8.00–8.08 (m, 3H, Ar-H), 8.26 (d, 1H, J = 7.4 Hz, Ar-H), 8.76 (d, 1H, J = 7.2 Hz, Ar-H), 8.80 (s, 1H, CH=); MS (EI) *m/z*: 284 (M^+, 100 %), 283 ([M-H]$^+$, 49 %), 256 ([M-CO]$^+$, 14 %), 255 ([M-H-CO]$^+$, 67 %), 227 ([M-H-2CO]$^+$, 19 %).

Anal. Calcd. for $C_{20}H_{12}O_2$ (M_r = 284.31): C 84.49, H 4.25; found: C 84.19, H 4.17.

2-[1-(4-Methylsulfanyl)naphthylmethylene]indane-1,3-dione (***5e***) was obtained from **2e** as red-orange pellets, 1.57 g (48 %); m.p. 191–191.5 °C; IR(KBr) ν_{max}/cm^{-1}: 3077, 2985 (CH), 1714, 1670 (CO), 1587, 1542, 1508 (C=C), 1317 (S-CH_3); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.66 (s, 3H, CH_3), 7.36 (d, 1H, J = 8.1 Hz, Ar-H), 7.54–7.68 (m, 2H, Ar-H), 7.77–7.80 (m, 2H, Ar-H), 7.94–8.03 (m, 2H, Ar-H), 8.24 (d, 1H, J = 8.4 Hz, Ar-H), 8.31 (d, 1H, J = 8.1 Hz, Ar-H), 8.74 (s, 1H, CH=), 8.94 (d, 1H, J = 8.4 Hz, Ar-H); MS (EI) *m/z*: 330 (M^+, 100 %), 315 ([M-CH_3]$^+$, 67 %), 283 ([M-SCH_3]$^+$, 89 %), 255 ([M-SCH_3-CO]$^+$, 48 %), 227 ([M-SCH_3-2CO]$^+$, 45 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{21}H_{14}O_2S$ (M_r = 330.40): C 76.34, H 4.27; found: C 76.44, H 4.17.

2-[1-(4-Ethylsulfanyl)naphthylmethylene]indane-1,3-dione (***5f***) was obtained from **2f** as red-orange pellets, 2.44 g (71 %); m.p. 145–146 °C; IR(KBr) ν_{max}/cm^{-1}: 3074, 2965 (CH), 1720, 1685 (CO), 1591, 1556, 1508 (C=C), 1319 (S-C_2H_5); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.50 (t, 3H, J = 7.3 Hz, CH_3), 3.25 (q, 2H, J = 7.3 Hz, CH_2), 7.35 (d, 1H, J = 8.0 Hz, Ar-H), 7.55–7.70 (m, 2H, Ar-H), 7.78–7.82 (m, 2H, Ar-H), 7.92–8.00 (m, 2H, Ar-H), 8.26–8.33 (m, 2H, Ar-H), 8.75 (s, 1H, CH=), 8.92 (d, 1H, J = 8.2 Hz, Ar-H); MS (EI) *m/z*: 344 (M^+, 100 %), 315 ([M-C_2H_5]$^+$, 65 %), 283 ([M-SC_2H_5]$^+$, 65 %), 255 ([M-SC_2H_5-CO]$^+$, 20 %), 227 ([M-SC_2H_5-2CO]$^+$, 65 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{22}H_{16}O_2S$ (M_r = 344.43): C 76.72, H 4.68; found: C 76.57, H 4.29.

2-(2-Furylmethylene)indane-1,3-dione (***5g***) was obtained from **2g** as green-yellow needles, 1.56 g (70 %); m.p. 209.5–211 °C; IR(KBr) ν_{max}/cm^{-1}: 3140, 3102, 2922 (CH), 1727, 1691 (CO), 1607, 1589 (C=C), 1160 (C-O-C); ^{1}H NMR ($CDCl_3$) δ/ppm: 6.71–6.73 (m, 1H, Ar-H), 7.74 (s, 1H, CH=), 7.76–7.79 (m, 3H, Ar-H), 7.95–7.98 (m, 2H, Ar-H), 8.57 (d, 1H, J = 3.7 Hz, Ar-H); MS (ESI+) *m/z*: 225 ([M+H]$^+$, 100 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{14}H_8O_3$ (M_r = 224.05): C 75.00, H 3.60; found: C 75.01, H 3.57.

Reduction of 2-Arylmethyleneindane-1,3-diones (***5a–g***) *to 2-Arylmethylindane-1,3-diones* (***6a–g***)

General Procedure. To a solution of **5** (3.0 mmol) in pyridine (15–20 mL), warmed up to 50 °C, a solution of $NaBH_4$ (0.11 g, 3.0 mmol) in 5 mL of water was added dropwise. After the addition was complete, a few pieces of ice were added and the mixture was acidified to pH = 1 with concentrated hydrochloric acid. The organic product was isolated by extraction with chloroform. The extract was dried with anhydrous sodium sulfate and the solvent was evaporated. The residue was purified by column chromatography (silica gel-chloroform) to give **6**, which was recrystallized from an appropriate solvent.

2-Benzylindane-1,3-dione (***6a***) was obtained from **5a** as light-yellow needles (from *n*-octane), 0.43 g (61 %); m.p. 97–99 °C; IR(KBr) ν_{max}/cm^{-1}: 3083, 3058, 3028, 2929 (CH), 1742, 1708 (CO), 1601, 1590, 1492 (C=C); ^{1}H NMR ($CDCl_3$) δ/ppm: 3.34 (s, 3H, CH-CH_2), 7.14–7.16 (m, 5H, Ar-H), 7.73–7.76 (m, 2H, Ar-H), 7.87–7.90 (m, 2H, Ar-H); MS (EI) *m/z*: 236 (M^+, 100 %), 219 ([M-OH]$^+$, 55 %), 208 ([M-CO]$^+$, 27 %), 191 ([M-CO-OH]$^+$, 10 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{16}H_{12}O_2$ (M_r = 236.27): C 81.34, H 5.12; found: C 81.10, H 5.07.

2-(4-Methoxybenzyl)indane-1,3-dione (***6b***) was obtained from **5b** as light-yellow needles (from *n*-octane), 0.49 g (62 %); m.p. 96–97.5 °C; IR(KBr) ν_{max}/cm^{-1}: 3076, 3037, 2930 (CH), 1737, 1708 (CO), 1613, 1594, 1513 (C=C), 1253 (O-CH_3); ^{1}H NMR ($CDCl_3$) δ/ppm: 3.29 (s, 3H, CH-CH_2), 3.67 (s, 3H, CH_3), 6.68 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.06 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.73–7.76 (m, 2H, Ar-H), 7.86–7.89 (m, 2H, Ar-H); MS (ESI+) *m/z*: 267 ([M+H]$^+$, 100 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{17}H_{14}O_3$ (M_r = 266.30): C 76.68, H 5.30; found: C 76.97, H 5.23.

2-(4-Methylsulfanylbenzyl)indane-1,3-dione (***6c***) was obtained from **5c** as light-yellow needles (from *n*-heptane), 0.57 g (57 %); m.p. 70–71 °C; IR(KBr) ν_{max}/cm^{-1}: 3081, 3037, 2981 (CH), 1744, 1707 (CO), 1591, 1495 (C=C), 1325 (S-CH_3); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.38 (s, 3H, CH_3), 3.31 (s, 3H, CH-CH_2), 7.03–7.10 (m, 4H, Ar-H), 7.75–7.78 (m, 2H, Ar-H), 7.87–7.90 (m, 2H, Ar-H); MS (EI) *m/z*: 282 (M^+, 90 %), 235 ([M-SCH_3]$^+$, 50 %), 207 ([M-SCH_3-CO]$^+$, 18 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{17}H_{14}O_2S$ (M_r = 282.36): C 72.32, H 5.00; found: C 71.94, H 4.92.

2-(1-Naphthylmethyl)indane-1,3-dione (***6d***) was obtained from **5d** as light-yellow plates (from *n*-octane), 0.53 g (62 %); m.p. 136–138 °C; IR(KBr) ν_{max}/cm^{-1}: 3076, 3048, 3018 (CH), 1747, 1703 (CO), 1594, 1510 (C=C); ^{1}H NMR ($CDCl_3$) δ/ppm: 3.46 (t, 1H, *J* = 6.0 Hz, CH), 3.67 (d, 2H, *J* = 6.0 Hz, CH_2), 7.37–7.51 (m, 3H, Ar-H), 7.55–7.60 (m, 1H, Ar-H), 7.74 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.79–7.86 (m, 3H, Ar-H), 7.92–7.96 (m, 2H, Ar-H), 8.25 (d, 1H, *J* = 8.4 Hz, Ar-H); MS (EI) *m/z*: 286 (M^+, 100 %), 269 ([M-OH]$^+$, 26 %), 268 ([M-H_2O]$^+$, 94 %), 258 ([M-CO]$^+$, 13 %), 240 ([M-H_2O-CO]$^+$, 45 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{20}H_{14}O_2$ (M_r = 286.33): C 83.90, H 4.93; found: C 83.60, H 4.84.

2-[1-(4-Methylsulfanyl)naphthylmethyl]indane-1,3-dione (***6e***) was obtained from **5e** as yellow plates (from *n*-octane), 0.77 g (76 %); m.p. 125–126 °C; IR(KBr) ν_{max}/cm^{-1}: 3087, 3056, 2930 (CH), 1742, 1704 (CO), 1589, 1566, 1510 (C=C), 1326 (S-CH_3); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.54 (s, 3H, CH_3), 3.42 (t, 1H, *J* = 5.9 Hz, CH), 3.62 (d, 1H, *J* = 5.9 Hz, CH_2), 7.29 (d, 1H, *J* = 7.5 Hz, Ar-H), 7.40 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.51–7.63 (m, 2H, Ar-H), 7.78–7.81 (m, 2H, Ar-H), 7.91–7.94 (m, 2H, Ar-H), 8.24–8.31 (m, 2H, Ar-H); MS (EI) *m/z*: 332 (M^+, 44 %), 317 ([M-CH_3]$^+$, 1 %), 285 ([M-SCH_3]$^+$, 4 %), 257 ([M-SCH_3-CO]$^+$, 2 %) 229 ([M-SCH_3-2CO]$^+$, 2 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{21}H_{16}O_2S$ (M_r = 332.42): C 75.88, H 4.85; found: C 75.62, H 4.74.

2-[1-(4-Ethylsulfanyl)naphthylmethyl]indane-1,3-dione (***6f***) was obtained from **5f** as orange needles (from *n*-octane), 0.20 g (26 %); m.p. 69–70 °C; IR(KBr) ν_{max}/cm^{-1}: 3060, 2974 (CH), 1741, 1703 (CO), 1589, 1565, 1510 (C=C), 1324 (S-C_2H_5); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.29 (t, 3H, J = 7.1 Hz, CH_3), 2.98 (q, 2H, J = 7.1 Hz, CH_2), 3.40 (t, 1H, J = 5.9 Hz, CH), 3.61 (d, 1H, J = 5.9 Hz, CH_2), 7.27 (d, 1H, J = 7.4 Hz, Ar-H), 7.40 (d, 1H, J = 7.5 Hz, Ar-H), 7.50–7.63 (m, 2H, Ar-H), 7.78–7.82 (m, 2H, Ar-H), 7.91–7.94 (m, 2H, Ar-H), 8.23–8.29 (m, 2H, Ar-H); MS (EI) *m/z*: 346 (M^+, 50 %), 317 ($[M-C_2H_5]^+$, 3 %), 285 ($[M-SC_2H_5]^+$, 4 %), 257 ($[M-SC_2H_5-CO]^+$, 1 %), 229 ($[M-SC_2H_5-2CO]^+$, 3 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{22}H_{18}O_2S$ (M_r = 346.44): C 76.27, H 5.24; found: C 76.55, H 5.39.

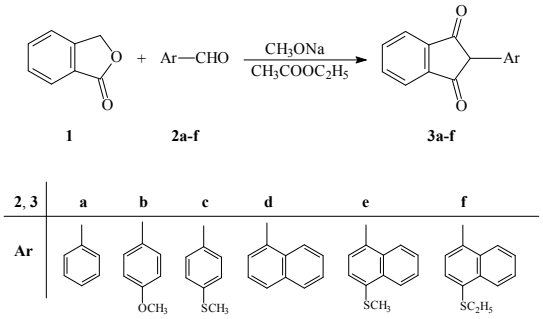
2-(2-Furylmethyl)indane-1,3-dione (***6g***) was obtained from **5g** as yellow plates (from *n*-heptane), 0.48 g (70 %); m.p. 97.5–98 °C; IR(KBr) ν_{max}/cm^{-1}: 3081, 2919 (CH), 1747, 1704 (CO), 1591, 1507 (C=C), 1164 (C-O-C); ^{1}H NMR ($CDCl_3$) δ/ppm: 3.31 (t, 1H, J = 5.7 Hz, CH), 3.38 (d, 2H, J = 5.5 Hz, CH_2), 5.98–6.00 (m, 1H, Ar-H), 6.12–6.13 (m, 1H, Ar-H), 7.08–7.09 (m, 1H, Ar-H), 7.78–7.84 (m, 2H, Ar-H), 7.92–7.98 (m, 2H, Ar-H); MS (ESI+) *m/z*: 227 ($[M+H]^+$, 100 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{14}H_{10}O_3$ (M_r = 226.23): C 74.33, H 4.46; found: C 74.21, H 4.54.

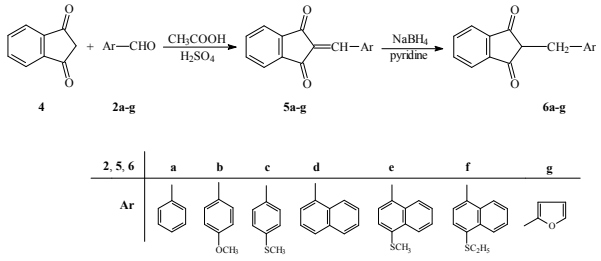
Results and discussion

2-Arylindane-1,3-diones (**3a**–**f**) have been prepared by condensing phthalide (**1**) with appropriate arylaldehydes (**2a**–**f**), in the presence of sodium methoxide in ethyl acetate (Scheme 1). By this method 2-arylindane-1,3-diones (**3a**, **c**–**f**) as well as the anisindione (**3b**) approved as an anticoagulant drug, were obtained.[12, 13]

In order to check the influence of modification of 2-arylindane-1,3-diones (**3a**–**f**) on their anticoagulant properties 2-arylmethylene- (**5a**–**g**) and 2-arylmethylindane-1,3-diones (**6a**–**g**), possessing the additional methine and methylene units respectively, were also obtained (Scheme 2). The compounds **5a**–**g** were prepared by condensation of indane-1,3-dione (**4**) with the corresponding arylaldehydes **2a**–**g**. The reactions were carried out in glacial acetic acid in the presence of concentrated sulfuric acid as a catalyst at room temperature. The 2-arylmethyleneindane-1,3-diones (**5a**–**g**) were prepared in acceptable yields (Scheme 2).[14] All of the 2-arylmethyleneindane-1,3-diones were colored crystalline solids with sharp melting points.



Scheme 1.



Scheme 2.

The compounds **5a**–**g** were converted into their saturated analogues **6a**–**g** by reduction of **5a**–**g** with sodium tetrahydroborate in pyridine at 50 °C (Scheme 2).[15] It is worth pointing out that under the reduction conditions applied, only the reduction of C=C bonds in **5a**–**g** took place, without any effect on the C=O bonds. It is a well known fact that $NaBH_4$ has a great tendency to effect double reduction of conjugated C=C–C=O systems. Moreover, even in the case of single reduction the product of C=O bond reduction is usually formed in a larger amount. In our investigations, we did not isolate any products of C=O bond reduction in any case.

The structure of the compounds **5a**–**g** and **6a**–**g** was identified by analytical and spectroscopic data. In all the ^{1}H NMR spectra of **5a**–**g**, protons of –CH= were within the aromatic region. Unexpectedly, in the ^{1}H NMR spectra of **6a**–**c,** protons of CH–CH_2 units were observed as singlets at δ 3.29–3.34 ppm. This phenomenon may be attributed to structural symmetry of **6a**–**c**. Inspection of the heteronuclear ^{1}H-^{13}C correlation spectra of **6a**–**c**, showed cross-peaks between protons at two different C-atoms. For example, Figure 1 shows ^{1}H-^{13}C COSY spectrum of **6b** – the proton signal at δ 3.29 ppm, gives a cross-peak with the composite signal of carbons in CH_2 (δ 31.42 ppm) and CH (δ 55.26 ppm). In the ^{1}H NMR spectra of structurally unsymmetrical derivatives **6d**–**g** protons in CH–CH_2 units appear in a ABX pattern.

BIOLOGICAL ASSAY

Methods

Drugs

All of the synthesized compounds **3**, **5** and **6** were screened for their anticoagulant activity, and the data obtained were compared with anticoagulant effect of warfarin. Warfarin potasium was purchased from LG Promochem. All agents were suspended in 100 % olive oil.

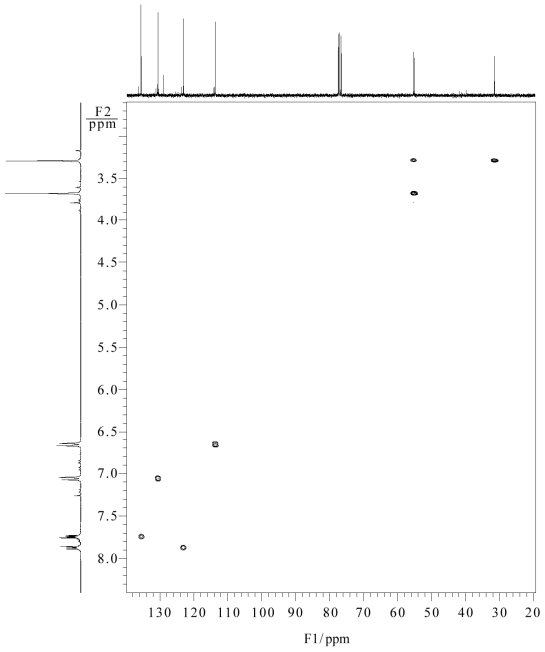


Figure 1. ^{1}H-^{13}C COSY spectrum of 2-{[(4-methoxy)phenyl]-methyl}indane-1,3-dione (**6b**).

Drugs Administration

The compounds tested and warfarin were administered orally to male mice (Balb/c) at doses 10 and 50 mg/kg for compounds **3**, **5** and **6** and 1 mg/kg for warfarin, for four consecutive days (at 9:00 am). On the day 5, 18–20 hours after the last drug administration, blood samples were collected by aspiration from the inferior vena cava of mice, previously anaesthetized with ketha-mine/xilazine mixture (vol. ratio 87:13). As an anticoa-gulant, 3 % *w* sodium citrate was used. Platelets-poor plasma was separated by centrifugation at 5000 *g* for 7 minutes at room temperature.

PT Assay

Anticoagulant activity in plasma samples was measured by microplate-based blood coagulation assay[16] using kinetic microplate reader (FluoroStar Optima, BMG). To measure prothrombin time (PT) 100 μL of plasma was incubated for 1 min at 37 °C and the coagulation was induced by addition of 200 μL of PT reagent (HemoStat THROMBOPLASTIN-SI, HUMAN). Clot-ting time was determined by change in absorbance at 405 nm. The method was previously validated using normal and abnormal human plasmas (HUMAN).

Statistical Analysis

All PT data represent mean values ± SD. Statistical analysis was performed by ANOVA test. A *P* value of less then 0.05 was considered as significant.

Results and discussion

All of the orally given compounds at 10 mg/kg doses did not prolong prothrombin time after 4 days of conti-

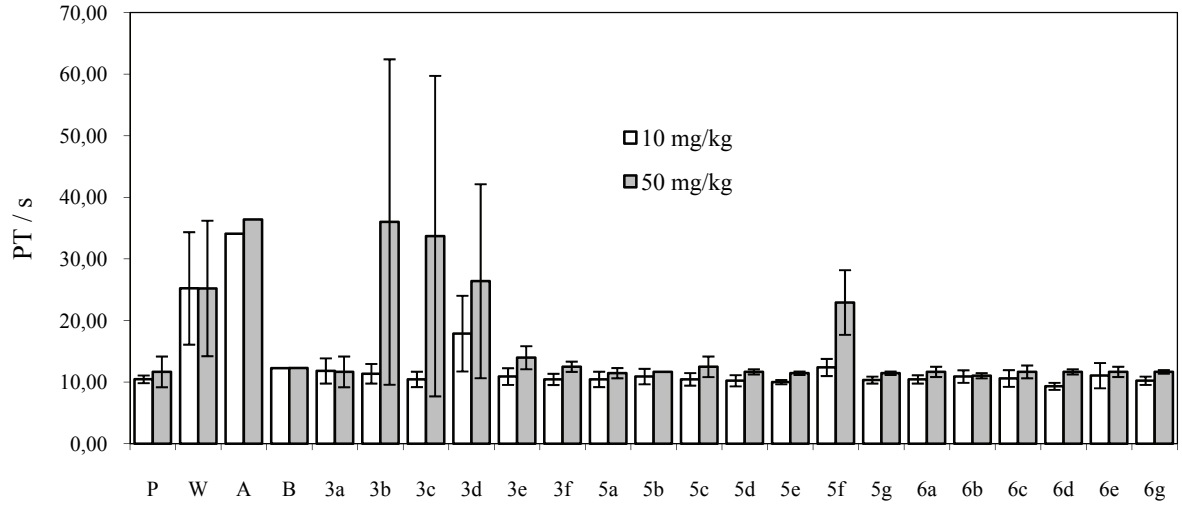


Figure 2. Anticoagulation effect of the compounds **3**, **5** and **6** and warfarin in mice. The drugs and warfarin were given orally for 4 consecutive days at the doses 10 mg/kg, 50 mg/kg and 1 mg/kg, respectively. The data represent the prothrombin time in plas-mas $n = 6$ with standard deviation. P – placebo, W – warfarin (1 mg/kg), A – abnormal human plasma control, B – normal human plasma control.

nuous feeding, except for **3d** and to a very little extent **5f**, for which the measured PT was (17.88 ± 6.14) s and (12.39 ± 1.39) s, respectively. The controls were: placebo – (10.46 ± 0.62) s and warfarin (1 mg/kg) – (25.22 ± 9.13) s. For **3d** and **5f** $P < 0.05$, for warfarin $P < 0.01$, both compared with placebo group. The compounds studied were also tested at the doses 50 mg/kg given orally for 4 consecutive days. Only **3b**, **3c**, **3d**, **5f**, and **3e** affected the coagulation, significantly prolonging the clotting time. The results of PT were 36.0 (± 26.42); 33.71 (± 26.01); 26.39 (± 15.75); 22.93 (± 5.25); 13.97 (± 1.87) s respectively. *P* values for active compounds were < 0.05, compared with placebo group. All the remaining compounds induced very small anticoagulation effect (Figure 2).

Amongst the obtained compounds screened for their anticoagulant activity, the highest prothrombin time was found for 2-(4-methylsulfanylphenyl)indane-1,3-dione (**3c**) (PT = 33.71 (± 26.01) s), which is very close to the PT of the drug anisindione (**3b**) (PT = 36.0 (± 26.42) s). Hence, the replacement of the methoxy substituent in the structure of 2-(4-methoxyphenyl)-indane-1,3-dione (**3b**) with a methylsulfanyl group, practically does not affect the protrombin times of the compounds studied. Both these compounds, however, are *ca.* five times less active than warfarin. Moreover, the data obtained indicate that generally the presence of additional methine (compounds **5**) and methylene (compounds **6**) units in the structure of 2-arylindane-1,3-dione induces a very small anti-coagulation effect.

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SAŽETAK

Sinteza novih derivata indan-1,3-diona i biološko ispitivanje njihovog antikoagulacijskog djelovanja

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Pripravljeni su 2-supstituirani derivati indan-1,3-diona **3a**–**f**, **5a**–**g**, **6a**-**g** i ispitivano njihovo antikoagulacijsko djelovanje. 2-Arilindan-1,3-dioni (**3**) dobiveni su reakcijom ftalida s odgovarajućim aldehidima. 2-Arilmetilenindan-1,3-dioni (**5**) pripravljeni su kondenzacijom indan-1,3-diona s odgovarajućim aldehidima. Redukcijom s natrijevim tetrahidroboranom spojevi **5** prevedeni su u metil-analoge **6**. Svim spojevima ispitivana je antikoagulacijska aktivnost. Najduže protrombinsko vrijeme nađeno je za 2-[4-(metilsulfanil)fenil]indan-1,3-dion (**3c**) (PT = 33,71 (± 26,01) s), što je vrlo slično PT-u lijeka anisindiona (PT = 36,0 (± 26,42) s)