Supplementary Material

Molecular targets of Bis (7)-cognitin and its relevance in neurological disorders: A systematic review

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# Supplementary Tables

**Table 1. Pre-defined search terms and database search strategy**

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| **ID** | **Search terms** |
|  | Bis 7 cognitin |
|  | B7C |
|  | Bis heptyl cognitin |
|  | Bis 7 tacrine  |
|  | B7T |
|  | 1 OR 2 OR 3 OR 4 OR 5  |

Table 2. Description of *in vitro* and *in vivo* studies on B7C

| Reference | *In vitro* study |  | *In vivo* study |  | Molecular target, biological process or behavioral test |  | Conclusion |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Cell line | B7C concentration |  | Animal species/strain | B7C dose or concentration |  |  |
| (Wang et al., 1999b) | NA | NA |  | Female and male Sprague-Dawley rats | 1, 3, 5, 9.5, 19, 38 nM |  | AChE and BChE |  | - B7C inhibited both AChE (IC50= 5.1 nM) and BChE (IC50= 159 nM). |
| (Ros et al., 2001) | NA | NA |  | *Torpedo marmorata* (fish) electric organOocytes from mature females of *Xenopus laevis* (frog) | 100 nM |  | ACh release by recording spontaneous synaptic activity |  | - B7C increased spontaneous quantal release from cholinergic terminals and blocked the AChE induced currents in a lower concentration than tacrine. |
| (Hu et al., 2002) | NA | NA |  | NA | Different concentrations |  | AChE and BChE  |  | - B7C inhibited both AChE (IC50= 2.7 nM) and BChE (IC50= 2.6 nM). |
| (Fu et al., 2006) | Hippocampal pyramidal neurons (isolated from Wistar rats) | 0.1, 1, 5, 10, 100, and 500 nM |  | NA | NA |  | AChE, L-type voltage-dependent Ca2+ channels |  | - B7C reduced the inward calcium current induced by Aβ induced Ca2+ which attenuated neuronal apoptosis. |
| (Bolognesi et al., 2010) | NA | NA |  | NA | Different concentrations |  | AChE and BChE (human recombinant enzymes) BACE-1, Aβ |  | - B7C inhibited both AChE (IC50= 0.81 nM) and BChE (IC50= 5.66 nM). |
| (Rizzo et al., 2011) | NA | NA |  | NA | Different concentrations |  | AChE and BChE (human recombinant enzymes), BACE-1 |  | - B7C inhibited both AChE (IC50= 0.81 nM) and BChE (IC50= 5.66 nM) (results were taken as reference from (Bolognesi et al., 2010)).- B7C inhibited BACE-1 (IC50= 7.5µM), and inhibited AChE-induced Aβ aggregation (results were taken as reference from (Fu et al., 2008)) (68%) (results were taken as reference from (Bolognesi et al., 2010)). |
| (Qian et al., 2014) | Rat cortexhomogenate/serum | 0.5, 1 and 2nM |  | NA | NA |  | AChE and BChE |  | - B7C inhibited both AChE (IC50= 1.5 nM) and BChE (IC50= 328.9 nM). |
| (Hu et al., 2015b) | SH-SY5S cells (human neuroblastoma cells) | 0.1, 0.3, 1, 3, 10 µM |  | Male Sprague-Dawley rats | 0.1 and 0.2 mg/kg |  | AChE, ChAT, Morris water maze test |  | - B7C inhibited Aβ fibrils formation and disaggregated pre-formed Aβ fibrils.- B7C reduced Aβ induced neurotoxicity.- B7C inhibited the memory impairment induced by infusion of Aβ in rats.- B7C reversed the dysfunction ChAT and AChE activity induced by Aβ in rats. |
| (Pan et al., 2009) | NA | NA |  | Male ICR mice | 0.25–20 micromol/kg |  | AChE, OFT, and Step-Through task (passive-avoidance response) |  | - B7C inhibited AChE activity in brain tissue and serum 15 min after drug administration.- B7C at 20 micromol/kg did not impair the open-field memory.- B7C reverted the scopolamine-induced learning and memory impairment at 1 micromol/kg.- B7C was more potent than tacrine improving scopolamine-induced cognitive dysfunction. |
| (Li et al., 1999) | Hippocampal neurons (isolated from mice) | 5, 25, 100 µM |  | NA | NA |  | AChE, GABAA |  | - B7C inhibited AChE (IC50= 1.5 nM).- B7C antagonizes the GABAA receptor in a competitive mode |
| (Yu et al., 2008) | NA | NA |  | Male ICR mice | 3.54 micromol/kg |  | AChE |  | - B7C inhibited AChE (46.3%). |
| (Fu et al., 2008) | Mouse Neuro2a neuroblastoma cells | 0.1, 0.3, 1, 2, 3 µM  |  | NA | NA |  | BACE-1 |  | - B7C inhibits BACE-1, therefore decreases the generation of Aβ- B7C activates α-secretase |
| (Fu et al., 2009) | Mouse Neuro2 neuroblastoma cells | 0.1, 0.3, 1 µM |  | NA | NA |  | BACE-1 and α-secretase |  | - B7C inhibits BACE-1 and activates α-secretase |
| (Li et al., 2007a) | DRG neurons (isolated from Sprague-Dawley rats) | Different concentrations |  | NA | NA |  | GABA receptor |  | - B7C binds to GABA receptor in a potent but reversible manner (IC50= 6.28µM). |
| (Zhou et al., 2009) | Hippocampal neurons (primary cell culture from Sprague-Dawley rats) | 1, 3, 5, 10, 30, 100 µM |  | NA | NA |  | GABAA receptor |  | - B7C is a competitive GABAA receptor antagonist. |
| (Luo et al., 2004) | TG neurons |  |  | NA | NA |  | 5-HT3 receptor |  | - B7C inhibited the 5-HT3 receptor current in a competitive manner. |
| (Nie et al., 2007) | DRG neurons (isolated from Sprague-Dawley rats) | 10-9M to 10-4 M |  | NA | NA |  | Kv4.2 potassium channels |  | - B7C inhibited the delayed rectifier potassium channel and inhibited the Kv4.2 potassium channels. (IC50= 0.72 µM). |
| (Li et al., 2010) | DRG (isolated from Sprague-Dawley rats) | 1 µM |  | NA | NA |  | Kv4.2 potassium channels |  | - B7C suppressed the Kv4.2 potassium channels in a concentration-dependent manner (IC50= 0.53 µM). |
| (Bai-fang et al., 2001) | Cortical cells (isolated from Sprague-Dawley rats) | 0.3-1 µM/L |  | NA | NA |  | NMDA receptor |  | - B7C reduced the NMDA-mediated activity and exhibited a protective effect against glutamate-induced excitotoxicity. |
| (Li et al., 2005) | CGN (primary cell culture from Sprague-Dawley rats) | 0.1, 1µM |  | NA | NA |  | NMDA receptor |  | - B7C prevents glutamate-induced neuronal apoptosis through blockade of the NMDA receptor.- B7C inhibits AChE and acts as NMDA antagonist.- B7C inhibits the MAPK and ERK pathway. |
| (Luo et al., 2007) | Hippocampal neurons (isolated from Sprague-Dawley rats) |  |  | NA | NA |  | NMDA receptor |  | - B7C inhibited the NMDA receptor in a pH-dependent manner by desensitizing the receptors to proton inhibition.  |
| (Liu et al., 2008a) | Hippocampal neurons (isolated from Sprague-Dawley rats) | 0.001-1 µM |  | NA | NA |  | NMDA receptor |  | - B7C inhibited the NMDA receptor (IC50= 0.68 µM).- B7C prevented the glutamate-induced excitotoxicity by inhibition of the NMDA receptor.  |
| (Liu et al., 2008b) | Hippocampal neurons (isolated from Sprague-Dawley rats) | 0.5 µM |  | NA | NA |  | NMDA receptor |  | - B7C inhibited the NMDA receptors in a non-competitive manner and showed a protective effect against glutamate-induced neurotoxicity. |
| (Zhang et al., 2011) | RGC (primary cell culture from male Sprague-Dawley rats) | 1µM |  | Male Sprague-Dawley rats | 0.05, 0.1 and 0.2 mg/kg |  | NMDA receptor |  | - B7C prevented NMDA-induced apoptosis in GCL.-B7C inhibitory effect of NMDA receptors confers neuroprotection.- B7C reduced the NMDA activated current in RGC which indicates that B7C is an antagonist of the NMDA receptor. |
| (Liu and Li, 2012) | HEK-293 | 1 µM |  | NA | NA |  | NR1, NR2A, and NR2B receptors |  | - B7C inhibited the NR1/NR2B receptor expressed in the cells in a slow onset, non-competitive and voltage-dependent manner which is similar to what is observed in rat hippocampal neurons expressing the NMDA receptors. |
| (Li et al., 2007b) | CGN (isolated from Sprague-Dawley rats) | Different concentrations |  | NA | NA |  | NMDA receptor, NOS |  | - B7C showed to be a moderated NMDA receptor antagonist and a selective NOS inhibitor. |
| (Li et al., 2006) | Cortical neurons (isolated from Sprague-Dawley rats) | 0.001, 1 µM |  | NA | NA |  | NOS |  | - B7C reduced cell death induced by glutamate, Aβ, and L-arginine.- B7C suppressed the activation of the NOS induced by glutamate.- B7C inhibited the activity of NOS. |
| (Liu et al., 2000) | NA | NA |  | Male Sprague-Dawley rats | 0.22, 0.44, and 0.89 micromol/kg /kg |  | ChAT, and the Morris water maze |  | - The induced learning and memory deficits were reversed by B7C in a dose-dependent manner. |
| (Chang et al., 2015) | Hippocampal neurons (isolated from Sprague-Dawley rats) | NA |  | Male ICR miceWistar rats | 0.1 or 0.2 mg/kg0.1 to 3 μM |  | Morris water mazeNeurite outgrowthLTP Aβ aggregation |  | - B7C reduced cognitive Aβ-induced cognitive impairment in mice at concentrations of 0.1 and 0.2 mg/kg.- B7C at a concentration of 0.1 to 0.3 μM prevented an Aβ-induced reduction in neurite length.-B7C inhibited the formation of Aβ oligomers and reduced the amount of pre-formed oligomers. - B7C showed a dose-dependent effect to prevent Aβ oligomer-induced inhibition of LTP with a threshold of 0.1 μM. |
| (Han et al., 2000) | Cortical astrocytes (isolated from ICR mice) | 0.3, 1, 10, 100 nM |  | NA | NA |  | Apoptosis |  | - B7C (1-10 nM) inhibited the ischemia-induced apoptosis. |
| (Fu et al., 2007) | CGN (isolated from Sprague-Dawley rats) | 0.001, 0.01, 0.1, 1 µM |  | NA | NA |  | Apoptosis |  | - B7C protected against the glutamate-induced excitotoxicity. |
| (Zhao et al., 2008) | NA | NA |  | Male Sprague-Dawley rats | 0.05, 0.1, 0.2 mg/kg |  | Apoptosis |  | - B7C showed anti apoptotic effect (0.2 mg/kg) |
| (Fang et al., 2010) | RGC (isolated from male Sprague-Dawley rats) | 1 µM |  | Male Sprague-Dawley rats | 0.05, 0.1, and 0.2 mg/kg |  | Apoptosis |  | - B7C inhibited glutamate-induced cell death (IC50= 0.028 µM).- B7C reduced glutamate-induced apoptosis in vivo (0.02 mg/kg). |
| (Xiao et al., 2000) | PC12 cells (rat pheochromocytoma cells) | 0.01, 0.1, 1, 10 µM |  | NA | NA |  | Cell toxicity |  | - B7C protected the cells against H2O2-induced cell toxicity improving the redox disequilibrium. |
| (Hu et al., 2015a) | PC12 cells (rat pheochromocytoma cells)Cortical neurons (isolated from Sprague-Dawley rats) | 0.03-0.5 µM |  | NA | NA |  | NeuritogenesisAβ neurotoxicity |  | - B7C induced neuritogenesis in PC12 and primary cortical neurons at concentrations of 0.1–0.5 µM and 0.1-0.3 µM respectively.- B7C (0.1-0.5 µM) provided neuroprotection against Aβ challenge in PC12 cells.- B7C (0.1-0.5 µM) reverted the Aβ-induced neurite shortening in PC12 cells.- B7C acted via the activation of alpha7-nicotinic acetylcholine receptor/ERK pathway. |
| (Li et al., 2014) | NA | NA |  | Male Sprague–Dawley rats | 0.2 mg/kg |  | Retinal ischemia |  | - B7C reduced ischemia-induced cell loss in the retinal ganglion cell layer showing a neuroprotective effect against ischaemic retinal damage. |
| (Shu et al., 2012) | NA | NA |  | Male Sprague-Dawley rats | 0.2 mg/kg |  | Morris water maze test |  | - B7C decreased hippocampal neural apoptosis (it increased neurogenesis) in rats with chronic ischemia- B7C reversed the chronic-ischemia-induced decreased spatial learning and memory.  |
| (Wang et al., 1999a) | NA | NA |  | Male Sprague-Dawley rats | 0.18, 0.35, 0.71 micromol/kg |  | Morris water maze test |  | - B7C ameliorated the memory deficits induced by scopolamine as observed in the decreased escape latency to levels compared to the ones in the control group in the Morris water maze test. |
| (Han et al., 2012) | NA | NA |  | Male Kunming strain mice | 0.4, 0.5, and 0.6 micromol/kg |  | Morris water maze and the NOR task to evaluate recognition memory formation |  | - B7C mitigated the learning and memory deficits induced by scopolamine. |
| (Pan et al., 2007)  | NA | NA |  | Male ICR mice | 0.06, 1.25, 2.5, 5, 5, 10, 20 micromol/kg |  | Passive avoidance response, spontaneous motor activity, hepatotoxicity |  | - B7C enhanced cognitive function at a high dose (20 micromol/kg) but produced motor dysfunction and hepatotoxicity. |
| (Pan et al., 2011) | NA | NA |  | Male ICR mice | 0.25, 1, 5, and 20 micromol/kg |  | OFT |  | - B7C did not affect locomotion in the OFT- B7C (1 micromol/kg) improved the cycloheximide-induced amnesia in mice. |

Abbreviations: Aβ , beta-amyloid; BACE-1, beta-secretase; BuChE, butyrylcholinesterase; ChAT, choline acetyltransferase; DRG, dorsal root ganglion; LTP, long-term potentiation; NA, not applicable; NOR, novel object recognition; NOS, nitric oxide synthase; OFT, open field test; RGC, retinal ganglion cells; TG, rat trigeminal ganglion; CGN, cerebellar granule neurons.