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New functionalized aminofurazans as potential antimitotic agents in the sea urchin embryo assay

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Experimental

Melting points were determined on a Gallenkamp melting point apparatus and they are not corrected. Infrared spectra were determined in KBr pellets on a Perkin-Elmer Model 577 spectrometer. Mass-spectra were recorded on a Varian MAT-311A instrument. ^{1}H, ^{13}C, and ^{14}N NMR spectra were recorded on a Bruker AM-300 instrument at 300.13, 75.47, and 21.68 MHz respectively. The chemical shift values (δ) are expressed relative to the chemical shift of the solvent-*d* or to external standard without correction (nitromethane, ^{14}N). Analytical TLC was conducted on precoated silica gel plates (Silufol UV_{254}). The plates were visualized under UV after development followed by spraying with DPA reagent (5% diphenylamine in hexane). Gas-liquid chromatography was performed on a Biochrom-5 instrument with a SE-54 capillary column (0.2 mm × 20 m).

Compounds **1**[24,25] and **2**[18,19] have been prepared as described in the literature.

4-Aminofurazan-3-carbox-N-(p-methoxyphenyl)amidoxime **3**: A solution of 4-aminofurazan-3-carbohydroxymoyl chloride **2** [18,19] (8.1 g, 50 mmol) in EtOH (100 ml) was added dropwise to a solution of *p*-anizidine (12 g, 97 mmol) and NEt_3 (6 g, 60 mmol) in *i*PrOH (50 ml) at 0 °C over 10 min. The mixture was stirred at room temperature for 3 h, the solvent was removed *in vacuo* and the residue was treated with 80 ml of water. The precipitate was filtered and washed with H_2O (2×50 ml) followed by benzene (30 ml). The solid residue (14 g, 86 mmol) was further

recrystallized to give **3** as a grey solid (13.3 g, 61.8%); mp 197–198 °C (from benzene/*i*PrOH). ^{1}H NMR (DMSO-d_6) δ 3.69 (s, 3H, MeO), 6.18 (s, 2H, NH_2), 6.80 (s, 4H, Ar), 8.4 (s, 1H, NH), 11.02 (s, 1H, NOH). ^{13}C NMR (DMSO-d_6) δ 55.1, 113.6, 123.7, 133.2, 140.3, 140.7, 155.3, 155.4. IR (KBr, cm^{-1}) 3472, 3372, 3272, 2964, 2840, 1652, 1612, 1568, 1536, 1516, 1440, 1400, 1304, 1252, 1156, 1108, 1044, 952. Calc. for $C_{10}H_{11}N_5O_3$ (%): C, 48.19; H, 4.45; N, 28.10. Found (%): C, 48.23; H, 4.51; N, 28.05.

4-Aminofurazan-3-carbox-N-(p-methoxybenzyl)amidoxime **4**. A solution of 4-aminofurazan-3-carbohydroxymoyl chloride **2** [18,19] (8.1 g, 50 mmol) in EtOH (100 ml) was added dropwise to a solution of *p*-methoxybenzylamine (7 g, 51 mmol) and NEt_3 (6 g, 60 mmol) in *i*PrOH (50 ml) at 0 °C over 10 min. The mixture was stirred at room temperature for 3 h, the solvent was removed *in vacuo* and the residue was treated with 80 ml of water. The precipitate was filtered and washed with H_2O (2×50 ml) followed by benzene (30 ml). Recrystallization from benzene afforded 7.3 g (58.7%) of the product as a light-brown solid: mp 140–142 °C. ^{1}H NMR (DMSO-d_6) δ 3.69 (s, 3H, MeO), 4.54 (d, J = 7.1 Hz, 2H, CH_2), 6.26 (s, 2H, NH_2), 6.83 (m, 3H), 7.12 (d, J = 8.4 Hz, 2H), 10.79 (s, 1H, NOH). ^{13}C NMR (DMSO-d_6) δ 45.8, 55.0, 113.7, 128.1, 132.8, 139.7, 144.8, 155.3, 158.2; MS (EI) m/z 263 (M^+). Found (%): C, 50.27; H, 5.01; N, 26.54. Calc. for $C_{11}H_{13}N_5O_3$ (%): C, 50.19; H, 4.98; N, 26.60.

4-(p-Methoxyphenylamino)furazan-3-carboxamidoxime **5**: A solution of compound **3** (13.3 g, 53 mmol) and KOH (2.92 g, 53 mmol) in ethylene glycol was refluxed for 4 h. The reaction mixture was cooled, diluted with water (30 ml) and neutralized with hydrochloric acid. The residue was filtered off and washed with H_2O (100 ml) followed by benzene (15 ml). Recrystallization from benzene/*i*PrOH afforded **5** as a light-brown solid (10.84 g, 81.7%); mp 190–191 °C (from benzene/*i*PrOH); ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H, MeO), 6.39 (s, 2H, NH_2), 6.97 (d, 2H, J = 8.6 Hz, 2H, Ar), 7.38 (d, 2H, J = 8.6 Hz, 2H, Ar), 8.79 (s, 1H, NH), 10.71 (s, 1H, NOH); ^{13}C NMR (DMSO-d_6) δ 55.2, 114.5, 118.4, 132.5, 139.7, 143.9, 151.0, 154.5. Found (%): C, 48.22; H, 4.49; N, 28.03. Calc. for $C_{10}H_{11}N_5O_3$ (%): C, 48.19; H, 4.45; N, 28.10.

4-(p-Methoxybenzylamino)furazan-3-carboxamidoxime **6**. A solution of compound **4** (5.2 g, 20 mmol) and KOH (1.1 g, 20 mmol) in ethylene glycol was refluxed for 4 h. The reaction mixture

was cooled, diluted with water (30 ml) and neutralized with hydrochloric acid. The residue was filtered off and washed with H_2O (100 ml) followed by benzene (15 ml). Recrystallization from benzene/*i*PrOH afforded 4 g (78%) of product as a grey solid: mp 113–117 °C. ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 3H, MeO), 4. 36 (d, J = 6.0 Hz, 2H, CH_2), 6.22 (s, 2H, NH_2), 6.4 (bs, 1H, NH), 6.89 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 10.45 (s, 1H, NOH). ^{13}C NMR (DMSO-d_6) δ 47.1, 55.1, 113.9, 129.2, 130.3, 139.7, 144.1, 154.9, 158.7. MS (EI) m/z 263 (M^+), calcd for $C_{11}H_{13}N_5O_3$ 263.26. Found (%): C, 50.25; H, 5.00; N, 26.52. Calc. for $C_{11}H_{13}N_5O_3$ (%): C, 50.19; H, 4.98; N, 26.60.

4-Amino-3-(5-o-methoxybenzylamino-1,2,4-oxadiazol-3-yl)furazan **8a**. A solution of compound **7**[23] (0.54 g, 2 mmol) and *p*-methoxybenzylamine (0.82 g, 6 mmol) in anhydrous THF (10 ml) refluxed for 30 min. The solvent was removed *in vacuo*. The residue was washed with water (2×30 ml) and *i*PrOH (5 ml). Purification by crystallization (3:1 *i*PrOH/MeCN) afforded 0.54 g (93.7%) of the product as a colorless solid: mp 179–181 °C. ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H, MeO), 4.51 (d, J = 6.2 Hz, 2H, CH_2), 6.38 (s, 2H, NH_2), 6.96 (m, 2H), 7.38 (m, 2H), 9.24 (t, J = 6.2 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 42.0, 55.4, 110.7, 120.2, 125.2, 128.2, 128.9, 137.4, 155.4, 156.9, 159.0, 171.7. IR (KBr, cm^{-1}) 3456, 3316, 3212, 3180, 2980, 1676, 1640, 1600, 1560, 1508, 1496, 1420, 1360, 1340, 1256, 1184, 1120, 1076, 1032, 972, 904, 748. MS (EI) m/z 288 (M^+). Found (%): C, 50.11; H, 4.24; N, 29.09. Calc. for $C_{12}H_{12}N_6O_3$ (%): C, 50.00; H, 4.20; N, 29.15.

Compounds **8b-r** were synthesized following protocol described for the preparation of **8a**.

4-Amino-3-(5-m-methoxybenzylamino-1,2,4-oxadiazol-3-yl)furazan **8b:**
Colorless solid, mp 175.5–177 °C (9:1 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H, MeO), 4.54 (d, J = 6.0 Hz, 2H, CH_2), 6.38 (s, 2H, NH_2), 6.9 (m, 3H), 7.36 (t, J = 8.5 Hz, 1H), 9.4 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 46.5, 55.0, 112.8, 113.0, 119.4, 129.6, 137.4, 139.5, 155.4, 159.0, 159.4, 171.7. IR (KBr, cm^{-1}) 3444, 3312, 3180, 3072, 1684, 1636, 1596, 1560, 1508, 1488, 1432, 1416, 1356, 1264, 1188, 1092, 1052, 972, 892, 880. MS (EI) m/z 288 (M^+). Found (%): C, 50.07; N, 29.14; H, 4.25. Calc. for $C_{12}H_{12}N_6O_3$ (%): C, 50.00; H, 4.20; N, 29.15.

4-Amino-3-(5-p-methoxybenzylamino-1,2,4-oxadiazol-3-yl)furazan **8c:**

Colorless solid, mp 217–219 °C (3:1 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 3H, MeO), 4.49 (d, *J* = 6.2 Hz, 2H, CH_2), 6.39 (s, 2H, NH_2), 6.91 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 9.36 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 46.1, 55.1, 113.9, 128.8, 129.8, 137.4, 155.4, 158.6, 159.0, 171.6. IR (KBr, cm^{-1}) 3448, 3324, 3076, 1676, 1632, 1560, 1516, 1500, 1408, 1364, 1304, 1252, 1240, 1184, 1084, 1040, 968. MS (EI) m/z 288 (M^+). Found (%): C, 50.06; H, 4.28; N, 29.11. Calc. for $C_{12}H_{12}N_6O_3$(%): C, 50.00; H, 4.20; N, 29.15.

4-Amino-3-{5-[2-(p-methoxyphenyl)ethylamino]-1,2,4-oxadiazol-3-yl}furazan **8d:**

Colorless solid, mp 231–233 °C (4:3:2 *i*PrOH/MeCN/$CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 2.82 (t, *J* = 7.1 Hz, 2H, $\underline{CH_2}$Ar), 3.50 (q, *J* = 6.6 Hz, 2H, $\underline{CH_2}$NH), 3.70 (s, 3H, MeO), 6.36 (s, 2H, NH_2), 6.84 (d, *J* = 8.5 Hz, 2H), 7.16 (d, *J* = 8.5 Hz, 2H), 8.94 (t, *J* = 6.6 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 33.9, 44.7, 55.0, 113.8, 129.7, 130.5, 137.4, 155.4, 157.9, 159.0, 171.5; IR (KBr, cm^{-1}) 3444, 3332, 3184, 3072, 2936, 1676, 1644, 1564, 1516, 1420, 1368, 1244, 1176, 1092, 1036, 972. MS (EI) m/z 302 (M^+). Found (%): C, 51.71; H, 4.69; N, 27.77. Calc. for $C_{13}H_{14}N_6O_3$ (%): C, 51.65; H, 4.67; N, 27.80.

4-Amino-3-[5-(3,4-dimethoxy)benzylamino-1,2,4-oxadiazol-3-yl]furazan **8e:**

Colorless solid: mp 209–213 °C (4:3 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 3H, MeO), 3.75 (s, 3H, MeO), 4.48 (d, *J* = 5.4 Hz, 2H, CH_2), 6.4 (s, 2H, NH_2), 6.89 (m, 2H), 7.00 (s, 1H), 9.34 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 46.5, 55.4, 55.5, 111.4, 111.7, 119.6, 130.2, 137.4, 148.2, 148.7, 155.4, 159.0, 171.6. IR (KBr, cm^{-1}) 3440, 3332, 3180, 2936, 1684, 1636, 1596, 1556, 1524, 1504, 1464, 1448, 1360, 1264, 1240, 1180, 1160, 1144, 1084, 1024, 972, 960. MS (EI) m/z 318 (M^+). Found (%): C, 49.11; H, 4.47; N, 26.34. Calc. for $C_{13}H_{14}N_6O_4$(%): C, 49.06; H, 4.43; N, 26.40.

4-Amino-3-[5-piperonylamino-1,2,4-oxadiazol-3-yl]furazan **8f:**

Colorless solid: mp 247–248 °C (1:5 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 4.45 (d, *J* = 6.0 Hz, 2H, CH_2), 5.99 (s, 2H, OCH_2O), 6.38 (s, 2H, NH_2), 6.85 (m, 2H), 6.93 (s, 1H), 9.34 (t, *J* = 6.0 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 46.4, 101.0, 108.1, 108.2, 120.8, 131.7, 137.4, 146.6, 147.4, 155.4, 159.0, 171.6. IR (KBr, cm^{-1}) 3464, 3336, 3212, 3180, 1672, 1648, 1600, 1560,

1504, 1488, 1448, 1372, 1352, 1256, 1184, 1044, 972. MS (EI) m/z 302 (M^+). Found (%): C, 47.74; H, 3.38; N, 27.73.Calc. for $C_{12}H_{10}N_6O_4$ (%): C, 47.69; H, 3.33 N, 27.80.

4-Amino-3-(5-o-chlorobenzylamino-1,2,4-oxadiazol-3-yl)furazan **8g:**

Light-cream solid, mp 220–222 °C (3:1 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 4.63 (d, J = 6.0 Hz, 2H, CH_2), 6.35 (s, 2H, NH_2), 7.37 (m, 2H), 7.48 (m, 2H), 9.44 (t, J = 6.0 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 44.5, 127.5, 129.4, 132.4, 134.8, 137.3, 155.4, 159.0, 171.7. MS (EI) m/z 291, 293 (M^+). Found (%): C, 45.21; H, 3.13; N, 28.65. Calc. for $C_{11}H_9Cl_1N_6O_2$ (292.68) (%): C, 45.14; H, 3.10; N, 28.71.

4-Amino-3-(5-p-fluorobenzylamino-1,2,4-oxadiazol-3-yl)furazan **8h:**

Colorless solid: mp 241–242 °C (1:1 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 4.54 (d, J = 6.2 Hz, 2H, CH_2), 6.37 (s, 2H, NH_2), 7.18 (bt, J = 9.0 Hz, 2H), 7.42 (dd, J = 5.5, 8.8 Hz, 2H), 9.4 (bt, J = 6.2 Hz, 1H, NH). ^{19}F NMR (DMSO-d_6) δ -114.7. ^{13}C NMR (DMSO-d_6) δ 45.8, 115.28 (d, J_{CF} = 21.3 Hz), 129.5 (d, J_{CF} = 8.3 Hz), 134.13 (d, J_{CF} = 2.4 Hz), 137.4, 155.4, 159.0, 161.5 (d, J_{CF} = 241.5 Hz), 171.7. IR (KBr, cm^{-1}) 3452, 3316, 3180, 1680, 1636, 1596, 1564, 1512, 1416, 1360, 1344, 1220, 1188, 1092, 972. Found (%): C, 47.88; H, 3.23; N, 30.45. Calc. for $C_{11}H_9F_1N_6O_2$ (%): C, 47.83; H, 3.28; N, 30.42.

4-Amino-3-[5-m-(trifluoromethyl)benzylamino-1,2,4-oxadiazol-3-yl]furazan **8i:**

Colorless solid, mp 199–201 °C ($CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 4.67 (d, J = 6.2 Hz, 2H, CH_2), 6.48 (s, 2H, NH_2), 7.7 (m, 4H), 9.48 (bt, J = 6.2 Hz, 1H, NH). ^{19}F NMR (DMSO-d_6) δ -60.7; ^{13}C NMR (DMSO-d_6) δ 46.0, 121.4, 124.0, 124.1, 128.9, 129.6, 131.5, 137.3, 139.5, 155.4, 159.0, 171.7. IR (KBr, cm^{-1}) 3456, 3336, 3220, 3072, 1664, 1648, 1604, 1560, 1504, 1420, 1360, 1328, 1200, 1172, 1116, 1076, 972, 920, 900, 872. MS (EI) m/z 326 (M^+). Found (%): C, 44.09; H, 2.82; N, 25.66. Calc. for $C_{12}H_9F_3N_6O_2$ (%): C, 44.18; H, 2.78; N, 25.76.

4-Amino-3-(5-o-picolylamino-1,2,4-oxadiazol-3-yl)furazan **8j:**

Colorless solid, mp 210–211 °C (1:1 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 4.67 (d, J = 6.1 Hz, 2H, CH_2), 6.33 (s, 2H, NH_2), 7.29 (dd, J = 6.8, 4.8 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.79 (dd, J = 8.0,6.8 Hz, 1H), 8.54 (d, J = 4.8 Hz,, 1H), 9.42 (t, J = 6.1 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6)

δ 48.1, 121.2, 122.5, 136.8, 137.2, 149.0, 155.2, 156.8, 158.9, 171.8. IR (KBr, cm^{-1}) 3448, 3268, 3160, 3060, 1700, 1632, 1556, 1496, 1484, 1430, 1412, 1372, 1188, 1104, 1056, 1020, 1004, 996, 992, 976. MS (EI) m/z 259 (M^+). Calc. for $C_{10}H_9N_7O_2$ (%): C, 47.83; H, 3.28; N, 30.42. Found (%): C, 47.91; H, 3.35 N, 30.38.

4-Amino-3-(5-m-picolylamino-1,2,4-oxadiazol-3-yl)furazan **8k:**

Colorless solid, mp 218–220 °C (3:2 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 4.61 (d, *J* = 6.1 Hz, 2H, CH_2), 6.38 (s, 2H, NH_2), 7.38 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 8.48 (d, *J* = 4.8 Hz, 1H), 8.62 (s, 1H), 9.47 (t, *J* = 6.1 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 44.2, 123.6, 133.5, 135.3, 137.3, 148.7, 148.9, 155.4, 159.0, 171.7. IR (KBr, cm^{-1}) 3452, 3064, 1680, 1636, 1560, 1508, 1480, 1432, 1420, 1364, 1188, 1100, 1008, 972. MS (EI) m/z 259 (M^+). Found (%): C, 47.89; H, 3.33; N, 30.37. Calc. for $C_{10}H_9N_7O_2$ (%): C, 47.83; H, 3.28; N, 30.42.

4-Amino-3-(5-p-picolylamino-1,2,4-oxadiazol-3-yl)furazan **8l:**

Light-cream solid: mp 224–225.5 °C (3:2 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 4.63 (d, *J* = 5.9 Hz, 2H, CH_2), 6.32 (s, 2H, NH_2), 7.37 (d, *J* = 6.0 Hz, 2H), 8.53 (d, *J* = 6.0 Hz, 2H), 9.47 (t, *J* = 5.9 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 45.4, 122.1, 137.3, 147.0, 149.8, 155.4, 159.1, 171.8. IR (KBr, cm^{-1}) 3452, 3316, 3208, 1676, 1636, 1600, 1564, 1504, 1416, 1368, 1324, 1228, 1184, 1092, 972. MS (EI) m/z 259 (M^+). Found (%): C, 47.88; H, 3.23; N, 30.45. Calc. for $C_{10}H_9N_7O_2$ (%): C, 47.83; H, 3.28; N, 30.42.

4-Amino-3-[5-(tetrahydrofur-2-yl)methylamino-1,2,4-oxadiazol-3-yl]furazan **8m:**

Colorless solid, mp 169–170 °C (*i*PrOH). ^{1}H NMR (DMSO-d_6) δ 1.5-2.0 (m, 4H), 3.38 (m, 2H), 3.82 (m, 2H), 4.01 (m, 1H), 6.37 (s, 2H, NH_2), 9.02 (t, *J* = 5.9 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 25.2, 28.4, 47.2, 67.3, 76.6, 137.4, 155.4, 159.0, 171.9. IR (KBr, cm^{-1}) 3444, 3324, 3220, 3184, 3064, 2976, 2948, 2876, 1668, 1636, 1612, 1560, 1500, 1408, 1376, 1344, 1184, 1112, 1072, 976. MS (EI) m/z 252 (M^+). Found (%): C, 42.95; H, 4.84; N, 33.24. Calc. for $C_9H_{12}N_6O_3$ (%): C, 42.86; H, 4.80; N, 33.32.

4-Amino-3-[5-(thyen-2-yl)methylamino-1,2,4-oxadiazol-3-yl]furazan **8n:**

Light-cream solid, mp 190–192 °C (*i*PrOH). ^{1}H NMR (DMSO-d_6) δ 4.73 (d, *J* = 6.0 Hz, 2H, CH_2), 6.35 (s, 2H, NH_2), 6.97 (dd, *J* = 5.1, 3.3 Hz, 1H), 7.12 (d, *J* = 3.3 Hz, 1H), 7.44 (d, *J* = 5.1 Hz, 1H), 9.45 (t, *J* = 6.0 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 41.6, 125.8, 126.4, 127.0, 137.3, 140.5, 155.5, 159.0, 171.5. IR (KBr, cm^{-1}) 3448, 3312, 3208, 1676, 1636, 1592, 1564, 1500, 1432, 1412, 1368, 1332, 1184, 1160, 1084, 1072, 1024, 968. MS (EI) m/z 264 (M^+). Found (%): C, 41.00; H, 3.09; N, 31.74. Calc. for $C_9H_8N_6O_2S_1$ (%): C, 40.91; H, 3.05; N, 31.80.

4-Amino-3-[5-(1-methylpyrazol-4-yl)methylamino-1,2,4-oxadiazol-3-yl]furazan **8o:**

Colorless solid, mp 202–203 °C (3:1 *i*PrOH/MeCN). ^{1}H NMR (DMSO-d_6) δ 3.63 (s, 3H, Me), 4.23 (d, *J* = 5.9 Hz, 2H, CH_2), 6.17 (s, 2H, NH_2), 7.25(s, 1H), 7.52(s, 1H), 8.98 (t, *J* = 5.9 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 37.4, 38.6, 117.4, 129.7, 137.4, 138.0, 155.4, 159.0, 171.5. IR (KBr, cm^{-1}) 3468, 3320, 3252, 3212, 3176, 3068, 2944, 1684, 1640, 1596, 1564, 1504, 1408, 1368, 1340, 1328, 1192, 1184, 1160, 1076, 1016, 988, 968, 948. MS (EI) m/z 262 (M^+). Found (%): C, 41.31; H, 3.80; N, 42.65. Calc. for $C_9H_{10}N_8O_2$ (%): C, 41.22; H, 3.84; N, 42.73.

4-Amino-3-[5-(1-ethylpyrazol-4-yl)methylamino-1,2,4-oxadiazol-3-yl]furazan **8p:**

Colorless solid, mp 165–166.5 °C (*i*PrOH). ^{1}H NMR (DMSO-d_6) δ 1.16 (t, *J* = 6.6 Hz, 3H, Me), 3.91 (d, *J* = 6.6 Hz, 2H, CH_2), 4.24 (d, *J* = 5.8 Hz, 2H, CH_2), 6.22 (s, 2H, NH_2), 7.27(s, 1H), 7.57(s, 1H), 9.02 (t, *J* = 5.8 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 15.5, 37.5, 46.1, 117.1, 128.2, 137.4, 137.9, 155.4, 159.0, 171.5. IR (KBr, cm^{-1}) 3328, 3212, 3180, 3096, 1672, 1636, 1600, 1560, 1504, 1408, 1372, 1344, 1320, 1180, 1168, 1080, 1016, 1000, 984, 968. MS (EI) m/z 276 (M^+). Found (%): C, 43.53; H, 4.43; N, 40.49. Calc. for $C_{10}H_{12}N_8O_2$ (%): C, 43.48; H, 4.38; N, 40.56.

4-Amino-3-[5-(3-phenylpyrazol-4-yl)methylamino-1,2,4-oxadiazol-3-yl]furazan **8q:**

Light-brown solid, mp 210–212 °C (*i*PrOH). ^{1}H NMR (DMSO-d_6) δ 4.54 (d, *J* = 6.0 Hz, 2H, CH_2), 6.61 (s, 2H, NH_2), 7.49 (d, *J* = 7.8 Hz, 2H), 7.74(m,3H), 10.03 (t, *J* = 6.0 Hz, 1H, NH), 12.95(bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 34.3, 114.0, 128.7, 129.1, 132.3, 136.3, 152.3, 155.7, 159.0, 169.6. MS (EI) m/z 324 (M^+). Found (%): C, 51.92; H, 3.81; N, 34.44. Calc. for $C_{14}H_{12}N_8O_2$ (%): C, 51.85; H, 3.73; N, 34.55.

4-Amino-3-[5-(2-phenyl-1,2,3-triazol-4-yl)methylamino-1,2,4-oxadiazol-3-yl]furazan **8r:**
Light-brown solid, mp 198–200 °C (*i*PrOH). ^{1}H NMR (DMSO-d_6) δ 4.74 (d, J = 5.9 Hz, 2H, CH_2), 6.39 (s, 2H, NH_2), 7.39- 7.55 (m, 3H), 7.96 (d, J = 7.8 Hz, 2H),, 8.09 (s,1H), 9.51 (t, J = 5.9 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 38.2, 118.3, 127.7, 129.7, 135.2, 139.1, 146.7, 155.4, 159.0, 171.6. MS (EI) m/z 325 (M^+). Found (%): C, 48.11; H, 3.37; N, 38.69. Calc. for $C_{13}H_{11}N_9O_2$ (%): C, 48.00; H, 3.41; N, 38.75.

3-(p-Methoxyphenylamino)-4-(5-trichloromethyl-1,2,4-oxadiazol-3-yl)furazan **9:**
A mixture of compound **5** (5 g, 20 mmol) and trichloroacetyl chloride (2.73 g, 15 mmol) in butyl acetate (25 ml) was refluxed for 4 h. The solvent was removed *in vacuo*. The residue was washed with water (70 ml), saturated solution of $NaHCO_3$ (2×80 ml), water (70 ml) and hexanes (2×30 ml). Recrystallization furnished a light-yellow solid (5.05 g, 66.6%); mp 128–129 °C (from hexane/$CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, MeO), 6.96 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 8.50 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 55.2, 82.1, 114.3, 119.7, 132.5, 136.9, 152.4, 154.9, 159.2, 174.4. Found (%): C, 38.32; H, 2.16; N, 18.63. Calc. for $C_{12}Cl_3H_8N_5O_3$ (%): C, 38.27; H, 2.14; N, 18.60.

3-(p-Methoxybenzylamino)-4-(5-trichloromethyl-1,2,4-oxadiazol-3-yl)furazan **10:**
A mixture of compound **6** (3 g, 11.4 mmol) and trichloroacetyl chloride (2.73 g, 15 mmol) in butyl acetate (25 ml) was refluxed for 4 h. The solvent was removed *in vacuo*. The residue was washed with water (70 ml), saturated solution of $NaHCO_3$ (2×80 ml), water (70 ml) and hexanes (2×30 ml). Recrystallization from hexanes/$CHCl_3$ afforded 3.14 g (70.6%) of the product as a light-cream plates: mp 131–132.5 °C. ^{1}H NMR ($CDCl_3$) δ 3.81 (s, 3H, MeO), 4.53 (d, J = 5.6 Hz, 2H, CH_2), 6.48 (bs, 1H, NH), 6.90 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 8.7 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 48.2, 55.3, 114.1, 129.1, 129.2, 135.1, 155.3, 159.3, 160.3, 175.4. IR (KBr, cm^{-1}) 3404, 2976, 2840, 1624, 1596, 1584, 1576, 1508, 1444, 1364, 1288, 1248, 1172, 1152, 1028, 988, 968, 940, 852, 820, 804, 764, 720. Found (%): C, 40.04; H, 2.60; N, 17.83. Calc. for $C_{13}Cl_3H_{10}N_5O_3$ (%): C, 39.97; H, 2.58; N, 17.93.

3-(p-Methoxybenzylamino)-4-(5-amino-1,2,4-oxadiazol-3-yl)furazan **11a:**
Anhydrous ammonia was bubbled through a suspension of **10** (0.39 g, 1 mmol) in MeOH (20 ml) for 5 min. The resulting yellow solution was kept at room temperature for 12 h, solvent was removed *in vacuo* and the residue was treated with water (30 ml). The precipitate was filtered off, washed with H_2O (20 ml) followed by Pr^iOH (15 ml) and recrystallization from *i*PrOH to afford 0.21 g (72.9%) of product as a light-yellow solid: mp 177–180 °C. ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 3H, MeO), 4.38 (d, J = 5.9 Hz, 2H, CH_2), 6.49 (t, J = 5.9 Hz, 1H, NH), 6.89 (d, J = 8.3 Hz, 2H), 7.31 (d, J = 8.3 Hz, 2H), 8.30 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ 46.9, 55.0, 113.7, 128.9, 130.2, 137.0, 155.3, 158.4, 158.9, 172.2. IR (KBr, cm^{-1}) 3404, 3356, 3180, 2936, 1684, 1620, 1576, 1516, 1488, 1460, 1368, 1304, 1248, 1180, 1112, 1036, 1024, 968. Found (%): C, 50.09; H, 4.24; N, 29.09. Calc. for $C_{12}H_{12}N_6O_3$ (%): C, 50.00; H, 4.20; N, 29.15.

3-(p-Methoxybenzylamino)-4-(5-p-methoxybenzylamino-1,2,4-oxadiazol-3-yl)furazan **11b**
A solution of **10** (0.39 g, 1 mmol) and *p*-methoxybenzylamine (0.41 g, 3 mmol) in anhydrous THF (10 ml) was stirred at room temperature for 12 h. Solvent was removed *in vacuo*, the residue was treated with water (30 ml) and *i*PrOH (1 ml). The precipitate was filtered off, washed with H_2O (50 ml) followed by MeOH (15 ml) and recrystallized from *i*PrOH/MeCN to furnish 0.31 g (76%) of product. ^{1}H NMR (DMSO-d_6) δ 3.54 (s, 6H, MeO), 4.22 (d, J = 5.5 Hz, 2H, CH_2), 4.31 (d, J = 5.6 Hz, 2H, CH_2), 6.47 (bs, 1H, NH), 6.72 (m, 4H), 7.16 (m, 4H), 9.23 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 46.1, 46.9, 55.0, 113.7, 113.9, 128.89, 128.94, 129.8, 130.3, 137.0, 155.5, 158.5, 158.6, 158.8, 171.6 (two carbons missing due to overlap). Found (%): C, 58.86; H, 4.90; 20.51. Calc. for $C_{20}H_{20}N_6O_4$ (%): C, 58.82; H, 4.94; N, 20.58.

Compounds **11c-h** were synthesized following the protocol described for the preparation of **11b**.

3-(p-Methoxyphenylamino)-4-(5-p-methoxybenzylamino-1,2,4-oxadiazol-3-yl)furazan **11c:** prepared from **9** (0.5 g, 1.33 mmol), a light-yellow solid (0.44 g, 84%); mp 212–214 °C (from *i*PrOH). ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 6H, MeO), 4.52 (bs, 2H, CH_2), 6.93 (m, 4H), 7.35 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.6 Hz, 2H), 8.32 (s, 1H, NH), 8.41 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 46.1, 55.0, 55.2, 113.9, 114.3, 119.2, 128.8, 129.7, 132.5, 137.5, 152.2, 154.8, 158.4, 158.7,

171.6. IR (KBr, cm^{-1}) 3356, 3172, 3068, 2956, 1672, 1628, 1608, 1576, 1512, 1440, 1348, 1280, 1248, 1176, 1112, 1080, 1028, 968. Found (%): C, 57.90; H, 4.67; N, 21.25. Calc. for $C_{19}H_{18}N_6O_4$ (%): C, 57.86; H, 4.60; N, 21.31.

3-(p-Methoxyphenylamino)-4-[5-(3,4-dimethoxy)benzylamino-1,2,4-oxadiazol-3-yl]furazan **11d:** prepared from **9** (0.5 g, 1.33 mmol), a yellow solid (0.48 g, 85.7%); mp 215–217 °C (from *i*PrOH). ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 9H, MeO), 4.54 (bs, 2H, CH_2), 6.97 (m, 5H), 7.48 (d, J = 8.4 Hz, 2H), 8.34 (s, 1H, NH), 9.42 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 46.4, 55.2, 55.5, 55.6, 111.5, 111.8, 114.4, 119.3, 119.6, 130.1, 132.5, 137.5, 148.3, 148.8, 152.2, 154.8, 158.4, 171.6. IR (KBr, cm^{-1}) 3376, 3216, 2912, 2832, 1664, 1624, 1608, 1580, 1520, 1512, 1496, 1468, 1440, 1424, 1344, 1320, 1308, 1288, 1268, 1248, 1168, 1148, 1084, 1024, 996, 972. Calc. for $C_{20}H_{20}N_6O_5$ (%): C, 56.60; H, 4.75; N, 19.80. Found (%): C, 56.65; H, 4.81; N, 19.72.

3-(p-Methoxyphenylamino)-4-[5-piperonylamino-1,2,4-oxadiazol-3-yl]furazan **11e:**
prepared from **9** (0.5 g, 1.33 mmol), a light-yellow solid (0.43 g, 79.6%); mp 221–222 °C (from *i*PrOH). ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H, MeO), 4.49 (bs, 2H, CH_2), 5.99 (s, 2H, OCH_2O), 6.92 (m, 6H), 7.46 (d, J = 8.7 Hz, 2H), 8.33 (s, 1H, NH), 9.45 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 46.4, 55.2, 101.0, 108.0, 108.2, 114.4, 119.3, 120.8, 131.6, 132.5, 137.5, 146.6, 147.5, 152.2, 154.8, 158.4, 171.6. IR (KBr, cm^{-1}) 3356, 3204, 3172, 3072, 2900, 1672, 1604, 1576, 1508, 1492, 1464, 1444, 1348, 1280, 1248, 1104, 1076, 1036, 964. Found (%): C, 55.96; H, 4.01; N, 20.54. Calc. for $C_{19}H_{16}N_6O_5$ (%): C, 55.88; H, 3.95; N, 20.58.

3-(p-Methoxyphenylamino)-4-(5-p-fluorobenzylamino-1,2,4-oxadiazol-3-yl)furazan **11f**:
prepared from **9** (0.5 g, 1.33 mmol), a light-brown solid (0.41 g, 80.7%); mp 212–213 °C (from MeCN). ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, MeO), 4.58 (bs, 2H), 6.96 (d, J = 8.6 Hz, 2H), 7.19 (bt, J = 8.0 Hz, 2H), 7.49 (m, 4H), 8.32 (s, 1H, NH), 9.47(bs, 1H, NH). ^{19}F NMR (DMSO-d_6) δ -114.4. ^{13}C NMR (DMSO-d_6) δ 45.8, 55.2, 114.3, 115.2 (d, J_{CF} = 21.1 Hz), 119.2, 129.3 (d, J_{CF} = 8.1 Hz), 132.4, 134.1 (d, J_{CF} = 3.4 Hz), 137.4, 152.1, 154.7, 158.3, 161.5 (d, J_{CF} = 242.2 Hz), 171.5. Found (%): C, 55.93; H, 4.19; N, 26.80. Calc. for $C_{17}H_{15}N_7O_3$ (%): C, 55.89; H, 4.14; N, 26.84.

3-(p-Methoxyphenylamino)-4-(5-m-picolylamino-1,2,4-oxadiazol-3-yl)furazan **11g:**
prepared from **9** (0.5 g, 1.33 mmol), a light-cream solid (0.4 g, 82.5%); mp 206–207 °C (from *i*PrOH). ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H, MeO), 4.65 (bs, 2H, CH_2), 6.97 (d, *J* = 8.8 Hz, 2H), 7.41 (dd, *J* = 7.4, 4.6 Hz, 1H), 7.46 (d, *J* = 8.8 Hz, 1H), 7.83 (d, *J* = 7.4 Hz, 1H), 8.28 (s, 1H, NH), 8.53 (d, *J* = 4.6 Hz, 1H), 8.67 (s, 1H), 9.49 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 44.2, 55.2, 114.3, 119.3, 123.7, 132.5, 133.4, 135.3, 137.5, 148.8, 148.9, 152.2, 154.8, 158.4, 171.6. IR (KBr, cm^{-1}) 3368, 3236, 3164, 2956, 2892, 2840, 2776, 2724, 1672, 1604, 1576, 1512, 1480, 1432, 1424, 1344, 1288, 1256, 1168, 1116, 1096, 1032, 984, 964. Found (%): C, 55.97; H, 4.20; N, 26.78. Calc. for $C_{17}H_{15}N_7O_3$ (%): C, 55.89; H, 4.14; N, 26.84.

3-(p-Methoxyphenylamino)-4-(5-p-picolylamino-1,2,4-oxadiazol-3-yl)furazan **11h:**
prepared from **9** (0.5 g, 1.33 mmol), a light-yellow solid (0.37 g, 76.3%); mp 195–196 °C (from *i*PrOH). ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H, MeO), 4.67 (bs, 2H, CH_2), 6.96 (d, *J* = 8.8 Hz, 2H), 7.44 (m, 4H), 8.29 (s, 1H, NH), 8.58 (bs, 2H), 9.52 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 45.5, 55.2, 114.3, 119.3, 122.1, 132.5, 137.5, 146.8, 149.8, 152.2, 154.8, 158.4, 171.8. Found (%): C, 55.97; H, 4.20; N, 26.78. Calc. for $C_{17}H_{15}N_7O_3$ (%): C, 55.89; H, 4.14; N, 26.84.

3-Nitro-4-(p-picolylamino)furazan **13**. A solution of 3,4-dinitrofurazan **12**[29] (2.52 g, 16 mmol) in CH_2Cl_2 (45 ml) was added dropwise over 15 min to a stirred solution of 4-(aminomethyl)pyridine (1.84 g, 17 mmol) and NEt_3 (2 g, 20 mmol) in CH_2Cl_2 (60 ml) at –10°C. The reaction mixture was stirred for additional 30 min at this temperature, warmed to room temperature, stirred for 2 h and quenched with saturated $NaHCO_3$ (70 ml). Organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (2×50 ml). The combined organic extracts were washed with H_2O (2×50 ml), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by recrystallization from hexanes to give **13** as a solid in 94% yield; mp 132–133 °C. ^{1}H NMR (DMSO-d_6) δ 4.51 (d, *J* = 6.0 Hz, 2H), 7.38 (d, *J* = 5.2 Hz, 2H), 7.88 (t, *J* = 6.0 Hz, 1H), 8.52 (d, *J* = 5.2 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ 46.0, 122.1, 147.0, 149.4, 151.3, 153.0. Found (%): C, 43.50; H, 3.23; N, 31.59. Calc. for $C_8H_7N_5O_3$ (%): C, 43.44; H, 3.19; N, 31.66.

3-(p-Methoxyphenoxy)-4-(m-picolylamino)furazan **14:**

Compound **13** (1.11 g, 5 mmol) was added in three portions over 30 min to a slurry of 4-methoxyphenol (0.7 g, 5.2 mmol) and K_2CO_3 (1.38 g, 10 mmol) in DMSO (5 ml) at 80 °C. The mixture was stirred for 1.5 h at 100 °C, cooled down to room temperature, stirred for additional 2 h and quenched with saturated $NaHCO_3$ (10 ml). The precipitate was filtered, solid residue was washed with water and recrystallized from *i*PrOH to give **14** (1.11 g, 75%) as a grey solid; mp 153–155 °C. ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3H, MeO), 4.43 (d, J = 6.2 Hz, 2H), 7.01 (d, J = 8.2 Hz, 2H), 7.34 (m, 4H), 7.47 (t, J = 6.2 Hz, 1H), 8.52 (d, J = 6.0 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ 46.0, 55.4, 114.8, 120.2, 122.2, 147.4, 147.5, 149.4, 156.7, 156.8. IR (KBr, cm^{-1}) 3212, 3000, 2840, 1620, 1604, 1568, 1508, 1416, 1360, 1292, 1272, 1248, 1188, 1104, 1060, 1032, 996, 988. Found (%): C, 60.48; H, 4.75; N, 18.69. Calc. for $C_{15}H_{14}N_4O_3$ (%): C, 60.40; H, 4.73; N, 18.78.

Biological Methods

Sea urchin embryo assay[16]**:** Adult sea urchins *Paracentrotus lividus* were collected from Mediterranean Sea at Cyprus coast and kept in aerated seawater tank. Gametes were obtained by intracoelomic injection of 0.5 M KCl. Eggs were washed with filtered sea water and fertilized by adding drops of a diluted sperm. Embryos were cultured at room temperature under gentle agitation with a motor-driven plastic paddle (60 rpm) in filtered sea water. The embryos were observed with light microscope Biolam LOMO. Electronic images were obtained using a digital camera (Olympus Camedia C-760 Ultra Zoom with microscope adaptor Optica M). For compound treatment, 5 ml aliquots of embryo suspension were transferred to 6-well plates and incubated as a monolayer at a concentration up to 3000 embryos/ml. The antiproliferative activity was assessed by exposing fertilized eggs (10–20 min after fertilization, 45–55 min before the first mitotic cycle completion) to 2-fold decreasing concentrations of a compound. Cleavage alteration and arrest were clearly detected at 3–6 h after fertilization (Figure 2). The effects were quantitatively estimated as a threshold concentration resulting in cleavage alteration and embryo death before hatching or full mitotic arrest. For tubulin destabilizing activity, the compounds were tested on free-swimming blastulae just after hatching (9–12 h after fertilization), originated from the same embryo culture. Embryo spinning was observed after 0.5–20 h of treatment, depending on the nature and concentration of the compound. Both spinning and lack of forward movement were interpreted to be the result of the tubulin destabilizing activity of a molecule.