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Regioselective synthesis and evaluation of 3-alkylidene-1, 3-dihydroisobenzofurans as potential antidepressant agents

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Abstract. 3-Alkylidene-1,3-dihydroisobenzofurans exhibited moderate antidepressant activity as evaluated by forced swim and tail suspension test methods. Virtual screening was carried out by docking the designed compounds into the serotonin binding sites of arabinase protein to predict the analogue binding mode of the compounds to the SSRIs.

Keywords. Phthalans; regioselectivity; antidepressant; tail suspension test; forced swim test; molecular docking.

1. Introduction

Depression is a serious disorder with estimates of lifetime prevalence as high as 21% of the general population in some developed countries.[1] Treatment for this disease is possible with antidepressant medications and psychotherapy for certain patients.[2] Tricyclics, monoamine oxidase (MAO) inhibitors, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, norepinephrine-dopamine reuptake inhibitors, norepinephrine reuptake inhibitors, serotonin modulators and norepinephrine-serotonin modulators are the major antidepressant drug classes used for the treatment of depressive disorders.[3] Although antidepressants have been used in the clinic for several decades, most of them are inadequate in efficiency and have many adverse side effects.[4] Therefore, studies for discovering and developing new antidepressant drugs with greater effectiveness and lower adverse effects are still desirable.[5,6] The introduction of selective serotonin reuptake inhibitors (SSRIs) as second generation antidepressants was a breakthrough in the treatment of depression. The SSRIs are characterized by significantly reduced disadvantageous properties. These drugs produce notably fewer adverse drug reactions (ADRs) compared with older drugs such as tricyclic antidepressants (TCAs) or non-selective monoamine oxidase inhibitors (MAOIs). However, SSRIs also have several drawbacks including lower clinical effectiveness, ADRs and relatively long onset of action which led to the idea to develop newer, more efficacious and safer antidepressants.[7–9] In view of getting broad spectrum of activity with more potency, a series of 3- alkylidene-1,3-dihydroisobenzofurans have been synthesized and screened for their antidepressant activity. These series comprise the derivatized phthalan (1,3-dihydroisobenzofuran) pharmacophore that are structurally related to the standard antidepressant drug, citalopram (SSRIs). The thrust of efforts in the derivatization of such type of compounds focused mainly on the aryl moiety as well as on dihydrobenzofuran scaffold. In the present study, the substitution pattern on the aryl moiety and dihydroisobenzofuran pharmacophore was selected so as to confer a different electronic environment that would affect the lipophilicity and hence the affinity of the target molecules (figure 1).

The objective of forming these hybrids is an attempt to reach an active antidepressant agent with potential activity and selectivity towards serotonin (5-hydroxytryptamine). Moreover, drug-likeness and molecular docking were used to identify the structural features required for antidepressant properties of these new series of phthalan derivatives. The results of docking studies could support the postulation that the more active compounds may act on the same receptor target, where the animal study conformed the

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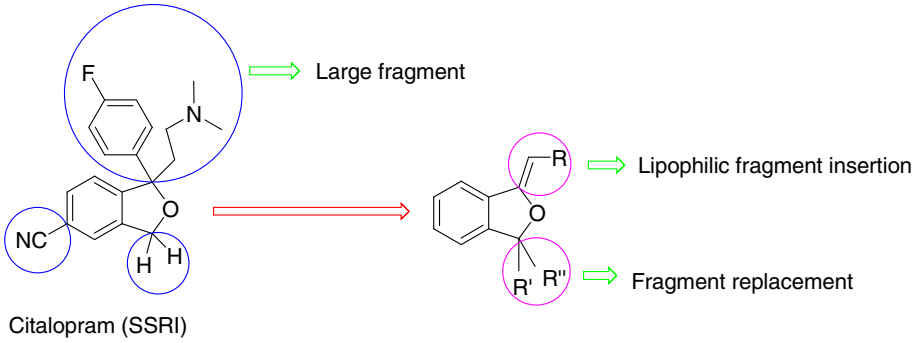


Figure 1. Reported and proposed antidepressant (SSRI) phthalan derivatives.

molecular design of the reported class of antidepressant agents.

2. Experimental

2.1 *Materials, method and instruments*

Melting points were determined in capillary tubes and are uncorrected. IR spectra were taken as neat for liquid compounds and as KBr pellets for solids on a Perkin Elmer Spectrum RXI FT-IR. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded as $CDCl_3$ solution for compounds **2a–i**, **2n** and **2o** and as benzene-d_6 solution for compounds **2j–m** on a JEOL instrument. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in parts per million. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Coupling constants (J) are given in hertz. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX 6000 ESI spectrometer. Elemental analysis data were recorded using Thermo Finnigan FLASH EA 1112 CHN analyzer. All the compounds gave C, H and N analysis within ±0.5% of the theoretical values. Column chromatography was performed on silica gel (100–200 mesh, SRL, India). Analytical TLC was performed on precoated plastic sheets of silica gel G/UV-254 of 0.2 mm thickness (Macherey-Nagel, Germany) using analytical grade solvents and visualizing with iodine spray (10% (w/w) I_2 in silica gel or UV light ($\lambda = 254$ and 365 nm). UV-Visible spectra were recorded on *THERMAL* spectrophotometer. 2-(Ethynyl)benzyl alcohols were prepared by the Sonagashira reaction of 2-iodobenzyl alcohol with the corresponding terminal alkyne. Copper(II) trifluoromethanesulphonate was purchased from Sigma-Aldrich Ltd.

2.2 *General procedure for the synthesis of compounds* (**2a–o**)

2.2a *Representative procedure for the synthesis of compounds* (**2a–i**, **2n** *and* **2o**): To a degassed solution of (5-(2-phenylethynyl)benzo[*d*][1,3]dioxol-6-yl) methanol (**1i**, 252 mg, 1.0 mmol) in dry toluene (1 mL) under N_2 was added $Cu(OTf)_2$ (18.08 mg, 0.018 mmol) and the reaction mixture was stirred at 110°C for 30 min. After completion of the reaction as indicated by TLC, the reaction mixture was concentrated under reduced pressure and was purified by column chromatography over silica gel (100–200 mesh) to afford the pure product of **2i** in 85% (214 mg) yield. The same procedure was applied for the synthesis of compounds **2a**–**i**, **2n** and **2o**.

2.2b *Representative procedure for the synthesis of (Z)-3-alkylidene-phthalans* (**2j–m**) *in benzene-*d_6: To an NMR tube equipped with a screw cap containing benzene-d_6 (0.50 mL) under argon, 2-(2-(2-methoxyphenyl)ethynyl)phenylmethanol (**1m**, 50.0 mg, 0.21 mmol) and $Cu(OTf)_2$ (3.79 mg, 0.0105 mmol) were added. The resulting mixture was heated at 65°C and monitored by NMR until the starting material had been consumed. At completion of the reaction, this reaction mixture was filtered through a small plug of silica gel to remove the catalyst. The crude product was purified by silica gel column chromatography (petroleum ether/AcOEt 9.5:0.5) to afford the pure product of (*Z*)-1-(2-methoxy-benzylidene)-1,3-dihydro-isobenzofuran 2m in 87% (43 mg) yield. The same procedure was applied for the synthesis of compounds **2j–m**.

2.2c *(Z)-1-Benzylidene-1,3-dihydro-isobenzofuran* (**2a**): Colourless solid; Mp 120–122°C; IR (KBr): 3040,

1612, 1456, 1273, 1064, 817, 750, 685 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 5.24 (s, 2H); 6.49 (s, 1H); 7.08–7.12 (m, 2H); 7.19 (t, 1H, $J = 7.6$ Hz); 7.27 (t, 1H, $J = 6.9$ Hz); 7.34–7.37 (m, 1H); 7.39–7.42 (m, 2H); 7.75–7.77 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 69.1, 101.3, 123.5, 123.9, 125.1, 126.5, 128.1, 128.3, 128.4, 128.9, 132.0, 134.3. 154.1. MS (ESI): m/z = 209 $[M+H]^+$. Anal. Calcd. for $C_{15}H_{12}O$: C, 86.51; H, 5.81%. Found: C, 86.75; H, 5.75%.

2.2d *(Z)-1-(4-Methoxy-benzylidene)-1,3-dihydro-isobenzofuran* (**2b**): Colourless solid; Mp 122–124°C; IR (KBr): 3033, 2843, 2331, 1678, 1616, 1550, 1446, 1384, 1271, 1062, 906, 811, 755, 679 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 3.83 (s, 3H); 5.20 (s, 2H); 6.35 (s, 1H); 6.90 (d, 2H, $J = 9.1$ Hz); 7.05 (t, 2H, $J = 7.6$ Hz); 7.14 (t, 1H, $J = 7.6$ Hz); 7.24 (t, 1H, $J = 7.6$ Hz); 7.67 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 55.4, 69.1, 99.6, 113.8, 123.2, 123.8, 126.1, 126.7, 126.9, 127.8, 128.2, 132.3, 154.1, 160.3. MS (ESI): m/z = 239 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{14}O_2$: C, 80.65; H, 5.92%. Found: C, 80.88; H, 5.85%.

2.2e *(Z)-1-(3-Methyl-benzylidene)-1,3-dihydro-isobenzofuran* (**2c**): Pink solid; Mp 112–114°C; IR (KBr): 3042, 2840, 1946, 1698, 1602, 1448, 1383, 1283, 1175, 1069, 881, 782, 691 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 2.42 (s, 3H); 5.24 (s, 2H); 6.48 (s, 1H); 7.08–7.12 (m, 2H); 7.17–7.19 (m, 2H); 7.27–7.32 (m, 2H); 7.56–7.59 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 21.6, 69.1, 101.2, 122.3, 123.5, 123.8, 125.8, 126.5, 128.1, 128.3 (2C), 129.7, 132.1, 134.3, 138.0, 154.3. MS (ESI): m/z = 223 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{14}O$: C, 86.45; H, 6.35%. Found: C, 86.60; H, 6.30%.

2.2f *(Z)-1-(4-Methyl-benzylidene)-1,3-dihydro-isobenzofuran* (**2d**): Colourless solid; Mp 96–98°C; IR (KBr): 2846, 1910, 1605, 1448, 1270, 1193, 1061, 931, 802 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 2.39 (s, 3H); 5.22 (s, 2H); 6.43 (s, 1H); 7.08 (t, 2H, $J = 8.4$ Hz); 7.17 (t, 1H, $J = 7.6$ Hz); 7.20 (d, 2H, $J = 7.6$ Hz); 7.24 (t, 1H, $J = 8.4$ Hz) 7.64 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 21.4, 69.0, 100.5, 123.4, 123.8, 125.1, 126.3, 128.0, 128.2, 129.1, 131.5, 132.2, 139.0. 154.3. MS (ESI): m/z = 223 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{14}O$: C, 86.45; H, 6.35%. Found: C, 86.25; H, 6.40%.

2.2g *(Z)-1-Benzylidene-3-butyl-1,3-dihydroisobenzofuran* (**2e**): Yellow oil; IR (neat) 2955, 2929, 2869, 1655, 1464, 1365, 1307, 1049, 809, 761, 693, 517 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 0.91 (t, 3H, $J = 7.3$ Hz); 1.29–1.58 (m, 4H); 1.66–1.81 (m, 4H), 1.90–2.01 (m, 1H); 5.60 (dd, 1H, $J = 7.8$, 3.8 Hz); 5.88 (s, 1H); 7.10–7.18 (m, 1H); 7.19–7.23 (m, 1H); 7.24–7.37 (m, 1H); 7.46–7.52 (m, 1H); 7.72–7.80 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 14.0, 22.6, 27.1, 35.7, 86.1, 95.7, 119.9, 121.2, 125.1, 127.7, 128.1, 128.3, 128.6, 135.0, 136.7, 142.8, 155.8. MS (ESI): m/z = 265 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{20}O$: C, 86.32; H, 7.63%. Found: C, 86.15; H, 7.70%.

2.2h *(Z)-1,1-Diethyl-3-pentylidcene-1,3-dihydroisobenzofuran* (**2f**): Yellow oil; IR (neat): 2964, 2930, 2874, 2853, 1679, 1463, 1354, 1296, 1050, 752 cm^{-1}. ^1H NMR (500 MHz, Benzene-d_6): δ_H 0.75 (t, $J = 7.3$ Hz); 0.95 (t, 3H, $J = 7.3$ Hz); 1.36–1.76 (m, 6H); 1.80–1.95 (m, 2H); 2.54 (q, 3H. $J = 7.2$ Hz) 4.94 (t, 1H, $J = 7.2$ Hz); 6.71–6.77 (m, 1H); 7.01–7.06 (m, 2H); 7.22–7.28 (m, 1H). MS (ESI): m/z = 245 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{24}O$: C, 83.55; H, 9.90%. Found: C, 83.35; H, 9.95%.

2.2i *(Z)-3-Benzylidene-1,1-diethyl-1,3-dihydroisobenzofuran* (**2g**): Colourless solid; Mp 62–64°C; IR (KBr): 2960, 1649, 1462, 1358, 1096, 1051, 939, 820, 761 cm^{-1}. ^1H NMR (500 MHz, Benzene-d_6): δ_H 0.68 (t, 6H, $J = 7.3$ Hz); 1.53-1.66 (m, 2H); 1.80-1.94 (m, 2H); 5.95 (s, 1H); 6.66–6.74 (m, 1H); 7.00–7.14 (m, 3H); 7.20–7.26 (m, 1H); 7.30–7.40 (m, 2H); 8.00-8.05 (m, 2H). ^{13}C NMR (125 MHz, Benzene-d_6): δ_C 7.9, 33.0, 94.7, 96.0, 120.1, 121.1, 125.4, 128.0, 128.4, 128.6, 136.5, 137.6, 144.5, 156.0. MS (ESI): m/z = 265 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{20}O$: C, 86.32; H, 7.63%. Found: C, 86.50; H, 7.55%.

2.2j *(Z)-1-(3H-Isobenzofuran-1-ylidene)-propan-2-one* (**2h**): Yellow oil; IR (neat): 1671, 1624, 1590, 706 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 2.46 (s, 3H); 5.58 (s, 2H); 5.83 (s, 1H); 7.35–7.71 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 31.4, 77.0, 98.2, 121.8, 129.2, 132.0, 135.6, 141.8, 167.4, 197.6. MS (ESI): m/z = 175 $[M+H]^+$. Anal. Calcd. for $C_{11}H_{10}O_2$: C, 75.84; H, 5.79%. Found: C, 76.05; H, 5.71%.

2.2k *(Z)-5-Benzylidene-5,7-dihydro-furo[3′,4′:4,5]benzo[1,2-d][1,3]dioxole* (**2i**): Pale yellow solid; Mp 132–134°C; IR (KBr): 2898, 1733, 1632, 1481, 1367, 1291, 1152, 1035, 860, 757 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 5.11 (s, 2H); 5.93 (s, 2H); 6.36 (s, 1H); 6.60 (d, 2H, $J = 10.7$ Hz); 7.32–7.38 (m, 3H); 7.69 (d, 2H,

J = 6.8 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 69.0, 101.0, 101.4, 104.6, 105.2, 121.5, 124.8, 126.3, 128.4, 128.7, 134.2, 146.1, 147.5, 152.7. MS (ESI): m/z = 253 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79%. Found: C, 75.99; H, 4.85%.

2.2l *1-Methylene-1,3-dihydroisobenzofuran* (**2j**): ^{1}H NMR (500 MHz, Benzene-d_6): δ_H 4.54 (s, 1H); 4.71 (s, 1H); 4.75 (s, 2H); 6.62–6.65 (m, 1H); 6.91–6.96 (m, 2H); 7.19–7.21 (m, 1H). ^{13}C NMR (125 MHz, Benzene-d_6): δ_C 73.4, 78.0, 120.6, 121.2, 128.2, 128.7, 134.1, 140.6, 162.5.

2.2m *1-Methyl-3-methylene-1,3-dihydroisobenzofuran* (**2k**): ^{1}H NMR (500 MHz, Benzene-d_6): δ_H 1.18 (d, 3H, J = 6.8 Hz,); 4.55 (s, 1H); 4.72 (s, 1H); 5.17 (q, 1H, J = 5.9 Hz); 6.69 (t, 1H, J = 4.4 Hz); 6.96–7.00 (m, 2H); 7.20 (t, 1H, J = 4.4 Hz). ^{13}C NMR (125 MHz, Benzene-d_6): δ_C 21.2, 77.9, 80.7, 120.7, 121.0, 128.2, 128.8, 134.0, 145.0, 161.3.

2.2n *(3-Methylene-1,3-dihydroisobenzofuran-1-yl) methanamine* (**2l**): ^{1}H NMR (500 MHz, Benzene-d_6): δ_H 0.60 (brs, 2H); 2.59–2.64 (m, 1H); 2.74–2.77 (m, 1H); 4.54 (s, 1H); 4.70 (s, 1H); 5.04 (t, 1H, J = 4.4 Hz); 6.75 (t, 1H, J = 4.9 Hz); 6.95 (t, 2H, J = 3.9 Hz); 7.21 (t, 1H, J = 5.9 Hz). ^{13}C NMR (125 MHz, Benzene-d_6): δ_C 46.9, 18.0, 86.3, 120.7, 121.5, 128.2, 128.8, 135.0, 141.7, 161.7.

2.2o *(Z)-1-(2-Methoxy-benzylidene)-1,3-dihydro-isobenzofuran* (**2m**): Brown paste; IR (neat): 3308, 2938, 2189, 1635, 1427, 1327, 1200, 1076, 878 cm^{-1}. ^{1}H NMR (500 MHz, Benzene-d_6): δ_H 3.25 (s, 3H); 4.92 (s, 2H); 6.48–7.05 (m, 8H); 7.92–7.93 (m, 1H). ^{13}C NMR (125 MHz, Benzene-d_6): 54.8, 68.5, 107.0, 111.2, 120.4, 123.7, 123.8, 126.2, 128.3, 128.5, 128.8, 129.4, 129.6, 132.6, 151.4, 157.6. MS (ESI): m/z = 239 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{14}O_2$: C, 80.65; H, 5.92%. Found: C, 80.85; H, 5.86%.

2.2p *(Z)-2-(3H-Isobenzofuran-1ylidenemethyl)-pyridine* (**2n**): Yellow oil; IR (neat): 3401, 3358, 1612, 1430, 1300, 1211, 1076, 691 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.52 (s, 2H); 6.22 (s, 1H); 7.00 (dd, 1H, J = 8.1 Hz, J = 1.0 Hz); 7.31–7.38 (m, 3H); 7.60–7.65 (m, 2H); 8.06 (d, 1H, J = 8.0 Hz) 8.50 (d, 1H, J = 4.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 76.8, 89.4, 121.0, 122.7, 126.6, 127.1, 127.6, 128.2, 137.0, 143.4, 148.9, 152.7, 154.9, 159.9. MS (ESI): m/z = 210 $[M+H]^+$. Anal. Calcd. for $C_{14}H_{11}ON$: C, 80.36; H, 5.30; N, 6.69%. Found: C, 80.55; H, 5.27; N, 6.79%.

2.2q *(Z)-2-(3H-Isobenzofuran-1ylidenemethyl)-pyrazine* (**2o**): Yellow oil; IR (neat): 3411, 3351, 1602, 1420, 1310, 1210, 1077, 699 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.60 (s, 2H); 6.15 (s, 1H); 7.36–7.50 (m, 3H); 7.65 (dd, 1H, J = 8.0, 1.0 Hz); 8.25 (s, 1H); 8.47 (s, 1H); 9.31 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 76.5, 85.9, 119.7, 126.6, 127.1, 127.6, 128.2, 143.4, 152.7, 157.6, 160.1, 166.9. MS (ESI): m/z = 211 $[M+H]^+$. Anal. Calcd. for $C_{13}H_{10}ON_2$: C, 74.27; H, 4.79; N, 13.32%. Found: C, 74.55; H, 4.72; N, 13.22%.

2.3 *Animals and drug dosage*

2.3a *Animals*: Inbred albino mice (Swiss strain) of adult gender weighing 20–25 g were used for the study. The mice were kept in clean polypropylene cages with free access to standard pellet diet and water (*ab libitum*), under standardized housing conditions (natural light-dark cycle, temperature 23 ± 1°C, relative humidity 55 ± 5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to 12 experimental groups of 5 mice each. Each mouse was used only once. All tests were performed between 08:00 and 16:00 h. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures listed below conformed to the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Ethics Committee. Mice equivalent doses in mg/kg body weight of clinical doses were calculated as mg/kg body weight with the help of standard tables.[10]

2.3b *Chemical and administration of compounds*: The synthesized compounds (10 mg/kg), and citalopram as a reference antidepressant drug (10 mg/kg) were dissolved in DMSO. The drugs were injected intraperitoneally (ip) in a standard volume of 0.5 mL/20 g body weight, 1 h prior to the test.

2.4 *Evaluation of antidepressant activity*

2.4a *Porsolt's behavioural despair test (Forced swim test)*: The method described by Porsolt *et al*. was used in our study.[11] Each animal was placed individually in a 5 L glass beakers, filled with water up to a height of 15 cm and were observed for duration of 6 min. The duration of immobility was recorded during the last

4 min of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. Water was changed after each test.

2.4b *Tail suspension test*: Antidepressant-like activity of the test compounds was screened using the tail suspension test similar to that described by Steru *et al.*[12] Mice were dangled from their tail using adhesive tape placed approximately 1 cm from the tip of the tail attached to a applicator stick and hung approximately 30 cm above a table. Mice were considered immobile only when they fail to make any struggling movements and hung passively. Immobility time for each animal was scored by stopwatch during the last 4 min of a 6 min test.[12a]

2.5 *Statistical analysis*

The obtained data were analysed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. The results are presented as mean ± Standard error of means (SEM). Differences between data sets were considered as significant when $P < 0.001$.

2.6 *Molecular docking methodology*

2.6a *Preparation of the analogs and receptors*: All analogues were drawn as 2D structures with ChemDraw Ultra version 9.0. Before docking with the GLIDE and the GOLD each analog was prepared using LigPrep, an application that is available through Maestro 7.5. After employing the energy minimization through Macro model application, each 3D structure was saved as an SD file (*.spf), and combined into a single analogs library SD file, to be used for docking experiments.

2.6b *GLIDE docking*: Each homology receptor was prepared by the protein preparation mode of GLIDE and the receptor grid was generated by specifying Asp89 as a central residue and selecting extra precision docking within 20 Å of Asp89. After this step the G-scores were determined and the docking poses of each analogues were visually inspected using GLIDE pose viewer.

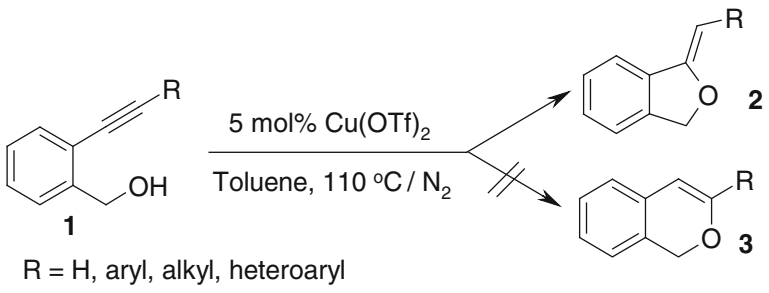
2.6c *GOLD docking*: As input file for the GOLD docking, both homology models were used as a PDB file. In the GOLD Wizard, set-up hydrogen was added and the binding site was defined as the residues that are falling within 15 Å of Asp89. The GOLD score was chosen as fitness function. After docking was finished the top-scoring receptor-analogues complexes were visually inspected.

2.6d *Visualization of selected docking posses*: All final receptor-analogues complexes, obtained from three different programs were visualized using PyMOL viewer only. Hydrogen bonds, hydrophobic interactions and distance for each hydrogen bond were indicated by dashed lines between the atoms involved, while hydrophobic contacts are represented by an arc with spokes radiating towards the ligand atoms they contact.

3. Results and discussion

3.1 *Chemistry*

3.1a *Synthesis*: As part of our ongoing research in the synthesis of novel heterocycles,[13] we have previously established, that 5 mol% of $Cu(OTf)_2$ in toluene was effective for the 5-*exo-dig* regioisomeric cycloisomerization of 2-(ethynyl)benzyl alcohols leading to phthalan derivatives.[13h] Various substituted 2-(ethynyl)benzyl alcohols reacted under this



Scheme 1. $Cu(OTf)_2$ catalysed synthesis of isobenzofuran **2**.

Table 1. Copper(II) catalysed synthesis of phthalans via cycloisomerisation of 2-(ethynyl)benzyl alcohols.[a]

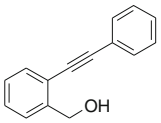
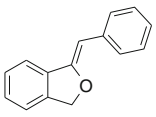
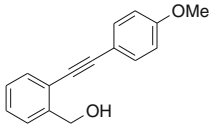
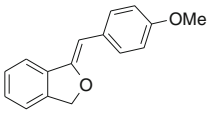
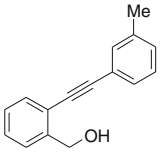
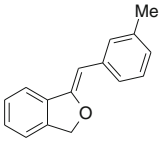
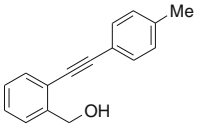
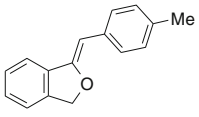
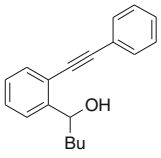
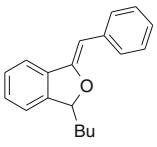
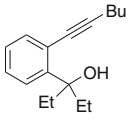
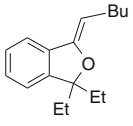
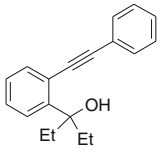
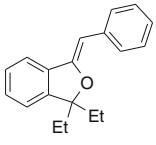
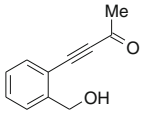
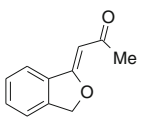
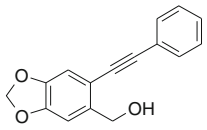
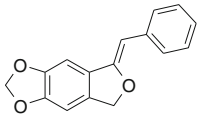
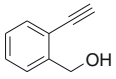
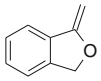
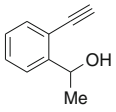
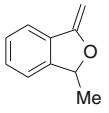
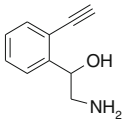
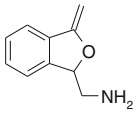
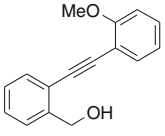
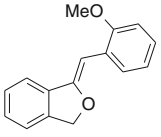
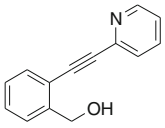
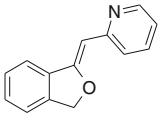
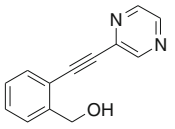
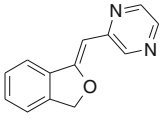
Entry	2-(Ethynyl)benzyl alcohol (**1**)	Phthalan (**2**)[b]	Time (min)	Yield (%)[c]
1	 **1a**	 **2a**	20	92
2	 **1b**	 **2b**	20	95
3	 **1c**	 **2c**	45	94
4	 **1d**	 **2d**	45	95
5	 **1e**	 **2e**	30	88
6	 **1f**	 **2f**	3.5	76
7	 **1g**	 **2g**	40	79
8	 **1h**	 **2h**	25	86

Table 1. (continued)

Entry	2-(Ethynyl)benzyl alcohol (**1**)	Phthalan (**2**)[b]	Time (min)	Yield (%)[c]
9	 **1i**	 **2i**	30	85
10	 **1j**	 **2j**	25	85
11	 **1k**	 **2k**	40	75
12	 **1l**	 **2l**	30	77
13	 **1m**	 **2m**	40	87
14	 **1n**	 **2n**	35	75
15	 **1o**	 **2o**	55	78

[a]All reactions were carried out at 110°C in toluene using 5 mol% of $Cu(OTf)_2$ under nitrogen atmosphere.
[b]All products were characterized by IR, ^1H NMR, ^{13}C NMR and mass spectroscopy.
[c]Isolated yield.
[d]The reaction was carried out in benzene-d_6 solvent at 65°C

condition and afforded the corresponding phthalan derivatives (**2a**–**2o**) in high yield (scheme 1, table 1).

The results revealed that alkynes possessing electron-rich groups enhance the cyclization and generally higher yields are obtained at a shorter reaction time

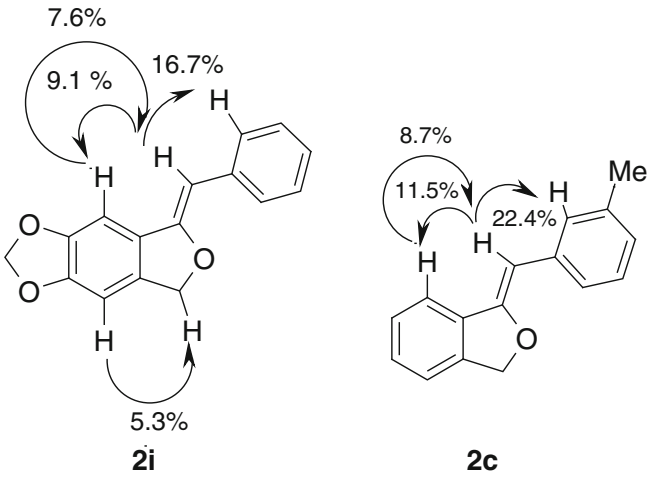


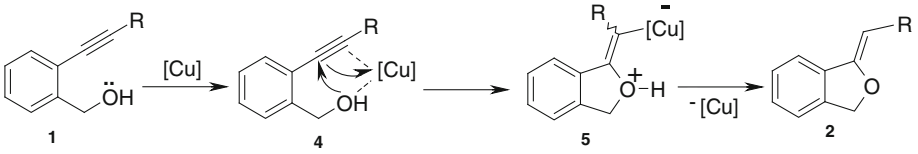
Figure 2. 1-D nOe enhancement of compounds **2i** and **2c**.

(entries 2–4). The presence of substituent at the benzylic position did have deleterious effects on the cyclization; primary alcohols underwent cyclization significantly (entries 1–4, 8–10 and 13–15) in terms of shorter reaction time and higher yields, than secondary alcohols (entries 5, 11 and 12), which in turn react more efficiently than their tertiary counterparts (entries 6 and 7). The protocol was found to be chemoselective. For example, when substrate **1l** was allowed to react with $Cu(OTf)_2$, it was found that only the hydroxyl group underwent cycloisomerization to afford the product **2l** leaving the amine group intact. A more complex heteroaromatic synthesis was also achieved under our reaction condition. For example, the piperanol derived substrate **1i** gave excellent yield of the product **2i** under the same reaction conditions. Substrates possessing heteroaromatic motif like pyridine **1n** and pyrazine **1o** were well tolerated under our reaction conditions (entries 14 and 15). A preparative scale-catalytic reaction was performed for the reaction of **2j**–**2m** in benzene-d_6, since these compounds readily underwent decomposition in $CDCl_3$ solvent.

3.1b *Structural and stereochemical assignment of (Z)-phthalan derivatives*: The *Z*-configuration of phthalan was assigned from comparison of the chemical shifts of the vinylic protons with those reported for similar compounds.[14] The (*Z*)-stereochemistry of phthalan **2i** has been assigned using a 1D-nOe experiment. Selective irradiation of the vinylic proton (δ_H = 6.36 ppm) effected the enhancement of the signals of C_4-H (δ_H = 6.62 ppm) by 9.1% and *ortho*-phenyl proton (δ_H = 7.69 ppm) by 16.7% respectively. Irradiation of C_4-H effected the enhancement of vinylic proton (7.5%) and no enhancement of *ortho*-phenyl proton. This observation confirmed that the vinylic proton is *cis* to C_4-H, thus the compound **2i** is *Z*-configured. Similarly, for compound **2c**, irradiation of vinylic proton (δ_H = 6.48 ppm) effected the enhancement of both C_4-H (δ_H = 7.12 ppm) by 11.5% and *ortho*-phenyl proton (δ_H = 7.59 ppm) by 22.4%. Irradiation of C_4-H proton however effected the enhancement of vinylic proton by 8.7% and no enhancement of the *ortho*-phenyl proton. These facts confirmed the *cis* relationship of the vinylic proton and C_4-H, thus favouring the *Z*-configuration (figure 2). The stereochemistry of other phthalans is assigned by analogy to **2i** and **2c**.

In IR spectra, a peak observed at 1620 to 1650 cm^{-1} for all compounds, revealed the presence of an ether linkage. In ^1H NMR spectra, all products exhibited a sharp singlet between δ_H 4.75 (for unsubstituted olefins) and 6.43 ppm (for substituted olefins), indicated the presence of vinylic proton. In ^{13}C NMR spectra, a peak at δc 120–125 ppm ascertained the presence of olefinic carbon, characteristic of the exocyclic alkylidene carbon of isobenzofuran (see supporting information). All these findings, confirmed the formation of phthalans.

3.1c *Mechanism*: The reason for the exclusive formation of the five-member ring over their six-member counterpart was not clear. However, a tentative mechanism, on the basis of the obtained results is proposed (scheme 2), according to which a six-member transition state **4** was formed via a bidentate complexation of [Cu] with the hydroxyl group and α-carbon of the alkyne of **1**. As a consequence, a partial depletion of electron density at the β-carbon of the acetylene function drives the nucleophilic attack of the pendant hydroxyl group towards the β-carbon leading to the five-member intermediate **5**. Subsequent protodecupration of **5** results in the formation of phthalan **2**.



Scheme 2. Plausible mechanism for the formation of isobenzofuran **2**.

Table 2. Antidepressant activity of compounds (**2a**–**2o**) by forced swim test.

S.No	Compound	Duration of immobility (s)	% change from control
1	**2a**	086.0 ± 1.41**	−57.0
2	**2b**	154.0 ± 1.41**	−23.0
3	**2c**	135.0 ± 5.09**	−32.5
4	**2d**	104.0 ± 1.07**	−48.0
5	**2e**	85.0 ± 1.41**	−57.5
6	**2f**	035.0 ± 1.41**	−82.5
7	**2g**	072.0 ± 0.71**	−64.0
8	**2h**	093.6 ± 1.07**	−53.2
9	**2i**	102.8 ± 1.71**	−49.0
10	**2j**	195.8 ± 1.28**	−02.1
11	**2k**	180.0 ± 1.41**	−10.0
12	**2l**	145.0 ± 1.14**	−27.5
13	**2m**	151.0 ± 1.00**	−24.5
14	**2n**	073.0 ± 0.71**	−63.5
15	**2o**	071.0 ± 0.71**	−64.5
16	Citalopram (10 mg/kg)	057.0 ± 0.73**	−71.5
17	Control (Vehicle)	200.0 ± 6.88**	-

Data were analysed by one way ANNOVA followed by Dunnet's t test.
P values: **<0.001.
**are more significant

4. Pharmacology

4.1 *Evaluation of antidepressant activity*

All the synthesized compounds (**2a**–**2o**) were evaluated for their antidepressant activity by forced swim and tail suspension tests.[11,12] The results were shown in tables 2 and 3 for forced swim test and tail suspension test, respectively.

As shown in table 2, the reference drug citalopram (10 mg/kg) and the test compounds (10 mg/kg) significantly shortened the immobility time of mice in forced swim test. Similarly in tail suspension test, citalopram

Table 3. Antidepressant activity of compounds (**2a**–**2o**) by tail suspension test.

S.No	Compounds	Duration of immobility (s)	% change from control
1	**2a**	080.0 ± 0.71*	−54.29
2	**2b**	125.8 ± 0.83*	−28.11
3	**2c**	134.4 ± 1.20*	−23.20
4	**2d**	139.6 ± 1.21*	−20.23
5	**2e**	081.6 ± 1.07*	−53.37
6	**2f**	040.0 ± 1.41*	−77.14
7	**2g**	070.8 ± 1.23*	−59.54
8	**2h**	083.8 ± 1.28*	−52.11
9	**2i**	095.8 ± 0.87*	−45.23
10	**2j**	180.4 ± 0.92**	+03.09
11	**2k**	169.2 ± 3.78**	−07.31
12	**2l**	139.0 ± 1.00*	−20.57
13	**2m**	129.2 ± 1.65*	−26.17
14	**2n**	071.0 ± 0.71**	−59.43
15	**2o**	0.78.2 ± 0.87*	−55.31
16	Citalopram (10 mg/kg)	068.0 ± 1.25*	−61.14
17	Control (Vehicle)	175.0 ± 4.79*	-

Data were analysed by one way ANNOVA followed by Dunnet's t test.
P values: *<0.5, **<0.001.
**are more significant

Table 4. Calculated Lipinski's rule of five for the compounds (**2a**–**2o**).

S.No	Compounds	M.W[a]	log p[b]	n ON[c]	n ONH[d]
1	**2a**	208.26	3.755	1	0
2	**2b**	238.29	3.812	2	0
3	**2c**	222.29	4.179	1	0
4	**2d**	222.29	4.203	1	0
5	**2e**	264.37	5.685	1	0
6	**2f**	244.38	5.918	1	0
7	**2g**	264.37	5.57	1	0
8	**2h**	174.20	1.468	2	0
9	**2i**	252.27	3.621	3	0
10	**2j**	132.16	2.048	1	0
11	**2k**	146.19	2.411	1	0
12	**2l**	161.21	0.840	2	2
13	**2m**	238.29	3.584	2	0
14	**2n**	209.25	2.405	2	0
15	**2o**	210.24	2.108	3	0
16	Citalopram	324.39	3.17	3	0

[a]Molecular weight.
[b]Calculated lipophilicity.
[c]Number of hydrogen bond acceptor.
[d]Number of hydrogen bond donor

and all test compounds shortened the immobility time of mice (table 3). The results of the animal study indicated that all the compounds showed significant activity except compound **2j**. Compound **2f** showed more potent activity than citalopram.

4.2 *Lipinski rule of five and drug-likeness profile*

All the compounds were submitted for the analysis of Lipinski rule of five that indicates if a compound could be an orally active drug in human.[15] Our *in vivo* results showed that all active compounds fulfilled this rule, similar to the clinically used drug, citalopram (molecular weight = 208.26 to 264.37 g/mol, log p = 2.05–5.6, nON = 1–3, and nOHNH = 0–2) (table 4). However a couple of points need to be noted. Compound **2i** which is closest to citalopram in log p, nON, nONH and M.W values is less active. The most active compound of our study is **2f** which has the highest log p value in the series. Compounds **2e** and **2g** are slightly less lipophilic and less active. On the other hand, compound **2h** which is much less lipophilic (log p = 1.468) than **2e** does not lag far behind the latter in activity. There are many approaches that assess a compound's drug-likeness based on topological descriptors, fingerprints of molecular drug-likeness, structural keys or other properties like c log p and molecular weight.[16] In the present work, we used molinspiration program for calculating the fragment based drug-likeness of all the compounds and compared with citalopram (figure 3). Interestingly in this analysis compounds **2e**, **2f** and **2g** with a drug-likeness score of −0.25, −0.28

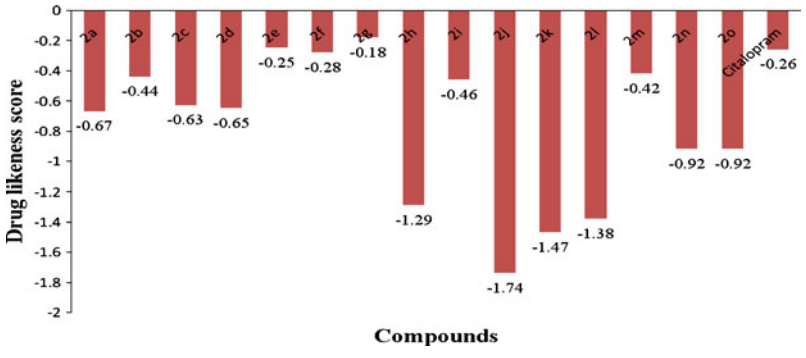


Figure 3. Drug-likeness score of compounds (**2a**–**2o**).

Table 5. Docking study of compounds (**2a**–**2o**) on serotonin binding site of arabinase receptor model (PDB code: 2WRZ).

S.No	Compound	Gold score	Glide score	Docking score
1	**2a**	38.08	−38.20	−6.34
2	**2b**	36.51	−40.96	−5.96
3	**2c**	39.72	−40.07	−6.14
4	**2d**	34.90	−38.97	−6.08
5	**2e**	42.51	−40.87	−6.05
6	**2f**	43.39	−36.22	−5.88
7	**2g**	43.04	−43.14	−6.11
8	**2h**	33.96	−34.35	−5.36
9	**2i**	42.23	−45.36	−6.25
10	**2j**	36.17	−27.83	−5.26
11	**2k**	40.07	−28.99	−5.37
12	**2l**	38.11	−43.51	−6.62
13	**2m**	42.44	−40.56	−6.18
14	**2n**	45.36	−37.47	−6.12
15	**2o**	41.02	−37.78	−5.89
16	Citalopram	48.32	−46.56	−6.93

and −0.18, respectively resemble citalopram with a score of −0.26. However, the drug-likeness scores of **2e**, **2f** and **2g** do not parallel strictly with their *in vivo* biological activites.

4.3 *Docking studies*

The level of antidepressant activity of compounds (**2a**–**2o**) was studied by *in silico* modelling techniques by automated docking of ligands to the serotonin binding site of arabinase (table 5).[17]

The docking studies revealed that compounds **2b**, **2c**, **2e**, **2g**, **2i**, **2l** and **2m** have the highest negative glide score ranging from −45 to −40 (citalopram −46.56). These compounds are expected to form more stable drug-receptor complexes. However, these were less active than compound **2f** with a score of −36.22. All the compounds were analysed for hydrophobic and Vanderwaals interactions, since very few compounds were found to exhibit hydrogen bonding with the receptor. Other compounds were found to exhibit no hydrogen bonding with the receptor.

5. Conclusion

The present work led to the discovery of antidepressant molecules containing 1,3 dihydroisobenzofuran (phthalan) pharmacophore of the reputed drug, citalopram, but with far less structural embellishments. Antidepressant activity was evaluated for all the proposed compounds by using forced swim test and tail suspension test. Compound **2f** exhibited potent activity than the reference drug, citalopram. Other compounds showed moderate to good antidepressant activity. The synthesized compounds showed antidepressant activity without having the usual aminoalkyl side chain characteristic of CNS active molecule, citalopram.

Supplementary information

The electronic supporting information can be seen in www.ias.ac.in/chemsci.

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References

1. Wong M L and Licinio J 2001 *Nat. Rev. Neurosci.* **2** 343
2. Little A 2009 *Am. Fam. Physician* **80** 167
3. (a) Richard C and Shelton M D 2003 *J. Clin. Psychiatry* **5** 27; (b) Koenig A M and Thase M E 2009 *Pol. Arch. Med. Wewn.* **119** 478; (c) Inoue T, Kusumi I and Yoshioka M 2002 *Curr. Drug Targets: CNS Neurol. Disord.* **1** 519
4. Stahl S M 2006 *Essential psychopharmacology: The prescriber's guide* (New York: Cambridge University Press)
5. Berton O and Nestler E J 2006 *Nat. Rev. Neurosci.* **7** 137
6. (a) Zhu W, Ma S, Qu R and Kang D 2006 *Life Sci.* **79** 74; (b) Petersen P V, Lassen N, Hansen V, Huld T, Hjortkjaer J, Holmblad J, Nielsen I M, Nymar M,

Pedersen V, Jorgensen A and Hougs W 1966 *Acta Pharmacol. Toxicol.* **24** 121; (c) Petersen P V, Lassen N, Norgaard J and Huld T 1969 U.S. Patent 345765; (d) Bigler A J, Bogeso K P, Toft A and Hansen V 1977 *Eur. J. Med. Chem.* **12** 289
7. Feighner J P 1999 *J. Clin. Psychiatry (supp. 4)* **60** 4
8. Briner K and Dodel R C 1998 *Curr. Pharm. Des.* **4** 291
9. Labbate L A, Grimes J B and Arana G W 1998 *Biol. Psychiatry* **43** 904
10. Ghosh M N 2005 *Fundamentals of experimental pharmacology*, 3rd Edition (Hilton and company, Kolkatta) pp. 190–197
11. Porsolt R D, Bertin A and Jalfre M 1977 *Arch. Int. Pharmacodyn. Ther.* **229** 327
12. (a) Steru L, Chermat R, Thierry B and Simon P 1985 *Psychopharmacology* (Berl) **85** 367; (b) Nielsen D M, Carey G J and Gold L H 2004 *Eur. J. Pharmacol.* **499** 135
13. (a) Praveen C, Sagayaraj Y W and Perumal P T 2009 *Tetrahedron Lett.* **50** 644; (b) Praveen C, Kiruthiga P and Perumal P T 2009 *Synlett.* 1990; (c) Praveen C, Karthikeyan K and Perumal P T 2009 *Tetrahedron* **65** 9244; (d) Praveen C, Jegatheesan S and Perumal P T 2009 *Synlett.* 2795; (e) Praveen C, Kalyanasundaram A and Perumal P T 2010 *Synlett.* 777; (f) Praveen C, Kumar K H, Muralidharan D and Perumal P T 2008 *Tetrahedron* **64** 2369; (g) Praveen C, Parthasarathy K and Perumal P T 2010 *Synlett.* 1635. (h) Praveen C, Iyyappan C and Perumal P T 2010 *Tetrahedron Lett.* **51** 4767; (i) Praveen C, Dheenkumar P and Perumal P T 2010 *Bioorg. Med. Chem. Lett.* **20** 7292; (j) Praveen C and Perumal P T 2011 *Synlett.* 521; (k) Praveen C, Ayyanar A and Perumal P T 2011 *Bioorg. Med. Chem. Lett.* **21** 4170
14. Hiroya K, Jouke R, Kameda M, Yasuhara A and Sakamoto T 2001 *Tetrahedron* **57** 9697
15. Lipinski C A, Lombardo F, Dominy B W and Feeney P J 2001 *Adv. Drug Deliv. Rev.* **46** 3
16. Tetko I V 2005 *Drug Discov. Today* **10** 1497
17. We didn't make any homology modelling for the receptor serotonin (5–HT)