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Pharmacology, Biochemistry and Behavior 86 (2007) 678–685
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Sedative–hypnotic profile of novel isatin ketals

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Received 12 May 2006; received in revised form 13 February 2007; accepted 13 February 2007
Available online 22 February 2007

Abstract

Isatin (1*H*-indol-2,3-dione) is an endogenous compound found in many tissues and fluids. Isatin and its derivatives exert pharmacological effects on the central nervous system, including anxiogenic, sedative and anticonvulsant activities. Two new groups of isatin derivatives were synthesized (nine dioxolane ketals and nine dioxane ketals) and studied for their sedative, hypnotic and anesthetic effects using pentobarbital-induced sleeping time, locomotor activity evaluation and intravenous infusion. The dioxolane ketals were more potent than dioxane ketals for inducing sedative–hypnotic states, causing up to a three-fold increase in pentobarbital hypnosis. The dioxolane ketals produced sedation, demonstrated by decreased spontaneous locomotor activity in an open field. Hypnosis and anesthesia were observed during intravenous infusion of 5′-chlorospiro-[1,3-dioxolane-2,3′-indolin]-2′-one (T3) in conscious Wistar rats. Complete recovery from hypnosis and anesthesia required 39.1±7.3 and 6.8±2.4 min, respectively. Changes in hemodynamic parameters after infusion of 5.0 mg/kg/min were minimal. These findings suggest that these new isatin derivatives represent potential candidates for the development of new drugs that act on the central nervous system and may lead to a new centrally acting anesthetic with no toxic effects on the cardiovascular or respiratory systems.

Keywords: Isatin; Derivative; Sedation; Hypnosis; Locomotor activity; Anesthesia

1. Introduction

Isatin (1*H*-indol-2,3-dione) is an endogenous compound that is widely distributed in mammalian tissues and body fluids. It has been identified in urine, blood and tissues using gas chromatography–mass spectrometry (Halket et al., 1991) and, more recently, high performance liquid chromatography with an ultraviolet detector (Hamaue et al., 1998). Isatin has a distinctive regional distribution in rat tissues, with the highest concentration in seminal vesicles (1.6 μg/g) and vas deferens (3.4 μg/g) (Watkins et al., 1990). There is also a discontinuous distribution within the rat brain (Crumeyrolle-Arias et al., 2003), especially in the hippocampus (0.13 μg/g) (Watkins et al., 1990). Isatin readily crosses the blood–brain barrier, suggesting its possible action on the central nervous system (CNS) (Bhattacharya et al., 1991).

Isatin has been identified as a selective inhibitor of monoamine oxidase B (MAOB), with IC_{50} values of about 5 μM (Glover et al., 1988). Hamaue (2000), Hamaue et al. (2004) suggested that isatin, as an MAOB inhibitor, could be an alternative for treatment of Parkinson's disease, by increasing dopamine levels in the striatum.

Isatin is also considered an endogenous marker of stress and anxiety (Bhattacharya et al., 1991). Acute stress can increase isatin levels in tissues (brain and heart) and serum, which is greater in males than in females (Igosheva et al., 2004). Urinary excretion of isatin is also increased following stress (Tozawa et al., 1998). Behavioral effects of isatin are dose dependent. Isatin can inhibit the facilitatory action of natriuretic peptides on memory consolidation in rats (Telegdy et al., 2000), and decrease food intake in mice (Morley et al., 1996). Several studies show that isatin has anxiogenic effects

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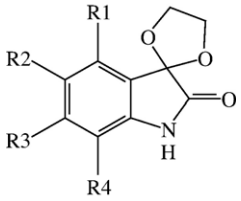
doi:10.1016/j.pbb.2007.02.013

Table 1
Chemical characteristics of dioxolane compounds



No.	R1, R2, R3, R4, formula (MW)	MP	MS (70 eV); IR (KBr)	^{1}H NMR; ^{13}C NMR
T1	H, H, H, H, $C_{10}H_9NO_3$ (191.19)	130–132 °C	191 (12), 163 (100), 146 (4), 136 (4), 119 (57), 104 (6), 92 (25), 76 (8), 64 (7); 3428, 3213, 3115, 2894, 2846, 1741, 1622, 1474, 1217, 1085, 759, 622, 486	(200 MHz, $CDCl_3$): 4.28 a 4.40 (2 H, m); 4.48 a 4.60 (2 H, m); 6.82 (1 H, d, J=7.8 Hz); 7.04 (1 H, t, J=7.8 Hz); 7.27 (2 H, m); 8.67 (1 H, bs); (75 MHz, $CDCl_3$): 66.0; 102.6; 110.9; 123.4; 124.6; 125.2; 131.8; 142.0; 175.9.
T3	H, Cl, H, H, $C_{10}H_8NClO_3$ (225.63)	173–176 °C	227 (6), 225 (17), 197 (100), 180 (4), 166 (2), 153 (58), 138 (6), 126 (15), 110 (4), 98 (6), 90 (8), 75 (8), 63 (15); 3425, 3183, 3140, 3059, 2881, 1746, 1625, 1481, 1218, 1101, 1029, 995, 948, 813, 755, 643, 538.	200 MHz, $CDCl_3$/acetone-d_6): 4.28 a 4.48 (4 H, m); 6.94 (1 H, dd, J=8.8 e 2.3 Hz); 7.35 (2 H, m); 9.67 (1 H, sl); (75 MHz, $CDCl_3$/acetone-d_6): 66.7; 102.6; 112.9; 126.0; 127.9; 128.1; 132.2; 142.7; 175.3.
T5	H, H, H, Cl, $C_{10}H_8NClO_3$ (225.63)	175–178 °C	227 (4), 225 (11), 197 (100), 180 (3), 169 (2), 153 (68), 138 (5), 125 (13), 118 (9), 102 (3), 90 (13), 75 (9), 63 (13); 3425, 3183, 3140, 3059, 2881, 1746, 1625, 1481, 1218, 1101, 1029, 948, 813, 755, 553.	(200 MHz, $CDCl_3$/acetone-d_6): 4.27–4.49 (4 H, m); 7.07 (1 H, t, J=7.5 Hz); 7.35 (2 H, m); 9.86 (1 H, bs); (75 MHz, $CDCl_3$/acetone-d_6): 66.7; 103.0; 116.1; 124.4; 124.7; 127.8; 132.3; 141.5; 175.2.
T7	H, H, H, CF3, $C_{11}H_8NF_3O_3$ (259.19)	139–141 °C	259 (11), 231 (100), 212 (8), 204 (4), 187 (56), 167 (11), 160 (14), 139 (10), 132 (11), 120 (2), 97 (7), 88 (3), 63 (6); 3458, 3195, 3133, 3040, 2981, 2911, 1753, 1624, 1457, 1308, 1193, 1120, 991, 801, 753, 509.	(200 MHz, $CDCl_3$/acetone-d_6): 4.30–4.50 (4 H, m); 7.25 (1 H, t, J=7.5 Hz); 7.64 (2 H, d, J=7.5 Hz); 9.92 (1 H, bs); (75 MHz, $CDCl_3$/methanol-d_4): 66.0; 100.9; 112.7; 120.8; 123.1; 126.2; 128.3; 128.7; 139.9; 174.9.
T9	Cl, H, H, OMe, $C_{11}H_{10}NClO_4$ (255.66)	167–169 °C	257 (30), 255 (88), 227 (100), 212 (12), 182 (40), 171 (63), 153 (85), 140 (36), 125 (11), 112 (14), 104 (10), 76 (11); 3431, 3193, 3095, 3014, 2970, 2912, 1731, 1629, 1495, 1280, 1055, 947, 800, 662, 562.	200 MHz, $CDCl_3$/acetone-d_6): 3.87 (3 H, s); 4.30–4.46 (4 H, m); 6.96 (1 H, d, J=8.8 Hz); 7.06 (1 H, d, J=8.8 Hz); 9.65 (1 H, bs); (75 MHz, $CDCl_3$/DMSO-d_6): 55.8; 66.0; 102.3; 114.1; 115.0; 122.1; 122.9; 133.3; 142.8; 173.9.
T11	H, Br, H, H, $C_{10}H_8NBrO_3$ (270.08)	187–189 °C	271 (17), 269 (17), 241 (100), 226 (4), 213 (2), 197 (39), 170 (14), 142 (2), 117 (5), 102 (4), 90 (17), 63 (24); 3432, 3314, 3053, 2985, 2907, 1739, 1621, 1467, 1210, 1087, 943, 822, 621, 536.	(200 MHz, $CDCl_3$/methanol-d_4): 4.24–4.53 (4 H, m); 6.68 (1 H, d, J=8.0 Hz); 7.38 (2 H, dd, J=8.0 and 2.0 Hz); 9.25 (1 H, bs); (75 MHz, $CDCl_3$/methanol-d_4): 66.1; 102.1; 112.4; 115.7; 126.6; 128.4; 134.5; 141.2; 175.4.
T13	Br, H, Br, H, $C_{10}H_7NBr_2O_3$ (348.98)	249–250 °C	351 (7), 349 (13), 347 (7), 321 (100), 294 (8), 277 (32), 250 (19), 196 (2), 168 (12), 141 (4), 116 (8), 100 (4), 88 (11), 62 (13); 3443, 3198, 3164, 3128, 3082, 2975, 2901, 1736, 1603, 1425, 1250, 1102, 1038, 935, 846, 764, 654.	(200 MHz, $CDCl_3$/acetona-d_6): 4.33–4.51 (4 H, m); 7.07 (1 H, d, J=1.4 Hz); 7.32 (1 H, d, J=1.4 Hz); 9.86 (1 H, bs); (75 MHz, $CDCl_3$/acetona-d_6): 67.2; 102.9; 113.9; 120.6; 123.1; 125.9; 129.0; 146.9; 174.5.
T15	H, Br, H, Br, $C_{10}H_7NBr_2O_3$ (348.98)	238–240 °C	351 (7), 349 (14), 347 (7), 321 (100), 304 (2), 277 (42), 249 (12), 225 (2), 196 (5), 168 (12), 141 (8), 117 (4), 100 (4), 88 (17), 62 (13); 3443, 3164, 3106, 2999, 2882, 1739, 1616, 1458, 1183, 1002, 945, 859, 733, 558.	(200 MHz, $CDCl_3$/acetona-d_6): 4.23–4.48 (4 H, m); 7.35 (1 H, d, J=1.7 Hz); 7.55 (1 H, d, J=1.7 Hz); 9.36 (1 H, bs); (75 MHz, $CDCl_3$/acetona-d_6): 66.6; 102.8; 104.7; 115.8; 127.8; 128.6; 136.8; 140.7; 174.4.
T17	H, F, H, H, $C_{10}H_8NFO_3$ (209.18)	164–166 °C	209 (14), 181 (100), 164 (4), 154 (2), 150 (3), 137 (69), 122 (9), 108 (23), 94 (10), 82 (15), 75 (2), 63 (4); 3422, 3193, 3155, 3072, 2980, 1736, 1630, 1494, 1218, 1184, 1077, 996, 949, 828, 590.	(200 MHz, $CDCl_3$/acetone-d_6): 4.27–4.38 (2 H, m); 4.49–4.60 (2 H, m); 6.82 (1 H, dd, J=8.3 and 5.5 Hz); 7.03 (2 H, m); 9.01 (1 H, bs); (75 MHz, $CDCl_3$/acetone-d_6): 65.8; 102.0; 111.4; 112.7 (J2CF=25 Hz); 117.8 (J^2CF=23 Hz); 125.8; 138.0; 159.0 (J^1CF=241 Hz); 175.2.

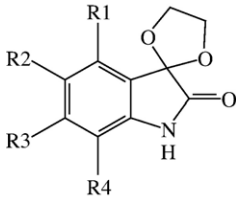
MW, molecular weight; MP, melting point; MS, mass spectrometry; IR, infrared; NMR, nuclear magnetic resonance.

at doses of 10–20 mg/kg (Bhattacharya and Acharya, 1993, Bhattacharya and Chakrabarti, 1998), in contrast to sedative and anticonvulsant effects at doses of 80–200 mg/kg (Chocholova and Kolinova, 1979, Bhattacharya and Chakrabarti, 1998, Pandeya et al., 2005). In this range, it can act as a sedative or reduce anxiety: It reduces movement in an open field (Abel, 1995). Administration of both anxiogenic and sedative doses of isatin (10–200 mg/kg) leads to an increase in monoamine neurotransmitter in the brain (Medvedev and Glover, 2004).

Other reports suggest that isatin interacts with natriuretic peptide receptors (NPR) coupled to guanylate cyclase, thereby decreasing cyclic guanosine monophosphate (cGMP) levels (Glover et al., 1995). Isatin acts as an antagonist of nitric oxide-stimulated guanylate cyclase activity in platelets (Medvedev et al., 2002). Bhattacharya et al. (1996) showed that isatin inhibits the anxiolytic effect of atrial natriuretic peptide (ANP) and that this effect is unaffected by flumazenil, a benzodiazepine receptor antagonist. Isatin abolishes natriuretic peptide-induced hyperthermia, suggesting that it antagonizes natriuretic peptide actions on the CNS (Pataki et al., 2000, 2002).

Many isatin derivatives have been synthesized and tested. Among their physiological effects are: 1. selective MAO

Table 2
Chemical characteristics of dioxane compounds



No.	R1, R2, R3, R4, formula (MW)	MP	MS (70 eV); IR (KBr).	^{1}H NMR; ^{13}C NMR
T2	H, H, H, H, $C_{11}H_{11}NO_3$ (205.21)	151–152 °C	205 (6), 191 (2), 177 (58), 163 (9), 148 (5),146 (6), 119 (100), 104 (6), 92 (32), 76 (6), 64 (16); 3383, 3183, 3154, 3090, 2977, 2896, 2840, 1709, 1624, 1471, 1214, 1116, 1086, 1027, 754, 743, 617.	(200 MHz, $CDCl_3$): 1.66 (1 H, dt, J=13.2 e 2.0 Hz); 2.26 a 2.50 (1 H, m); 3.95 a 4.04 (2 H, m); 4.98 (2 H, td, J=13.2 e 2.7 Hz); 6.76 (1 H, d, J=7.6 Hz); 7.04 (1 H, td, J=7.6 e 1.2 Hz); 7.26 (1 H, td, J=7.6 e 1.2 Hz); 7.43 (1 H, d, J=7.6 Hz), 8.33 (1 H, sl); (75 MHz, $CDCl_3$): 25.4; 61.3; 94.0; 110.3; 123.3; 124.5; 127.9; 131.0; 140.3; 174.0.
T4	H, Cl, H, H, $C_{11}H_{10}NClO_3$ (239.66)	184–186 °C	241 (4), 239 (12), 211 (83), 182 (7), 153 (100), 138 (2), 126 (16), 110 (2), 98 (6), 90 (9), 75 (6), 63 (16); 3447, 3301, 3056, 2989, 2917, 1733, 1626, 1485, 1472, 1265, 1129, 1030, 916, 818, 754, 621.	(200 MHz, $CDCl_3$/methanol-d_4): 1.64 (1 H, dt, J=13.3 e 2.1 Hz); 2.19 a 2.43 (1 H, m); 3.93 (2 H, ddd, J=13.3, 11.9 e 3.1 Hz); 4.90 (2 H, td, J=11.9 e 2.1 Hz); 6.72 (1 H, d, J=8.2 Hz); 7.20 (1 H, dd, J=8.2 e 2.2 Hz); 7.33 (1 H, d, J=2.2 Hz); 7.46 (1 H, s); (75 MHz, $CDCl_3$/methanol-d_4): 25.8; 61.7; 94.3; 111.9; 125.2; 128.6; 129.8; 131.3; 140.2; 174.3.
T6	H, H, H, Cl, $C_{11}H_{10}NClO_3$ (239.66)	165–166 °C	241 (4), 239 (11), 211 (90), 182 (8), 153 (100), 138 (2), 125 (14), 110 (2), 90 (17), 75 (7), 63 (14); 3408, 3253, 3094, 2975, 2891, 1717, 1623, 1474, 1461, 1322, 1185, 1145, 1030, 926, 781, 633.	(200 MHz, $CDCl_3$/acetone-d_6): 1.57 (1 H, d, J=13.6 Hz); 2.16–2.41 (1 H, m); 3.88 (2 H, ddd, J=13.6, 11.9 and 3.7 Hz); 4.89 (2 H, td, J=11.9 and 2.4 Hz); 6.92 (1 H, t, J=8.0 Hz); 7.21 (2 H, t, J=8.0 Hz); 8.82 (1 H, bs); (75 MHz, $CDCl_3$/methanol-d_4): 25.8; 61.7; 94.7; 115.9; 123.0; 124.3; 129.8; 131.4; 139.3; 174.0.
T8	H, H, H, CF_3, $C_{12}H_{10}NF_3O_3$ (273.21)	121–125 °C	273 (7), 245 (86), 226 (5), 214 (4), 196 (4), 187 (100), 160 (11), 148 (8), 132 (10), 120 (5), 97 (2), 88 (2), 63 (5); 3423, 3182, 3115, 2965, 2849, 1728, 1625, 1461, 1193, 1115, 1032, 919, 801, 753, 654.	(200 MHz, $CDCl_3$/methanol-d_4): 1.64 (1 H, dt, J=13.6 and 2.0 Hz); 2.20–2.44 (1 H, m); 3.94 (2 H, ddd, J=13.6, 11.9 and 2.0 Hz); 4.91 (2 H, td, J=11.9 and 2.7 Hz); 6.96 (1 H, dd, J=8.0 and 7.5 Hz); 7.25 (2 H, td, J=8.0 and 1.0 Hz); 7.46 (1 H, s); (75 MHz, $CDCl_3$/methanol-d_4): 25.3; 61.3; 92.5; 112.8; 120.9; 123.0; 126.3; 127.6; 127.6; 138.0; 173.2.
T10	Cl, H, H, OMe, $C_{12}H_{13}NClO_4$ (269.68)	160–163 °C	271 (14), 269 (44), 241 (100), 226 (5), 212 (20), 195 (10), 182 (72), 171 (39), 155 (83), 140 (23), 125 (14), 104 (9), 90 (7), 76 (15); 3409, 3301, 3087, 2980, 2943, 2903, 2843, 1716, 1626, 1493, 1275, 1069, 1037, 1019, 925, 797, 662, 579.	(200 MHz, $CDCl_3$/DMSO-d_6): 1.53 (1 H, dl, J=13.3 Hz); 2.17–2.41 (1 H, m); 3.76 (3 H, s); 3.86 (2 H, dd, J=10.6 and 5.1 Hz); 4.89 (2 H, td, J=13.3 and 2.4 Hz); 6.76 (1 H, d, J=7.9 Hz); 6.85 (1 H, d, J=7.9 Hz); 7.73 (1 H, s); (75 MHz, $CDCl_3$/DMSO-d_6): 24.6; 55.7; 65.9; 93.8; 114.2; 115.0; 122.1; 123.0; 131.8; 142.3; 172.1.
T12	H, Br, H, H, $C_{11}H_{10}NBrO_3$ (284.11)	205–207 °C	285 (17), 283 (17), 257 (100), 226 (9), 199 (82), 170 (18), 144 (2), 117 (4), 90 (18), 75 (8), 63 (27); 3447, 3311, 2984, 2915, 1737, 1621, 1473, 1264, 1199, 1097, 1029, 815, 753, 620.	(200 MHz, $CDCl_3$/methanol-d_4): 1.63 (1 H, ddd, J=13.3, 10.9 and 2.0 Hz); 2.21–2.45 (1 H, m); 3.95 (1 H, ddd, J=13.3, 11.6 and 2.0 Hz); 4.90 (1 H, td, J=13.3 and 2.0 Hz); 6.64 (1 H, d, J=8.2 Hz); 7.35 (1 H, dd, J=8.2 and 2.0 Hz); 7.51 (1 H, d, J=2.0 Hz); 9.05 (1 H, bs); (75 MHz, $CDCl_3$/methanol-d_4): 25.3; 61.3; 93.7; 111.8; 115.6; 127.7; 129.6; 133.8; 139.5; 173.7.
T14	Br, H, Br, H, $C_{11}H_9NBr_2O_3$ (363.01)	194–197 °C	365 (5), 363 (8), 361 (5), 335 (100), 304 (7), 277 (77), 250 (24), 222 (2), 198 (7), 168 (18), 141 (6), 116 (6), 88 (13), 62 (14); 3408, 3183, 3157, 3064, 2965, 2896, 1720, 1609, 1431, 1247, 1176, 1105, 1042, 918, 838, 770, 641.	(200 MHz, $CDCl_3$/DMSO-d_6): 1.52 (1 H, dl, J=13.3 Hz); 2.22–2.41 (1 H, m); 3.88 (2 H, dd, J=10.6 and 5.1 Hz); 4.85 (2 H, td, J=13.3 and 2.1 Hz); 6.84 (1 H, d, J=1.4 Hz); 7.16 (1 H, d, J=1.4 Hz); 10.4 (1 H, bs); (75 MHz, $CDCl_3$/DMSO-d_6): 24.6; 60.4; 93.3; 112.3; 119.2; 124.1; 124.3; 127.8; 144.2; 172.1.
T16	H, Br, H, Br, $C_{11}H_9NBr_2O_3$ (363.01)	192–195 °C	365 (7), 363 (14), 361 (7), 335 (100), 306 (7), 277 (80), 249 (16), 198 (8), 168 (15), 141 (9), 117 (3), 88 (19), 62 (13); 3459, 3168, 3110, 2969, 2874, 1750, 1610, 1459, 1177, 1043, 856, 742, 561.	(200 MHz, $CDCl_3$/DMSO-d_6): 1.58 (1 H, dl, J=13.3 Hz); 2.11–2.35 (1 H, m); 3.85 (2 H, ddd, J=11.9, 10.6 and 5.1 Hz); 4.81 (2 H, td, J=11.9 and 2.4 Hz); 7.34 (1 H, d, J=1.7 Hz); 7.49 (1 H, d, J=1.7 Hz); 10.6 (1 H, s); (75 MHz, $CDCl_3$/DMSO-d_6): 24.0; 59.7; 92.5; 102.4; 113.4; 125.1; 129.5; 134.3; 139.3; 171.5.
T18	H, F, H, H, $C_{11}H_{10}NFO_3$ (223.20)	165–167 °C	223 (12), 195 (81), 164 (6), 137 (100), 122 (3), 109 (23), 95 (4), 82 (17); 3391, 3174, 3135, 2961, 2900, 1716, 1631, 1491, 1471, 1212, 1081, 1030, 934, 869, 782, 596.	(200 MHz, $CDCl_3$/acetone-d_6): 1.66 (1 H, td, J=9.1 and 1.2 Hz); 2.28–2.45 (1 H, m); 3.98 (2 H, dt, J=9.8 and 1.2 Hz); 4.96 (2 H, ddd, J=9.1; 3.5 and 1.2 Hz); 6.77 (1 H, dd, J=8.5 and 5.0 Hz); 6.97 (1 H, td, J=8.5 and 1.8 Hz); 7.15 (1 H, dd, J=6.8 and 1.8 Hz); 8.94 (1 H, bs); (75 MHz, $CDCl_3$/acetone-d_6): 25.1; 61.0; 93.5; 110.9; 112.1 (J^2_{CF}=24 Hz); 117.0 (J^2_{CF}=23 Hz); 128.8; 136.2; 159.0 (J^1_{CF}=240 Hz); 173.5.

MW, molecular weight; MP, melting point; MS, mass spectrometry; IR, infrared; NMR, nuclear magnetic resonance.

inhibition (Medvedev et al., 1992, Crumeyrolle-Arias et al., 2004); 2. particulate guanylate cyclase coupled to NPR inhibition (Medvedev et al., 1999); 3. analgesic, antithermic and antiinflammatory activities (Sridhar and Ramesh, 2001); 4. parasite enzyme inhibition (Chiyanzu et al., 2003), 5. anticonvulsant action (Rajopadhye and Popp, 1988; Pandeya et al., 2002; Sridhar et al., 2002); and 6. anxiolytic (Geronikaki et al., 2004); antimicrobial (Pandeya et al., 1999), antiproliferative and proapoptotic effects (Cane et al., 2000).

Since endogenous isatin interferes with CNS function, it was of interest to synthesize new derivatives of isatin and test them as sedative, hypnotic and anesthetic agents. New hypnotic and sedative agents would be valuable because the classical benzodiazepines diazepam and flunitrazepam and the novel non-benzodiazepines zaleplon, zolpidem and idiplon used as hypnotics have been associated with side effects such as memory impairment (Wagner and Wagner, 2000). Results of the present work provide new insights into the CNS effects of novel isatin derivatives. All derivatives were investigated with the use of pentobarbital-induced-sleep and locomotor activity tests in Swiss mice. Also, 5′-chlorospiro-[1,3-dioxolane-2,3′-indolin]-2′-one was evaluated as a hypnotic and anesthetic agent in conscious Wistar rats.

2. Materials and methods

2.1. Chemistry — derivatives synthesis

Cyclic ketals of isatin were obtained using conventional procedures, which consisted in the use of one diol (ethylene glycol for the dioxolane group or 1,3-propanediol for the dioxane group), *p*-toluene-sulfuric acid as catalyst and toluene (or benzene). Briefly, 1 mmol isatin, with or without substitutions on the aromatic ring, was added to 20 mmol diol, 25 ml toluene and *p*-toluene sulfuric acid. The reaction mixture was stirred continuously and reflux performed with a Dean–Stark apparatus. Product synthesis during the reaction was followed by thin-layer chromatography (TLC). After total consumption of isatin, water was added to remove excess diol and the final product was extracted with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. After 6 h of reaction, isatin ketal (dioxolane) could be obtained in a yield of 79 to 88% following residue purification by flash column chromatography. Nine dioxolane ketals (T1, T3, T5, T7, T9, T11, T13, T15 and T17) (Table 1) and nine dioxane ketals of isatin (T2, T4, T6, T8, T10, T12, T14, T16 and T18) (Table 2) were synthesized and tested for biological activity.

2.2. Pentobarbital-induced sleeping time in mice

The Animal Care and Use Committee at Universidade Federal do Rio de Janeiro approved the protocols described as follows. Male Swiss mice (18–28 g) and Wistar rats (220–250 g) were housed with free access to food and water, and maintained on a 12/12 h light–dark cycle. In order to evaluate the potentiation of hypnosis induced by a barbiturate, mice were randomly divided into groups of 10 and each group was injected i.p. with either DMSO, isatin (20 mg/kg) or an isatin derivative (20 mg/kg) 30 min before the injection of sodium pentobarbital (25 mg/kg) into the tail vein. The low dose of isatin and its derivatives (20 mg/kg) was chosen based on a previous study which demonstrated that other derivatives had anxiolytic action at 10 mg/kg (Geronikaki et al., 2004). Sleeping time was defined as the loss of righting reflex, while time to recovery of this reflex determined the hypnosis endpoint. The time from the loss of righting reflex to recovery was considered as the sleeping time (Mora et al., 2005).

2.3. Locomotor activity evaluation in mice

The sedative properties of midazolam and isatin derivatives were compared using locomotor activity as an index of sedation. Spontaneous locomotor activity was determined in Swiss mice (18–28 g) which were placed in the center of an open field of 45×45 cm (LE 8811, Letica) in which 16 infrared photocells were positioned every 2.5 cm. Total locomotor activity was defined as the number of interruptions of the beams registered in a computer during a 40 min period. Activity was measured over 8 intervals of 5 min immediately after i.p. injection of 20 mg/kg isatin or an isatin derivative in a group of 10 mice. The data were expressed as number of movements per minute, averaged over 40 min (Abel, 1995).

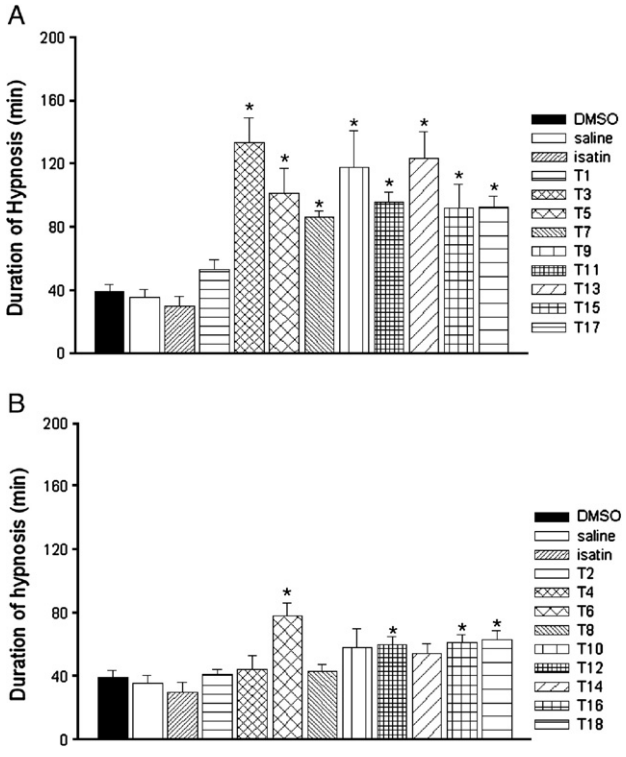


Fig. 1. Effects of new isatin ketals on the pentobarbital-induced sleep test. Dioxolane (A) and dioxane (B) derivatives were injected i.p. 30 min before intravenous injection of 25 mg/kg sodium pentobarbital. Data are expressed as the mean time between loss and recovery of the righting reflex. Error bars show± S.E.M for 10 animals. *$P<0.05$ vs. the control group (DMSO).

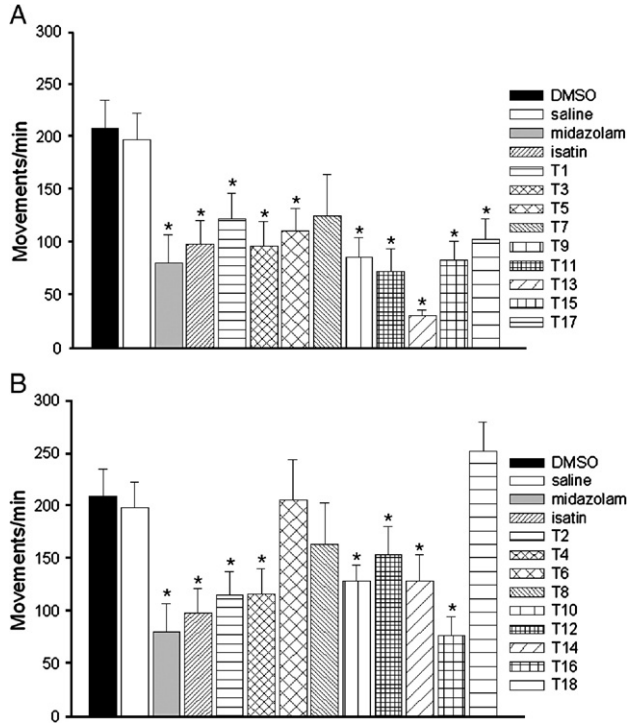


Fig. 2. Effects of midazolam and new isatin ketals spontaneous locomotor activity in mice. Dioxolane (A) and dioxane (B) derivatives were injected i.p. and motor activity in an open field was determined during the next 40 min. Data are expressed as the number of movements per minute (mean±S.E.M). *$P<0.05$ vs. the control group (DMSO).

2.4. Hypnotic and anesthetic activity in rats

Male Wistar rats (220–250 g) were used to investigate the hypnotic and anesthetic actions during continuous infusion of T3 because the surgical procedure was easier than in mice. Under ether anesthesia, the right carotid artery was dissected for arterial blood pressure (BP) measurement using a calibrated pressure transducer (Statham, P022). A catheter was placed in the jugular vein for intravenous infusion of T3 derivative. A pair of electrodes was placed on the chest for recording the electrocardiogram (EKG). Both BP and EKG were recorded continuously on a polygraph (AstroMed Grass, mod. 7400). One hour after surgery, rats received an intravenous infusion (Harvard pump mod. 1100) of T3 during 15 min at dose of 5 mg/kg/min. The following parameters were evaluated: 1. time to hypnosis and anesthesia (latency); 2. recovery from hypnosis and anesthesia; 3. respiratory and heart rates; and 4. systolic and diastolic pressures. Hypnosis was defined by the loss of mobility and anesthesia by the loss of the pinprick reflex. We have had considerable past experience with these experiments and have never observed alterations in BP, heart rate (HR) or respiratory rate 1 h after surgery when comparing with these parameters recorded before surgery. For that reason, we considered that 1 h was enough time to complete recovery of anesthesia (comparing with control values). The surgical procedure normally takes 10 min at which the animals are exposed to the anesthetic. Although the period of exposure to the anesthetic was short, we have performed control experiments in which we recorded continuously the BP and HR during anesthetic exposure (10–30 min) and after its interruption (after 1–5 h). No significant alterations were observed in systolic and diastolic pressure and heart rate from 1 to 5 h after surgery.

2.5. Statistical analysis

All data were expressed as mean±S.E.M. For the sleep and locomotor activity measurements, differences between those receiving isatin derivatives and controls (receiving DMSO) were assessed using Kruskal–Wallis ANOVA (analysis of variance) after ranking. Post-testing was performed by Dunn's test. Comparison among different isatin derivatives was performed using Kruskal–Wallis ANOVA on ranks followed by Dunn's test. Differences were considered statistically significant when $P<0.05$.

3. Results

3.1. Effects on the pentobarbital-induced sleeping time

Possible hypnotic activity of the derivatives was investigated using the pentobarbital-induced sleeping time. Fig. 1A–B shows the hypnotic effect of nine dioxolane ketals (T1, T3, T5, T7, T9, T11, T13, and T15) and nine dioxane ketals (T2, T4, T6, T8, T10, T12, T14 and T16). Isatin and compound T1, an isatin five-member ketal with no halogen on the aromatic ring, did not interfere with the duration of hypnosis induced by intravenous injection of pentobarbital. The duration of the pentobarbital-induced sleep was 39.7±4.4, 30.2±6.6 and 53.5±6.5 min for vehicle (DMSO), isatin and compound T1, respectively (Fig. 1A). All isatin dioxolane ketals produced an increase in the pentobarbital-induced sleeping time. Prior treatment with compounds T3 and T13 (20 mg/kg) significantly increased the duration of hypnosis to 134.0±15.7 and 123.7±16.9 min when compared to the non-substituted dioxolane T1 ($P<0.05$) (Fig. 1A). Not all of the dioxane ketals produced a significant increase in the duration of pentobarbital-induced sleep. Among the dioxane ketals tested, only compounds T6 (78.7±8.0 min), T12 (60.7±4.5 min), T16 (61.9±4.6 min) and T18 (63.8±5.6 min) significantly increased the time of pentobarbital-induced sleep when compared to vehicle ($P<0.05$). Compound T6 was more effective than the non-substituted dioxane T2 (an isatin six-member ketal with no halogen on the aromatic ring) (Fig. 1B).

Table 3
Time to hypnosis and anesthesia (latency) and recovery in Wistar rats during intravenous infusion of T3

	Latency (min)	Recovery (min)
Hypnosis	8.0±1.4	39.1±7.3
Anesthesia	9.1±2.9	6.8±2.4

Data are presented as mean±S.E.M.

Table 4
Changes in blood pressure, heart and respiratory rates during intravenous infusion of T3

	Control	5 min	10 min	15 min
HR (bpm)	430.0±10.0	410.0±10.0	370.0±12.6*	365.0±5.0*
RR (ipm)	87.0±4.0	76.0±5.5*	70.0±2.5*	72.0±3.1*
SP (mmHg)	129.2±5.7	130.8±5.4	125.8±4.9	125.0±4.1
DP (mmHg)	93.3±5.6	92.5±6.6	85.8±6.5	83.3±6.1

HR, heart rate; RR, respiratory rate; SP, systolic pressure; DP, diastolic pressure.
* $P<0.05$ compared to control.

3.2. Effects on spontaneous locomotor activity

Sedative activity of the dioxolane ketals was investigated by recording spontaneous locomotor activity of mice in an open field. In this test, compounds with sedative effects produce a decrease in the number of movements in an open field, interpreted as a decrease in curiosity about the new environment (Prut and Belzung, 2003). Fig. 2A–B shows the effects of dioxolane and dioxane isatin ketals on locomotor activity. Intraperitoneal injection of vehicle (DMSO) did not alter the motor activity when compared to the injection of the same volume of saline. Locomotor activity expressed as movements/min was 198.3±24.7 and 209.1±26.2 for saline and DMSO, respectively (Fig. 2). The reference benzodiazepinic agent, midazolam (2 mg/kg), significantly decreased motor activity to 80.9±26.6 movements/min ($P<0.05$). A single i.p. administration of each ketal derivative (20 mg/kg) produced a reduction of motor activity similar to that caused by midazolam at 2 mg/kg (Fig. 2). Among the ketals, only compound T13, which decreased motor activity to 31.5 movements/min, had a significantly greater effect ($P<0.05$) than midazolam and the other ketal derivatives (Fig. 2). Among the dioxane isatin ketals, T2, T4, T12, T14 and T16 significantly reduced the number of movements on the open field to 115.8±21.9; 116.3±24.4; 153.9±26.9; 129.1±2.6 and 77.3±17.6 movements/min ($P<0.05$), respectively, when compared to control (DMSO) (Fig. 2B).

3.3. Hypnotic and anesthetic activities in rats

Because 5′-chlorospiro-[1,3-dioxolane-2,3′-indolin]-2′-one (T3) produced a pronounced increase in pentobarbital-induced sleeping time and also, had a sedative effect, it was further evaluated as a hypnotic/anesthetic agent in rats. In addition to the reasons mentioned above, further evaluation was only performed with T3 because of the availability of great amount of this derivative. Continuous intravenous infusion in rats required a considerable amount of the derivative which was possible with T3.

Hypnosis and anesthesia were observed when rats received an intravenous infusion of T3. Table 3 shows the latency of hypnosis and anesthesia induced by T3. The times to reach complete recovery from hypnosis and anesthesia were 39.1±7.3 and 6.8±2.4 min, respectively. As shown in Table 4, no marked changes in hemodynamic parameters were observed during infusion of T3 of 5.0 mg/kg/min. The control heart rate of 430.0±10.0 was significantly reduced to 370.0±12.6 beats/min after 10 min of infusion, but there were no alterations in systolic or diastolic pressure. These findings indicate that T3 may serve as a centrally acting anesthetic with no toxic effects on the cardiovascular system. T3 reduced the respiratory rate from 87.0±4.0 to 72.0±3.1 ipm after 15 min of treatment (Table 4).

4. Discussion

Many reports have demonstrated that isatin and its derivatives have multiple biological and pharmacological effects in different tissues. Isatin, precursor for the synthesis of the compounds studied in this work, and semicarbazones of isatin have different effects on the CNS, including anticonvulsant activity (Pandeya et al., 2002; Sridhar et al., 2002; Pandeya et al., 2005). In the present study, we investigated the effects of two isatin ketal groups (dioxolane and dioxane) on the CNS through the evaluation of sedative and hypnotic activities. Isatin and non-substituted ketals (T1 and T2) did not interfere with the pentobarbital-induced sleeping time, but substituted ketals prolonged the duration of the barbiturate effect. All substituted ketals of the dioxolane group significantly increased the duration of hypnosis induced by pentobarbital but in contrast, only three ketals from the dioxane group (T6, T16, T18) promoted a similar effect. Hypnotic and anticonvulsant activities induced by derivatives of isatin have been attributed to their interaction with the benzodiazepine receptor (Evanno et al., 1999). The new ketals of isatin described here may be acting on the gamma-aminobutyric acid ($GABA_A$) receptor to increase the barbituric effects, since the $GABA_A$ receptor has several ligand sites at which different molecules can bind.

The marked hypnotic activity of substituted ketals when compared to T1 and T2 suggests that halogens on the aromatic ring may be responsible for that effect. Recently, it was shown that the substitution of hydrogen for a halogen atom in a molecule leads to a change in conformation that can increase or abolish its pharmacological effects (Zhao and Meng, 2006). Comparing dioxolane and dioxane ketal groups, we observe that dioxolanes have greater activity than dioxanes. These results can be explained based on a previous report that describes CNS actions of compounds that contain substructures such as an oxindole, a dioxolane moiety, a cyclic amide moiety and a tetrahedral carbon (Rajopadhye and Popp, 1988).

The effect of isatin on locomotor activity is dose dependent. Isatin is anxiogenic at low doses (15–20 mg/kg) and sedative at higher doses (50 mg/kg or above). The MAO inhibition cannot explain the behavioral effects of isatin because these effects were observed at doses which do not inhibit MAO A, and because MAO B inhibitors are neither anxiogenic nor sedative (Medvedev et al., 1996).

Similar to high doses of isatin (80 mg/kg, Abel, 1995), these new derivatives produced sedation, which was observed by a decrease spontaneous locomotor activity of mice. An open field generates anxiety behavior by two factors: individual factors (the animal is separated from its social group) and agoraphobia (as the arena is very large relative to the animal's natural environment). It is observed in rodents that live in social groups

and in small tunnels. On the open field, an increase in the number of movements reflects less fear, an anxiolytic effect, whereas a decrease reflects sedation or fear and anxiety (Prut and Belzung, 2003). In our study, animals showed a sedated behavior and slept during the experiment.

During intravenous infusion of T3 no alterations in systolic or diastolic pressures were observed, and respiratory depression was not as intense as that produced by other clinically used intravenous anesthetics such as propofol (2,6 di-isopropyl phenol) (Langley and Heel, 1998) and etomidate (D-ethyl-1(α-methyl-benzil)imidazol-5carboxilate). Propofol (Propovan®) was tested as a reference agent and we investigated its effects in the CNS, cardiovascular and respiratory system.

The derivatives tested may be interacting with *N*-methyl-D-aspartate (NMDA) receptors, since some isatin derivatives have been shown to bind to the glycine binding site of these receptors, inhibiting NMDA-induced-seizures in mice (Di Fabio et al., 1997). Ketamine is another intravenous anesthetic which induces sedation, hypnosis and analgesia by inhibiting NMDA receptors (Orser et al., 1997).

Although a recent study reported that isatin triggers cell death, it was observed only at high concentrations in the brain (200–400 μM). Bhattacharya et al. (1991) demonstrated that intraperitoneal injection of 100 mg/kg in rats achieves a concentration of 120 μM in the brain. This dose was 5-fold greater than that used in our work (20 mg/kg), so that brain concentrations were probably less than that found to cause cell death.

In conclusion, our results suggest that the new dioxolane ketal isatin derivatives have sedative/hypnotic properties. New isatin derivatives may have beneficial effects on the sleep disorders and could also be an alternative choice for pre-anesthesia or maintenance of anesthesia. Further investigations are necessary to elucidate the mechanisms of sedative, hypnotic and anesthetic actions of these new compounds, but some evidence suggests that they may act on CNS receptors interfering with GABA and/or NMDA systems.

Acknowledgements

This work was supported by CAPES, CNPq, FAPERJ and FUJB and fellowships CAPES, CNPq/UFRJ (LBP, DG, TCFM). We thank Instituto de Quimica for providing the derivatives. We also thank Dr. Thomas Nelson and Dr. Martha Sorenson for their positive critical analysis of the manuscript.

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