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Novel indolylmaleimide acts as GSK-3β inhibitor in human neural progenitor cells

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ABSTRACT

The Wnt pathway is involved in cellular processes linked to either proliferation or differentiation. Therefore small molecules offer an attractive opportunity to modulate this pathway, whereas the key enzyme GSK-3β is of special interest. In this study, non-symmetrically substituted indolylmaleimides have been synthesized and their ability to function as GSK-3β inhibitors has been investigated in a human neural progenitor cell line. Among the newly synthesized compounds, the substance IM-12 showed a significant activity in several biological tests which was comparable or even outplayed the effects of the known GSK-3β inhibitor SB-216763. Furthermore the treatment of human neural progenitor cells with IM-12 resulted in an increase of neuronal cells. Therefore we conclude that indolylmaleimides act via the canonical Wnt signalling pathway by inhibition of the key enzyme GSK-3β.

1. Introduction

Wnt signalling is connected to important cellular processes such as cell polarity, cell death, proliferation, self-renewal and morphogenic movements.[1] The involvement of Wnt signalling in neural stem cell differentiation includes numerous aspects such as migration,[2] synaptogenesis,[3] axon guidance[4] and neural induction.[5]

Wnts constitute a family of 19 secreted glycoproteins which are activators of at least three pathways: one canonical (β-catenin-dependent) and two non-canonical pathways (Wnt-planar (PCP) and Wnt/Ca^{2+}-pathway).[6] The canonical pathway is mainly characterized by the stabilization of β-catenin in the cytosol. In an inactive state, β-catenin is degraded by a complex formed of Adenomatous Polyposis Coli protein (APC), Axin and Glycogen synthase kinase-3β (GSK-3β).[7] Activation of the pathway induces the decay of the degradation complex, stabilizing β-catenin which then translocates into the nucleus where it binds to the T-cell factor (TCF)/lymphoid enhancer factor (LEF)-complex and regulates the transcription of Wnt-specific target genes.[8,9] The inhibition of the β-catenin degradation complex can be achieved in two ways: either by the binding of a Wnt protein to a complex of frizzled receptor and low-density lipoprotein receptor related protein (LRP) or by the direct inhibition of GSK-3β. So far, several pharmacological GSK-3 inhibitors have been described in the literature. The mechanism of inhibition varies from ATP-competition, as in the case with paullones, arylindolylmaleimides or indirubins, to Mg^{2+}-competition with lithium or beryllium ions.[10,11] Notably, GSK-3 plays a role in several diseases, such as diabetes,[12] Alzheimer's disease,[13] or bipolar disorders,[14] which makes it an attractive pharmacological target.

Another interesting aspect is the influence of canonical Wnt signalling on several processes linked to proliferation and differentiation of neural precursor cells. The absence of basic fibroblast growth factors (bFGF) enhances neuronal differentiation of neural precursor cells by canonical Wnt signalling.[15] Wnt 1, Wnt 3a and Wnt 5a (non-canonical) regulate proliferation and differentiation of neural precursor cells during dopaminergic neuronal development in the fetal ventral midbrain.[16] GSK-3 deletion strongly inhibits neurogenesis.[17] The influence of both, canonical and non-canonical Wnt signalling is stage and cellular-context dependent. Because of the manifold applications for new GSK-3β inhibitors, we decided to synthesize new small molecules, focusing in particular on their application in neurodegenerative diseases. Several drugs have been extensively characterized in this regard. A key

Abbreviations: bFGF, basic fibroblast growth factor; DAPI, 4′,6-diamidin-2′-phenylindolyldihydrochloride; DMEM, dulbecco's modified eagle medium; EGF, epidermal growth factor; ELISA, enzyme linked immunoabsorbent assay; KP, Kenpaullone; mAb, monoclonal antibody; NPC, neural progenitor cell; RIPA, radio immunoprecipitation assay; SB21, SB-216763; SV40, simian virus 40.

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substance is the GSK-3β inhibitor SB-216763 (SB-21, Fig. 1) which is an indolylmaleimide derivative that acts competitively with ATP and is generally specific to GSK-3β.[18] These attributes make SB-216763 an interesting lead structure for new active compounds which may inhibit GSK-3β as well.

The synthesized derivates are characterized with regards to their inhibitory potential on GSK-3β and the evolving effect on Wnt signalling in human neural progenitor cells (hNPCs). In this study, we used the human NPC line ReNcell VM (Millipore, USA) to investigate the biological function of the newly synthesized substances. Notably, this cell line can differentiate into neurons, astrocytes, and oligodendrocytes within a few days.[19,20] Beside this, the cell line shows a fast proliferation and can be cultured easily which makes it an appropriate model system to test the influence of GSK-3 inhibitors on neuronal differentiation. Furthermore only few studies deal with the differentiation of human neuronal progenitor cells. Following from a previous communication on selected catalytic and stoichiometric synthesis of non-symmetrically substituted 4-indolylmaleimides,[21] we here describe in detail chemical and biological data showing the effect on Wnt signalling on human NPCs. As a major result, one of the new substances showed significant biological effects on Wnt signalling in the same range as the known GSK-3β inhibitor SB-216763.

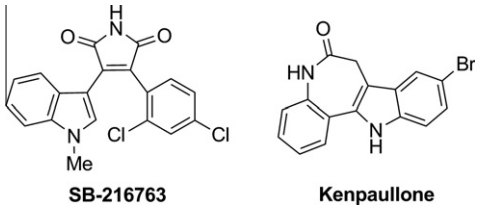

Figure 1. Chemical structures of the known GSK-3β inhibitors SB-216763 and Kenpaullone.

2. Results

2.1. Synthesis of substituted 4-indolylmaleimides

Indolylmaleimides (IM) 1–7 (Fig. 2) have been prepared by $Pd(OAc)_2$/cataCXium®A-catalyzed carbonylation of 3-bromo-1-methyl-4-(2-methyl-1*H*-indol-3-yl)-maleimide with carbon monoxide in the presence of alcohols or amines at 90–115 °C.[21] Thus, novel 3-alkoxycarbonyl- and 3-aminocarbonyl-4-indolylmaleimides were obtained in 25–70% yield. Alternatively, new 4-amino-3-indolylmaleimides 8–15 (Fig. 2) have been synthesized in good yields (69–91%) via stoichiometric amination of the same 3-bromo-1-methyl-4-(2-methyl-1*H*-indol-3-yl)-maleimides with corresponding amines.[21]

2.2. Treatment of ReNcell VM with SB-216763, Kenpaullone and indolylmaleimides increases the amount of total β-catenin

Initially, we investigated whether or not the application of SB-216763 or Kenpaullone to hNPCs could augment the level of total β-catenin. Thus, cells were cultivated under proliferation conditions until 70% confluence before differentiation was induced. The drugs were diluted in differentiation medium at appropriate concentrations. To determine the adequate time point for further studies, total cell extracts were harvested over 48 h and the amount of total β-catenin was measured using an ELISA specific for human total β-catenin. As expected, the change to differentiation condition resulted in an increase of β-catenin. This effect was potentiated by the addition of Kenpaullone or SB-216763 to the medium. Since the maximum of β-catenin accumulation is

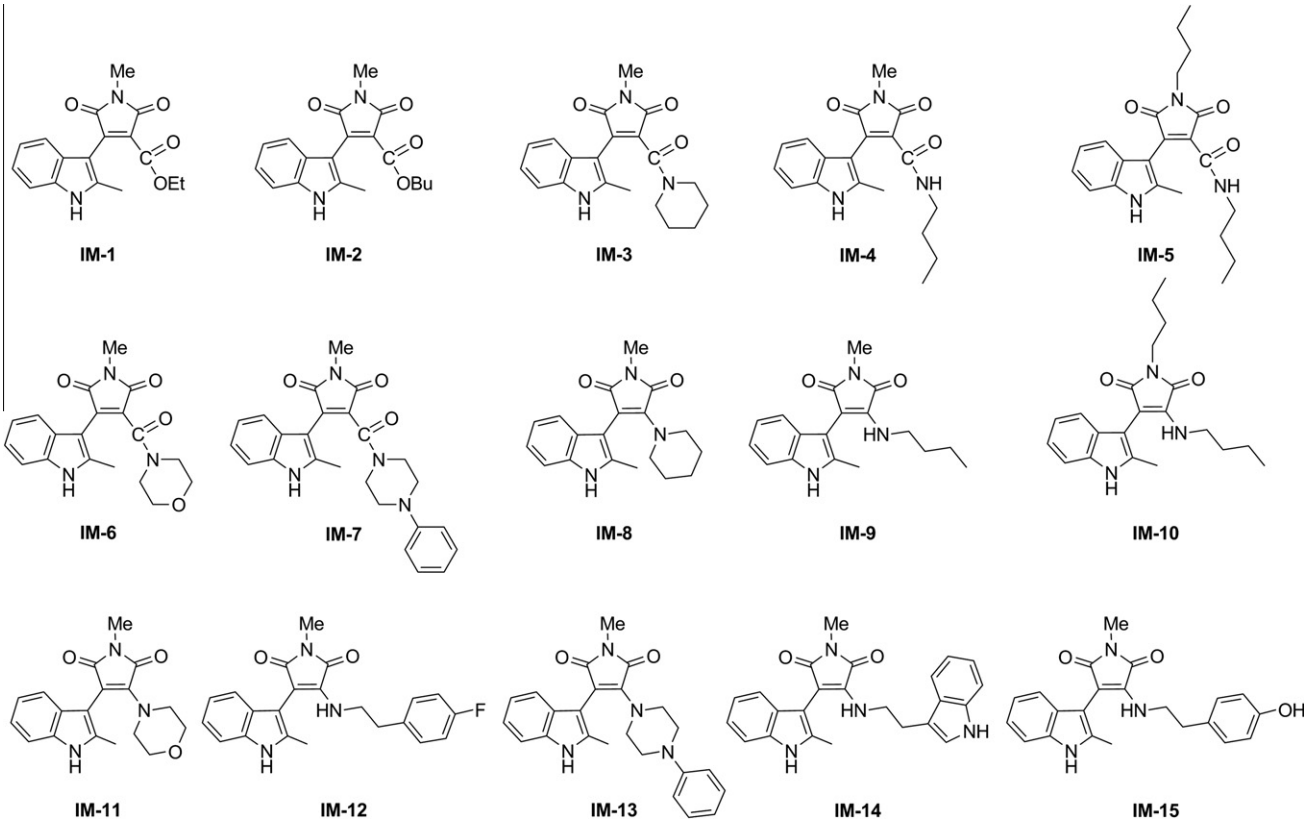

Figure 2. Chemical structures of various non-symmetrically substituted indolylmaleimides tested for biological activity.