

# Stem Cell Growth and Differentiation

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Kirsty Clarke and Victoria Christie are research scientists within the laboratory of Professor Stefan Przyborski at Durham University. Research in Professor Przyborski's group focuses on the development and application of technology to improve the growth, differentiation and function of cultured cells, including the use of small molecules that control stem cell fate.

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## Introduction

Small molecules are routinely used to manipulate signaling pathways during the *in vitro* culture of cells. Signaling pathways that control cell proliferation and differentiation are important targets for small molecules in the culture of stem cells. Targeting pathways such as the canonical Wnt, transforming growth factor- $\beta$  (TGF- $\beta$ ) and retinoic acid signaling pathways can be useful to enhance and maintain the proliferation of stem cells, or to guide stem cell fate toward specific lineages in controlled differentiation. This review provides a brief overview of the small molecules that interact with the primary signaling pathways that govern stem cell proliferation and differentiation to mediate stem cell behavior, along with the role of small molecules in the dedifferentiation of somatic cells to create populations of pluripotent stem cells.

Stem cells are characterized as having the ability to self-renew along with the potential to differentiate into defined cellular subtypes.<sup>1</sup> There are four main types of stem cell; embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, adult stem (AS) cells and cancer stem (CS) cells. ES cells are historically the most potent and are derived from the inner cell mass of the developing blastocyst. They are able to differentiate into any cell type representing all three of the developing germ layers upon exposure to developmental cues. The study of ES cells may provide useful therapeutic tools and insight into key developmental processes.<sup>2</sup> iPS cells are derived from the reprogramming of somatic cells through forced expression of transcription factors, or exposure to a multitude of molecules that revert them back to a stem cell-like phenotype.<sup>3</sup> They are thought to have a similar potency to ES cells as they are pluripotent and able to differentiate into cell types representing all three germ layers. This may provide therapeutic potential for autologous transplantation of iPS cell-derived cell types as treatment for degenerative diseases.

AS cells have a much more restricted differentiation potential and are typically responsible for the maintenance and repopulation of cell types found within specific niches in tissues. An example of an AS cell is the hematopoietic stem cell found within the bone marrow that can give rise to only cell types found within the blood.<sup>4</sup> The final category of stem cell, the CS cell, is responsible for cell proliferation within certain types of tumor. CS cells are thought to be implicated in cancer progression, initiation and metastasis, and therefore may provide a potential therapeutic target for anti-cancer drug development.<sup>5</sup>

Synthetic and naturally occurring molecules that interact with certain signaling pathways are an integral component of stem cell research. Compounds designed to interact with specific stages in developmental pathways can be utilized to invoke specific cellular responses, which can be modulated through changes in compound concentration. The selectivity of molecules

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to act only upon the desired pathway can allow for the controlled differentiation of stem cells into specific cellular lineages. This is important for *in vitro* modeling that can be used for basic research, drug screening and the study of pathological mechanisms. Custom design of molecular structures and screening of compound libraries are two approaches commonly used to discover small molecules that interact with key signaling pathways.

## Identification of Small Molecules

Small molecules that manipulate cell fate can be identified through a number of approaches (reviewed by Lyssiotis *et al*).<sup>6</sup> High-throughput cell-based phenotypic screening is one of the more common approaches and involves the screening of large chemical libraries using immortalized cell lines. Reporter-based cellular assays are a relatively simple example of high-throughput screening and involve the expression of a fluorescent reporter gene that is stimulated by the promoter of the gene of interest. An example of the use of reporter-based assays in stem cell research is work by Kumagai *et al*, who screened for small molecules that promote human ES cell self-renewal to maintain populations of undifferentiated cells.<sup>7</sup> This was achieved through observing green fluorescent protein expression driven by the Oct4 promoter, a transcription factor and marker of pluripotency, to ascertain if the small molecules tested affected cell fate.<sup>7</sup> Although this method is well suited for screening large libraries of molecules, it may produce a large number of false positives and results require robust further testing.<sup>6</sup>

Another high-throughput screening approach to small molecule identification is the multi-parametric high-content image-based assay, which involves the analysis of a desired phenotype at a single cell level but is time consuming and expensive.<sup>6</sup> Libraries screened using high-throughput approaches can range in size varying from large collections of >2 million compounds that are generally held by pharmaceutical companies, to smaller libraries of <10,000 compounds, which are usually used in academic research and are known to act on specific pathways. Smaller libraries are usually used when screening with cells that have a limited viability, for example primary cultures, whereas larger libraries tend to be screened using immortalized cell lines that can easily be applied to a 384 or 1536 well format.<sup>6</sup>

A more direct approach to the design of small molecule modulators may prove a profitable alternative to high-throughput screening, involving a more detailed study and assessment of the target with rational molecule design. To achieve this, a small group of compounds with known biological activity are analyzed to elucidate the mechanism by which they modulate specific pathways. Common assays used to elucidate such mechanisms include microarray gene expression analysis, protein expression analysis and affinity-based target assays. Once the mechanism of the molecule is known, it can be related to the structure and used to design more effective compounds for more focused trials. The development of EC 23, a synthetic retinoid compound and potent inducer of stem cell differentiation, was the outcome of structural design guided by biological activity.<sup>8</sup>

## Pathways that Modulate Stem Cell Activity

Modulating pathways that control stem cell proliferation and

differentiation is important to either maintain a pool of self-renewing, undifferentiated cells, or to guide stem cell fate down specific, desired lineages. Many developmental pathways can be manipulated to have an effect on stem cell proliferation and differentiation, particularly those involved in embryonic patterning, determination of cell fate and differentiation. Understanding the molecular mechanisms of such processes is important to either design molecules to act at specific stages in the pathway, or to screen a library of small molecules known to interact with the pathway.

Naturally occurring small molecules have been found to interact with important developmental pathways. Changing the concentration of such molecules can modulate the effects on cell fate, enabling controlled differentiation. Numerous synthetic small molecules have been developed to also act on primary developmental pathways to mediate stem cell behavior with great effect and increased potency. A collection of the primary developmental pathways targeted by small molecules and involved in stem cell proliferation and differentiation are outlined below.

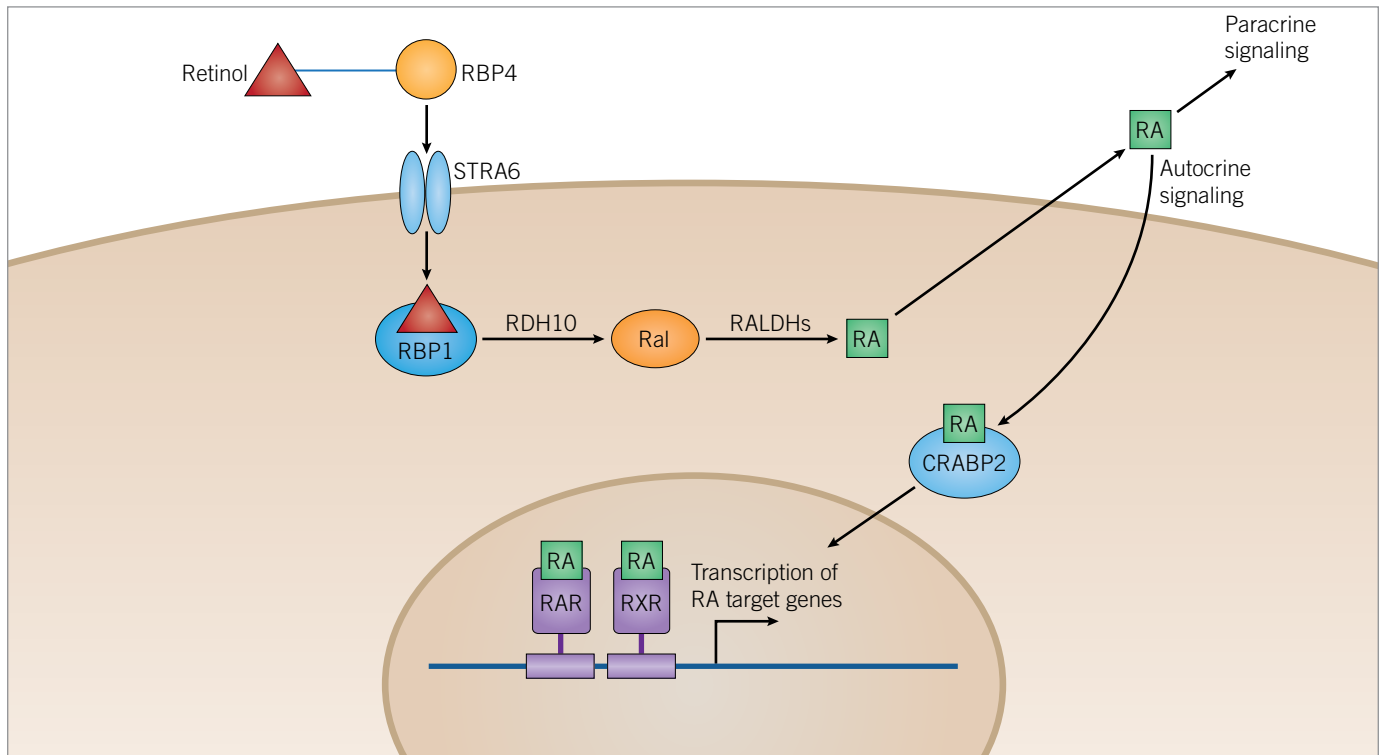
### Retinoic Acid Pathway

The retinoic acid (RA) pathway (Figure 1) is a major developmental pathway that has important roles in patterning and differentiation, particularly in the developing nervous system.<sup>9</sup> RA is an important patterning factor in the developing nervous system and acts in a concentration-dependent manner to contribute toward antero-posterior and dorsoventral patterning of the neural plate and neural tube.<sup>9</sup> Although RA is an important signaling molecule within the developing nervous system, it also has developmental roles in lung, pancreas and limb development, illustrating the importance of this pathway in embryonic development.<sup>10</sup>

RA is a metabolite of vitamin A obtained through dietary meats and vegetables, that is circulated as retinol in the blood stream bound to retinol-binding protein 4 (RBP4). Target cells obtain retinol through the action of the membrane receptor, STRA6, which promotes the entry of retinol into the cytoplasm of the target cell.<sup>9</sup> Once in the cytoplasm, retinol binds to retinol-binding protein 1 (RBP1) before being metabolized to *all-trans* retinoic acid (ATRA), in a two-step process involving the activity of the two enzymes retinol dehydrogenase 10 (RDH10) and retinaldehyde dehydrogenases (RALDHs). Newly synthesized ATRA is then bound to either cellular retinoic-acid-binding protein 1 or 2 (CRABP1, CRABP2) and can be released from the cytoplasm to act on target cells in a paracrine or autocrine manner.<sup>9</sup>

Translocation of RA to the nucleus is aided by CRABP2. Once in the nucleus RA binds to a transcription complex that includes a heterodimer of a retinoic acid receptor (RAR) and a retinoic X receptor (RXR).<sup>9</sup> There are various genes that encode RAR and RXR receptors including, *RARA*, *RARB* and *RARG*, and *RXRA*, *RXRB* and *RXRG* respectively. Combinations of these receptors bind a sequence of DNA known as the retinoic acid response element (RARE) to induce transcription of target genes. Following activation of RARs/RXRs, RA leaves the nucleus and is metabolized in the cytoplasm by the CYP26 class of enzymes.<sup>9</sup>

The role of RA in embryonic neural differentiation can be harnessed *in vitro*, with ATRA being used as a tool to differentiate stem cells in culture to form neural subtypes. An example of this is by Tanoury *et al* who used ATRA to induce neural differentiation

**Figure 1 | Retinoic Acid Pathway**

**Retinoic Acid Pathway:** Retinol is transported in the blood bound to retinol binding protein 4 (RBP4) and enters cells through the transmembrane receptor STRA6. Once in the cytoplasm retinol binds to retinol binding protein 1 (RBP1) and is metabolized to retinoic acid (RA) in a two step process catalyzed by retinol dehydrogenase 10 (RDH10) and retinaldehyde dehydrogenases (RALDHs), with retinaldehyde (Ral) as an intermediate. RA can be released from the cell and can either have a paracrine action on target cells or an autocrine action upon the cell that has metabolized it. RA is translocated into the nucleus of the target cell with the help of cellular retinoic-acid binding protein 2 (CRABP2) where it can bind retinoic acid receptors (RARs) and retinoid X receptors (RXRs) that can heterodimerize. Heterodimers of RXRs and RARs bind to DNA at a sequence known as the retinoic acid response element (RARE), activating the transcription of target genes.

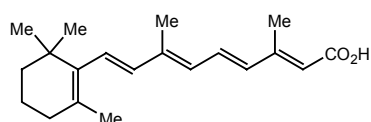
of mouse ES cells, observed through expression of the pan-neuronal marker  $\beta$ -III-tubulin; this allowed them to study the molecular mechanism of neuronal differentiation.<sup>11</sup> However, the use of naturally occurring ATRA *in vitro* is limited as the molecule is unstable and sensitive to light and heat, resulting in isomerization and breakdown of the molecule into other biologically active compounds.<sup>12</sup> This poses a problem when using ATRA for *in vitro* differentiation studies because exposure to light and changes in temperature cannot always be avoided in the laboratory. To avoid such variability, the synthetic retinoid EC 23 was developed as a stem cell differentiation tool, which is completely stable and mimics the biological activity of ATRA (Box 1).<sup>13</sup> EC 23 is a potent inducer of stem cell differentiation,<sup>13</sup> regulates neural development<sup>14</sup> and is known to activate key proteins involved in the retinoic acid signaling pathway without degrading upon exposure to light.<sup>8</sup>

## Hedgehog Pathway

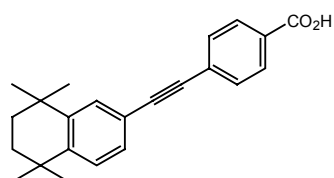
The Hedgehog (Hh) signaling pathway regulates many developmental processes, including neural cell fate and digit formation in a dose-dependent and tissue-specific manner (for details see review by Ingham and McMahon).<sup>15</sup> In the absence of a Hh ligand, Patched (Ptc 1) catalytically inhibits the translocation of Smoothened (Smo) to the membrane,<sup>16</sup> preventing it from inhibiting kinases such as protein kinase A (PKA), glycogen synthase kinase 3  $\beta$  (GSK-3 $\beta$ ) and casein kinase 1 (CK1). This

enables the phosphorylation of the transcription factors Gli1/2/3 by PKA, GSK-3 $\beta$  and CK1, resulting in proteasomal degradation of Gli2/3 to Gli2-R and Gli3-R, with Gli1 remaining at full length. Gli3-R is then translocated to the nucleus where it inhibits the transcription of Hh target genes, whilst the inhibitory protein Suppressor-of-Fused (SuFu) sequesters remaining Gli,<sup>17</sup> allowing only inactive Gli to travel to the nucleus. When Hh ligands are present they bind to and inhibit Ptc1, allowing Smo to be translocated to the membrane where it inhibits the phosphorylation of Gli1/2/3. This results in Gli1/2/3 being activated to Gli1/2/3-A, which then travels to the nucleus and stimulates the transcription of Hh target genes (Figure 2). Different modifications of the Hh ligand activate different developmental pathways, for example, in mammals Sonic Hh regulates neural cell fate whereas Indian Hh regulates digit formation.

There are many small molecules available that modulate the Hh pathway, the majority of which act on Smo (Box 2). The first small molecule discovered to affect the pathway was cyclopamine, a naturally occurring plant-derived steroidal alkaloid extracted from corn lily, which directly inhibits Smo.<sup>18,19</sup> Dysfunction of the Hh pathway has been implicated in certain types of cancer, therefore antagonists of Smo have been found to have potential chemotherapeutic effects. For example, the small molecule GDC 0449 has been demonstrated to be effective against basal cell carcinomas in phase 1 clinical trials.<sup>20</sup> Other antagonists of Smo have been discovered through high-throughput screens of

**Box 1 | Selected Small Molecules that Target the Retinoic Acid Signaling Pathway**

**ATRA (Retinoic Acid) (0695)**  
Endogenous retinoic acid receptor agonist



**EC 23 (4011)**  
Photostable synthetic retinoid

chemical libraries such as SANT1–4.<sup>22</sup> Smo agonists have also been discovered through high-throughput screening including purmorphamine, which was discovered during a screen of osteogenic compounds and has also been shown to modulate various neural patterning.<sup>23,24</sup> A family of Smo agonists (SAGs) have also been identified that promote neuronal differentiation from mouse

ES cells and can induce growth of hair on mouse skin.<sup>18</sup>

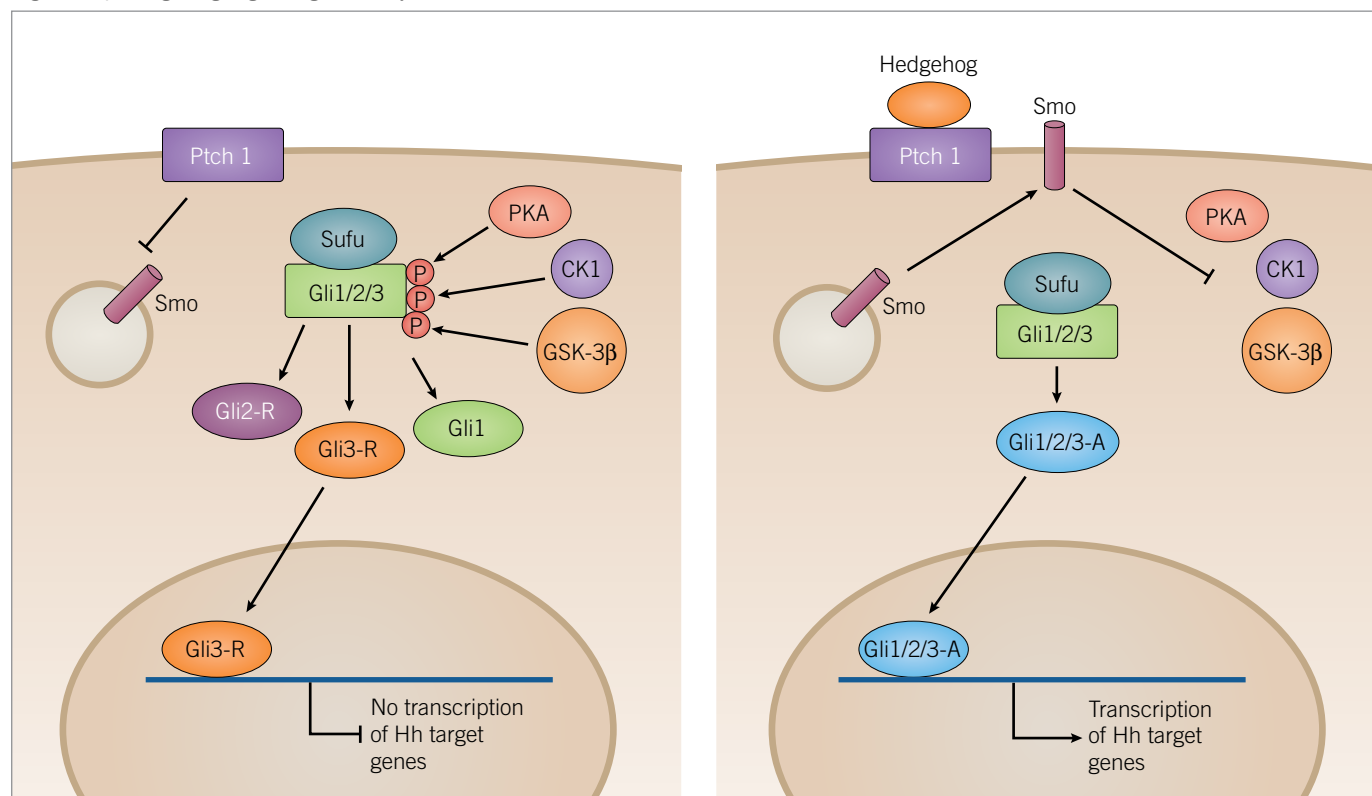
Small molecules that target the Hh pathway at alternative sites to Smo have also been identified, including Robotnikinin, one of the only molecules to act upstream of Smo.<sup>25</sup> Robotnikinin is a 12-membered macrocycle compound discovered during assays to identify recombinant Sonic Hh ligand binding molecules. Robotnikinin binds to the Hh ligand inducing a conformational change that prevents binding to Ptch 1, therefore inhibiting the Hh pathway.<sup>26</sup> Small molecules have also been identified that act downstream of Smo. JK 184 and GANT 61 are both Gli antagonists that inhibit Gli-dependent transcription of Hh target genes.

### Transforming Growth Factor- $\beta$ Superfamily

The TGF- $\beta$  superfamily includes more than 30 ligands that consist of TGF- $\beta$ , bone morphogenetic proteins (BMPs), activins, nodal ligands and related proteins. These ligands activate important developmental signaling cascades that regulate tissue differentiation and development through their roles in cell proliferation, differentiation and migration, along with having important roles in adult homeostasis.<sup>28</sup> Different subsets of ligands have different developmental roles; for example, nodal ligands are important in embryogenesis and the formation of the mesoderm and endoderm germ layers, whereas BMPs are more involved in the differentiation of skin, neurons and bone.<sup>29,30</sup>

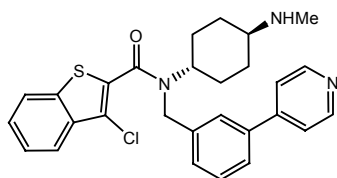
TGF- $\beta$  signaling is initiated by binding of a ligand to the TGF- $\beta$

**Figure 2 | Hedgehog Signaling Pathway**

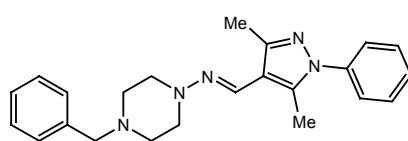


In the absence of ligand binding (left) Ptch1 inhibits translocation of Smoothened (Smo) to the membrane, resulting in the phosphorylation of the Sufu-Gli1/2/3 complex by the kinases, PKA, CK1 and GSK-3 $\beta$ . Phosphorylation results in the truncation of Gli1/2/3 forming inactive Gli2-R and Gli3-R, along with full length Gli1. Gli3-R is translocated to the nucleus and inhibits the transcription of target genes. In the presence of a Hedgehog (Hh) ligand (right) Ptch1 is inhibited and Smo is released to the membrane where it inhibits kinase activity. PKA, CK1 and GSK-3 $\beta$  are no longer able to phosphorylate Gli1/2/3 allowing it to remain in its full length active form. Active Gli1/2/3 then travels to the nucleus where it can induce transcription of Hh target genes.

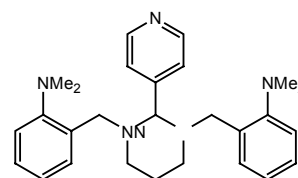
## Box 2 | Selected Small Molecules that Target the Hedgehog Signaling Pathway



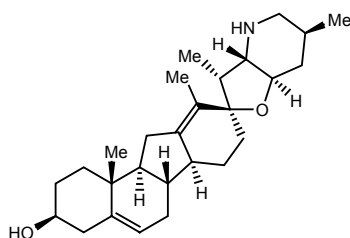
**SAG (4366)**  
Potent Smo agonist



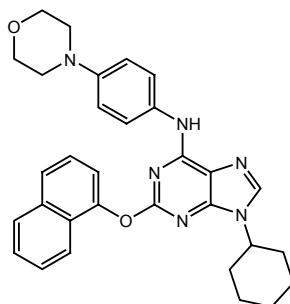
**SANT-1 (1974)**  
Potent Smo antagonist



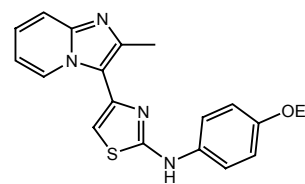
**GANT 61 (3191)**  
Gli 1/2 antagonist



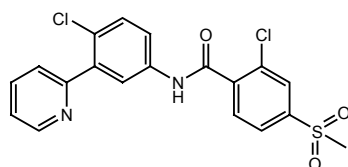
**Cyclopamine (1623)**  
Smo inhibitor



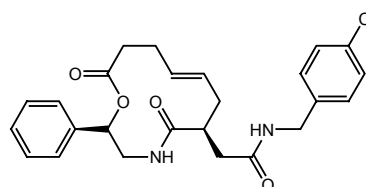
**Purmorphamine (4551)**  
Smo agonist



**JK 184 (3341)**  
Potent Gli antagonist



**GDC 0449**  
Smo inhibitor



**Robotnikinin**  
Inhibits Sonic Hh-Ptch 1 binding;  
induces Sonic Hh conformational change

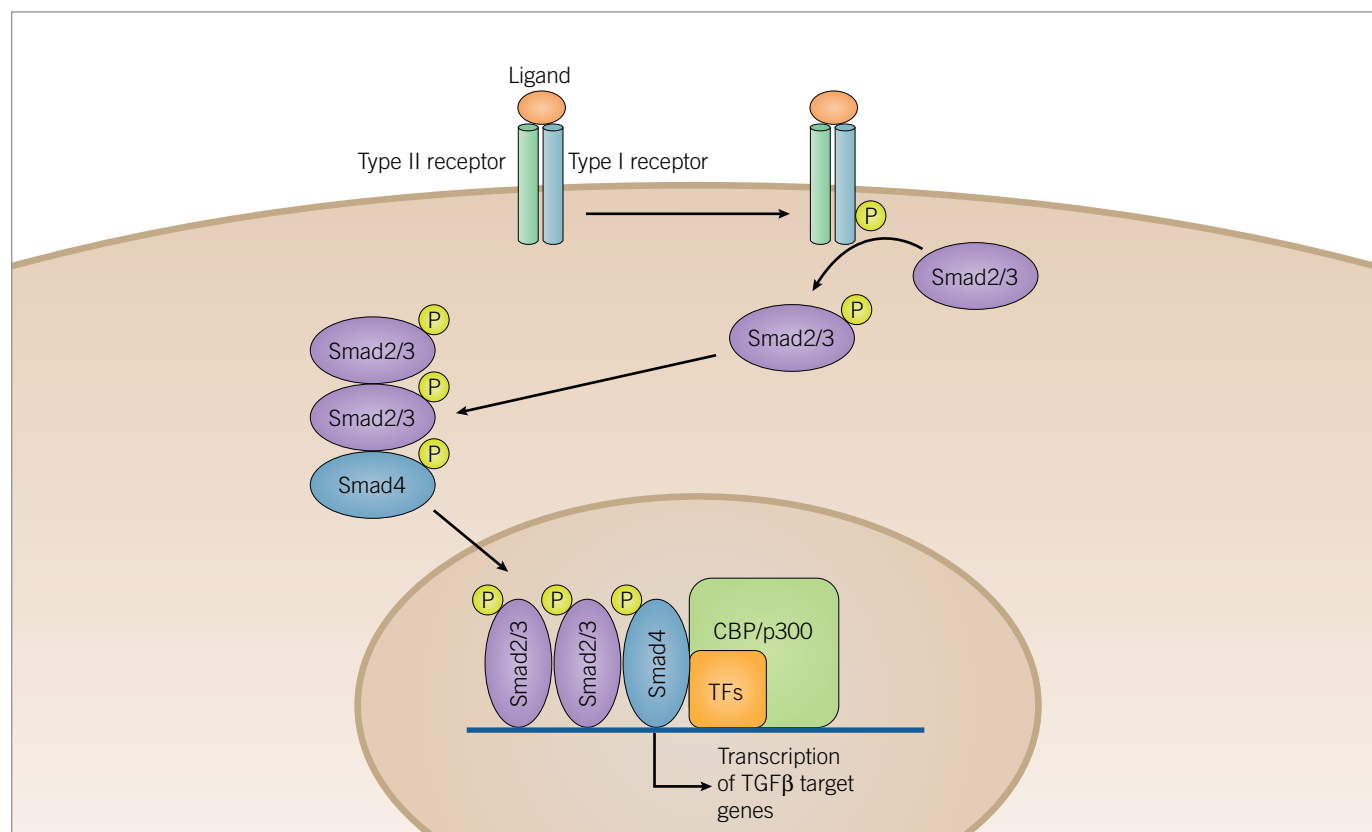
family complex of receptors at the cell surface that consist of two type I and two type II serine/threonine kinases. Ligand binding induces transphosphorylation of the type I receptor by the type II kinase activating it, and promotes the phosphorylation of Smad2/3, which then complexes with Smad4.<sup>28</sup> The heterooligomeric complex of Smads then translocates to the nucleus where it can regulate the transcription of TGF- $\beta$  target genes through association with transcription factors and co-activators such as CBP or p300 (Figure 3). This pathway can be negatively regulated by Smad6/7, which act as a negative feedback mechanism to prevent the phosphorylation of Smad2/3 and 4 by forming stable associations with activated type I receptors.<sup>31</sup>

Most of the small molecule inhibitors that target the TGF- $\beta$  superfamily pathway designed to date act on the type I kinase of the heterotrimeric receptor complex (Box 3). SB 431542 is a small molecule that has effects on many biological processes including proliferation, differentiation and promotion of sheet formation of endothelial cells derived from ES cells.<sup>32</sup> It achieves this through blocking the action of activin receptor-like kinases 4, 5 and 7 (ALK4, TGF- $\beta$ 1 and ALK7 respectively), which are type I receptor serine/threonine kinases, as do other small molecules including A 83-01 and SB 505124. SB 431542 has been shown to promote differentiation of glioblastoma CS cells<sup>33</sup> and replace one of the factors used to generate iPS cells,<sup>34</sup> therefore it is an important tool in many aspects of stem cell research. In contrast

to the majority of TGF- $\beta$  superfamily modulators, the small molecule ITD 1 acts through promoting the proteasomal degradation of the type II receptor, selectively targeting TGF- $\beta$  signaling rather than any of the other members of the TGF- $\beta$  superfamily. ITD 1 has been used *in vitro* to promote the differentiation of cardiomyocytes from ES cells.<sup>35</sup> Dorsomorphin is an antagonist of the pathway identified by a whole organism zebrafish developmental screen.<sup>36</sup> The compound blocks ALK 2, 3 and 6 which are associated with the BMP pathway and enhances myocardial differentiation from mouse ES cells. IDE 1 and 2 are alkyl hydrazone derivatives that are agonists of TGF- $\beta$  signaling and can induce differentiation of endoderm from ES cells through activation of TGF- $\beta$  signaling.<sup>37</sup>

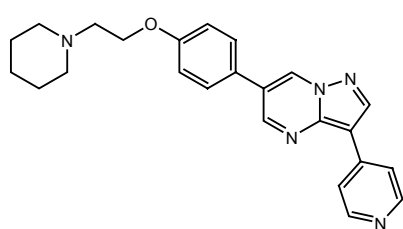
## Canonical Wnt Pathway

The canonical Wnt pathway is one of the most studied biological signaling pathways and has major roles in proliferation, self-renewal and differentiation of stem and progenitor cells during development.<sup>38</sup> In particular, the pathway has been shown to have a central role in bone formation, hematopoiesis and neural differentiation. However, dysregulation of the pathway can result in the onset of cancer, due to increased activation leading to increased cellular proliferation. The Wnt pathway has been implicated in various types of cancer including breast, brain and colon cancers, and has been proposed to play a role in their

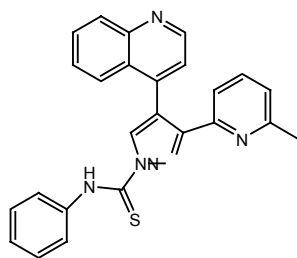
Figure 3 | TGF- $\beta$  Signaling Pathway

Ligands of the TGF- $\beta$  superfamily bind to a serine/threonine kinase receptor complex of type I and type II subunits. Activation of the receptor complex induces transphosphorylation of the type I receptor by the type II receptor. Smad2/3 is then phosphorylated by the activated type I receptor and complexes with Smad4 before being translocated to the nucleus. Once in the nucleus, activated Smad complexes regulate transcription of target genes through interaction with transcription factors (TFs) and co-activators such as CBP or p300.

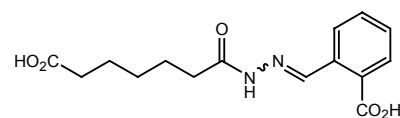
### Box 3 | Selected Small Molecules that Target the TGF- $\beta$ Signaling Pathway



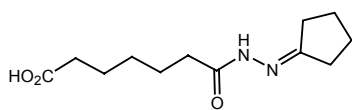
**Dorsomorphin (3093)**  
ALK2, ALK3 and ALK6 inhibitor



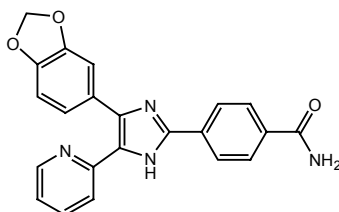
**A 83-01 (2939)**  
Selective inhibitor of TGF- $\beta$ RI,  
ALK4 and ALK 7



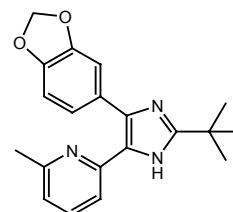
**IDE 1 (4015)**  
Induces Smad2 phosphorylation



**IDE 2 (4016)**  
Induces Smad2 phosphorylation



**SB 431542 (1614)**  
Potent and selective inhibitor  
of TGF- $\beta$ RI, ALK4 and ALK7



**SB 505124 (3263)**  
Selective inhibitor of TGF- $\beta$ RI,  
ALK4 and ALK7

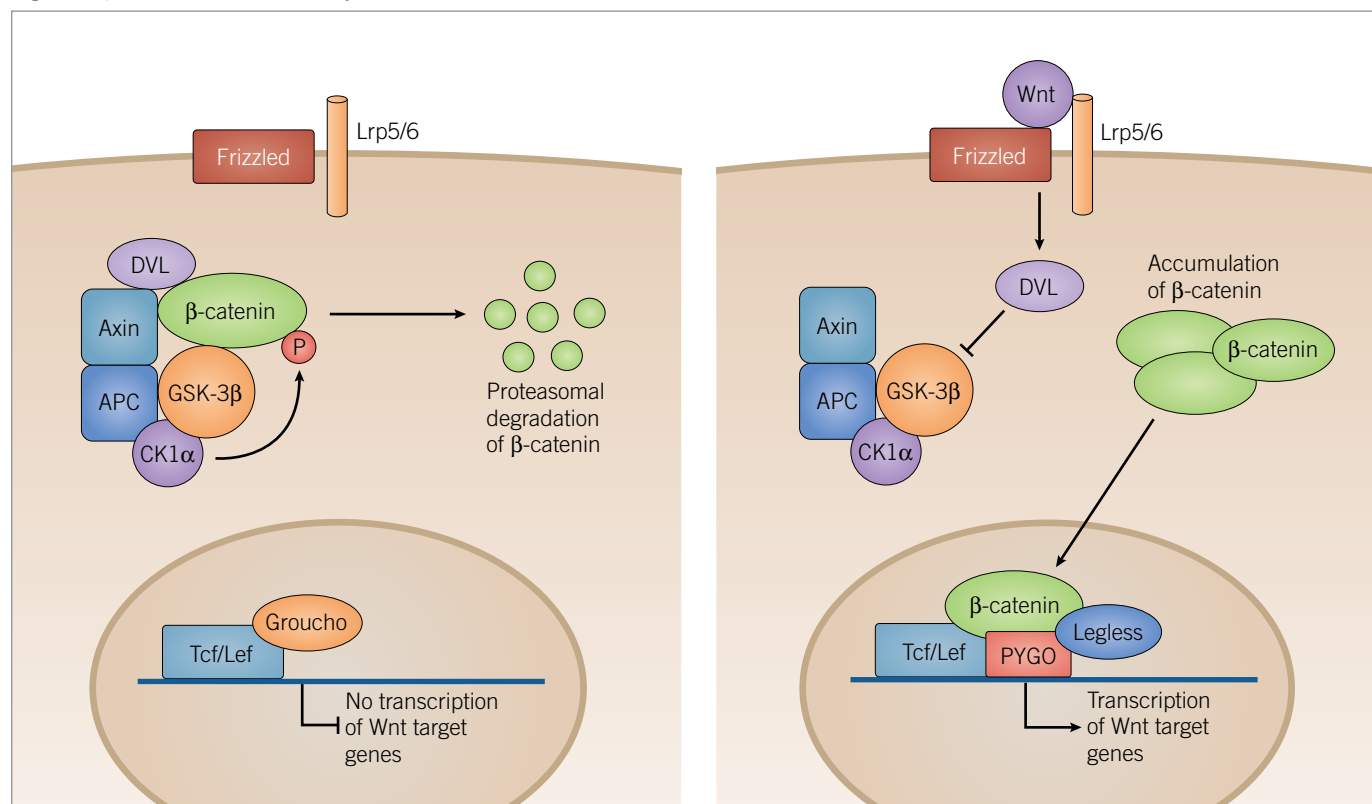
malignancy. Mutations to molecules central to the pathway are the most prevalent genetic alteration in colorectal carcinomas (reviewed by Reya and Clevers).<sup>38</sup> The central involvement of the canonical Wnt pathway in both stem cell biology and cancer pathogenesis makes it a desirable target for small molecule modulation.

Signaling of the canonical Wnt pathway (Figure 4) is stimulated by the binding of Wnt ligand to a receptor complex incorporating a member of the Frizzled family and a member of the LDL-receptor family (Lrp5/6).<sup>38</sup> When Wnt receptors are unoccupied, a destruction complex forms due to the association of the scaffolding proteins adenomatous polyposis coli (APC) and axin, and the kinases CK1 $\alpha$  and GSK-3 $\beta$ .  $\beta$ -catenin, the main cytoplasmic signaling molecule in the pathway, is bound to axin and sequentially phosphorylated by CK1 $\alpha$  and GSK-3 $\beta$ , targeting it for proteasomal degradation.<sup>38</sup> In the absence of Wnt, T-cell factor/lymphoid enhancer factor (Tcf/Lef) in the nucleus associates with co-repressors such as Groucho to inhibit the transcription of Wnt target genes, thus decreasing stem cell renewal and proliferation. In the presence of Wnt ligand, the action of the destruction complex is inhibited through a Dishevelled (DVL) dependent mechanism, resulting in a cytoplasmic accumulation of  $\beta$ -catenin.  $\beta$ -catenin is then trans-

located to the nucleus where it can engage Tcf/Lef, along with docking proteins such as Legless and co-activators from the PYGO family, transiently converting Tcf/Lef into transcriptional activators. The effector of the canonical Wnt pathway is the Tcf/Lef- $\beta$ -catenin-Legless-PYGO nuclear complex, which promotes transcription of target genes.<sup>39</sup>

Screening of chemical libraries has identified several small molecules that can inhibit or activate Wnt signaling (Box 4), with targets for modulation including CK1 $\alpha$ , GSK-3 $\beta$  and  $\beta$ -catenin itself. Benzothiazole derived compounds have been synthesized to antagonize the pathway and can be split into two separate groups relating to their mechanism: IWP compounds that inhibit Wnt processing and secretion through inactivation of Porcupine (PORCN), and IWR compounds that enhance the activity of the destruction complex, promoting the breakdown of  $\beta$ -catenin.<sup>40</sup> IWR compounds stabilize axin which is the concentration-limiting factor of the destruction complex. XAV 939 is a tankyrase inhibitor that antagonizes the pathway in a similar fashion.<sup>41</sup> XAV 939 has been shown to inhibit the growth of APC-deficient colorectal cancer cells, highlighting the therapeutic potential of this compound and the importance of understanding and targeting such pathways. Calphostin C (PKF 115584) is another small molecule that was identified in such a screen, and was found to target the interaction of  $\beta$ -catenin with Tcf/Lef to inhibit Wnt signaling.<sup>42</sup>

**Figure 4 | Canonical Wnt Pathway**



In the absence of Wnt signaling (left) adenomatous polyposis coli (APC) and axin complex and bind newly synthesized  $\beta$ -catenin, forming a destruction complex. Two kinases within the destruction complex, CK1 $\alpha$  and GSK-3 $\beta$ , phosphorylate  $\beta$ -catenin targeting it for proteasomal degradation. In the nucleus T-cell factor/lymphoid enhancer factor (Tcf/Lef) DNA-binding proteins repress target gene transcription through association with repressor proteins such as Groucho. In the presence of Wnt ligand (right) Frizzled and Lrp5/6 are activated and the destruction complex is inhibited through a Dishevelled (DVL) dependent mechanism, preventing the phosphorylation of  $\beta$ -catenin.  $\beta$ -catenin then accumulates in the cytoplasm and is translocated to the nucleus where it engages transcription factors, Tcf/Lef and docking proteins of the Legless family, while associating with members of the PYGO family of co-activators. The  $\beta$ -catenin-Tcf/Lef-Legless-PYGO nuclear complex promotes transcription of Wnt target genes.

GSK-3 $\beta$  inhibitors are the most common class of compounds that activate the Wnt pathway, an example of which is CHIR 99021.<sup>43</sup> They achieve this through the deactivation of the destruction complex, thus promoting  $\beta$ -catenin accumulation within the cytoplasm. GSK-3 $\beta$  is a desirable drug target, as upregulation of the kinase is associated with the pathogenesis of not only many cancers but also diseases such as type II diabetes and Alzheimer's disease. With this in mind, a chemical library of over 4,000 compounds was screened to identify the novel compound 3F8, which promotes Wnt signaling through inhibition of GSK-3 $\beta$ , with greater potency than other commonly used GSK-3 $\beta$  inhibitors.<sup>44</sup> Similarly, CK1 $\alpha$  can be inhibited by compounds such as D 4476 and (R)-CR8 to prevent degradation of  $\beta$ -catenin and promote Wnt signaling.  $\beta$ -catenin itself can also be stabilized through the action of the small molecule, prostaglandin E<sub>2</sub> that acts via cAMP/PKC activity.<sup>45</sup>

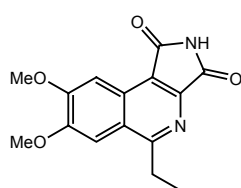
## Fibroblast Growth Factor and Notch Signaling Pathways

Similarly to the TGF- $\beta$  superfamily, fibroblast growth factors (FGFs) are a large family of secreted molecules that have a wide-range of biological roles in embryonic development and the homeostasis of adult cells.<sup>46</sup> A variety of signaling pathways can be activated by the complex expression of FGF ligands and such pathways are tightly regulated, as when dysfunction arises,

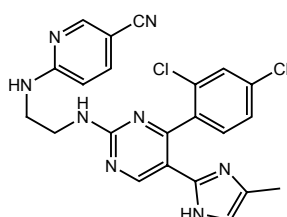
cancer can develop.<sup>47</sup> FGF ligands are commonly used *in vitro* in the culture of ES cells, either to maintain cells in an undifferentiated state<sup>48</sup> or to promote neural differentiation of cultures. FGF signal transduction begins with the binding of ligands to their specific receptors, resulting in dimerization and phosphorylation of multiple tyrosine residues on the receptors through the intrinsic tyrosine kinase activity of the receptors themselves. Receptor activation results in the recruitment of signaling complexes that in turn activate a cascade of phosphorylation events.<sup>49</sup> The specific molecules involved in this signaling cascade depend on the specific ligand and receptor activated, however there are three main pathways involved in FGF signaling including: phospholipase C  $\gamma$  (PLC $\gamma$ ), phosphoinositide-3-kinase (PI 3-K) and mitogen-activated protein kinase (MAPK) pathways.

Due to the diversity of FGF signaling and its interaction with other signaling pathways, small molecules usually target the initial stages of the pathway (Box 5). A range of small molecules have been designed to inhibit the FGF receptor, including PD 173074,<sup>50</sup> FIIN 1<sup>51</sup> and PD 161570.<sup>52</sup> Other targets for modulation are specific pathways within FGF signaling, for example, the MAPK pathway can be inhibited by the small molecule PD 0325901, which has undergone phase 2 clinical trials for use as a chemotherapeutic agent against certain types of cancer<sup>53</sup> and can also be used in the formation of iPS cells.<sup>54</sup>

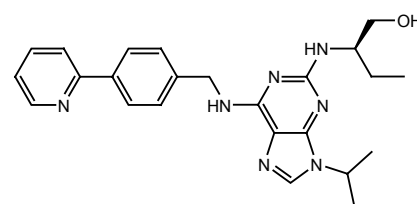
### Box 4 | Selected Small Molecules that Target the Wnt Signaling Pathway



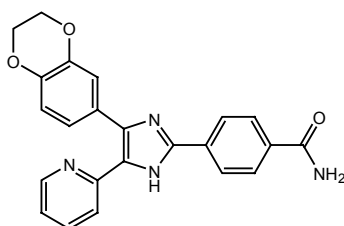
**3F8 (4083)**  
Potent and selective GSK-3 $\beta$   
inhibitor



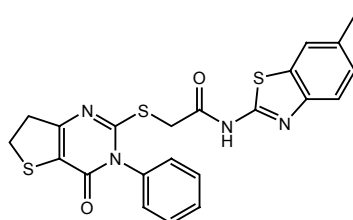
**CHIR 99021 (4423)**  
Potent and selective GSK-3 $\beta$   
inhibitor



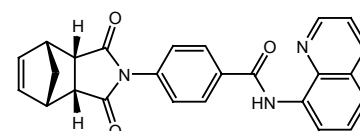
**(R)-CR8 (4015)**  
CK1 inhibitor



**D 4476 (2902)**  
CK1 inhibitor

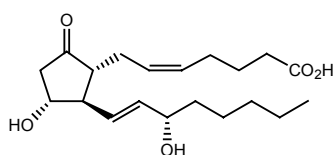


**IWP 2 (3533)**  
PORCN inhibitor

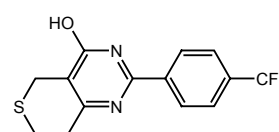


(and enantiomer)

**endo-IWR 1 (3532)**  
Axin stabilizer



**Prostaglandin E<sub>2</sub> (2296)**  
 $\beta$ -catenin stabilizer



**XAV 939 (3748)**  
Tankyrase inhibitor, stabilizes axin



The Notch signaling pathway is involved in the creation of specific cellular niches due to the ability of its ligands to only activate signaling in adjacent cells rather than the surrounding area. An example of this is the neural cell niche that contains a balance between differentiated neural cells and progenitor cells.<sup>55</sup> It has proven difficult to develop small molecules that modulate this pathway because many of the molecular targets are not unique to the Notch pathway. However, two antagonists of Notch are DAPT<sup>56</sup> and MRK 003,<sup>57</sup> which have been shown to reduce neural differentiation and promote programmed cell death respectively.

## Promotion of ES Cell Self-Renewal

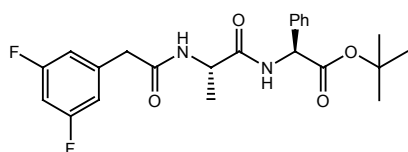
Spontaneous differentiation poses a problem in the long-term culture of ES cells *in vitro*, which is why a cocktail of proteins and exogenous factors are included in culture media to inhibit differentiation through the promotion of pathways such as the MAPK pathway. Usually, human ES cells require the addition of basic FGF into the medium along with either being cultured on a feeder layer of mouse embryonic fibroblasts (MEFs) or in conditioned media to promote their continued self-renewal. The use of MEFs as a feeder layer may restrict the use of human ES cells in regenerative medicine due to concern of xenogenic contamination; this would result in increased variability of results, along with limiting the use of ES cells on a large scale. This is why, although the exact identities of the active factors within the feeder layer remain unknown, efforts are being made to develop protocols to promote the proliferation of ES cells without the need for feeder layers.<sup>58</sup> Similarly, mouse ES cells require the addition of leukemia inhibitory factor (LIF) and BMP 4 to their culture media, which may result in variability due to the use of different batches. The use of serum products and feeder layers may also bias stem cell fate toward specific lineage types, through the activation of certain signaling pathways (reviewed by Xu *et al*),<sup>59</sup>

which has led many groups to turn to high-throughput screening of small molecules to overcome these issues (Box 6).

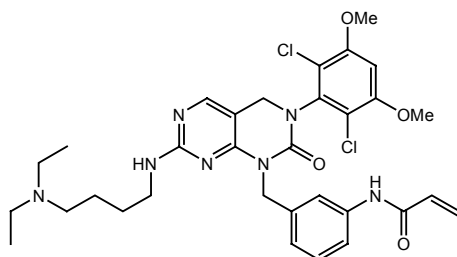
Compounds that have been found to promote self-renewal in mouse ES cells include CHIR 99021, which is a GSK-3 $\beta$  inhibitor that suppresses the Wnt pathway, and PD 0325901 which inhibits the MEK pathway. Pluripotin is a dihydropyrimidine that promotes the long-term maintenance of mouse ES cells without the need for feeder layers, LIF, BMPs or Wnt proteins.<sup>60</sup> It acts independently of the main stem cell signaling pathways including the Wnt and BMP pathways. Further structure-function investigations found that pluripotin binds to Ras GTPase activating protein (RasGAP) and extracellular signal-related kinase 1 (ERK1), two proteins that have differentiation-inducing activity. Pluripotin simultaneously inhibits RasGAP and ERK1, suppressing their roles in differentiation; this is sufficient for long term self-renewal of mouse ES cells. Similarly, ID 8 is an indole derivative that has been shown to promote mouse ES cell proliferation in serum-free conditions.<sup>61</sup>

A potent inhibitor of GSK-3 $\beta$ , 6-bromoindirubin-3'-oxime (BIO), has been found to promote self-renewal in human ES cells via modulation of the Wnt pathway.<sup>62</sup> However, another GSK-3 $\beta$  inhibitor, TWS 119, has been shown to induce neural differentiation of ES cells,<sup>63</sup> demonstrating the complexity of such signaling pathways. Another modulator of the Wnt pathway that has functions in the long term maintenance of ES cells is IQ 1, which has been shown to promote long term expansion of ES cells and prevent spontaneous differentiation.<sup>64</sup> The development of small molecules such as these that can maintain undifferentiated ES cells and promote their proliferation, is important to reduce variability of culture conditions and to eliminate animal products from culture media to advance research into the therapeutic benefit of stem cell therapy.

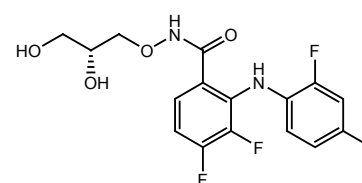
### Box 5 | Selected Small Molecules that Target the FGF and Notch Signaling Pathways



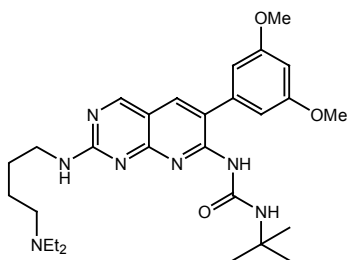
**DAPT (2634)**  
Notch pathway inhibitor



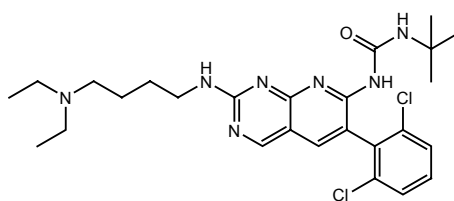
**FIN 1 (4002)**  
Potent FGF receptor inhibitor



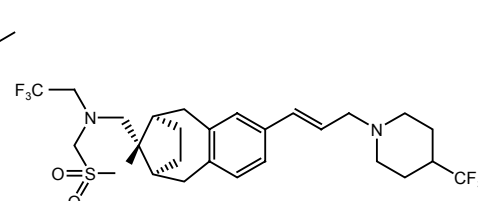
**PD 0325901 (4192)**  
Potent MEK inhibitor



**PD 173074 (3044)**  
Selective FGFR1/3 inhibitor



**PD 161570 (3724)**  
Selective FGF receptor inhibitor



**MRK 003**  
Notch pathway inhibitor

## Somatic Cell Reprogramming

In 2006, Yamanaka's group were able to reprogram mouse somatic cells into pluripotent populations of cells that could differentiate into cells representative of the three embryonic germ layers.<sup>65</sup> The reprogramming of somatic cells results in a phenotype similar to that of ES cells. The pluripotent populations formed have a similar capacity for differentiation and are termed iPS cells. iPS cells are thought to have potential therapeutic benefits as they could be derived from a patient's own somatic cells, reprogrammed and differentiated into specific cellular subtypes for use in regenerative therapies and autologous transplantation. This avenue of stem cell research could also have an impact on *in vitro* disease modeling, as somatic cells could be retrieved from an individual with a specific disease, reprogrammed and differentiated, with resultant cells being used to elucidate mechanisms of pathogenesis. Some of the ethical concerns regarding nuclear transfer and the use of ES cells could also be overcome with the development of iPS cells. However, before iPS cell-based therapies can be further developed, the reprogramming protocol needs to be optimized as the yield of iPS cells obtained is usually small, and it can take weeks to generate populations of cells.

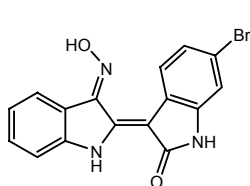
Traditional reprogramming protocols involve exogenous genetic manipulation of key pluripotency transcription factors such as Oct4, Sox2, Klf4 and c-Myc using retroviruses, known as the four-

factor method. However, for iPS cell-based therapies to be considered for use in medicine, the use of retroviruses and transcription factors associated with tumorigenesis must be eliminated. This is why chemically defined approaches to reprogramming somatic cells have been developed rather than genetic manipulation through transcription factors. To achieve this, screens of small molecules that have the potential to replace one or all of these transcription factors have been conducted (Box 7). One such screen studied the ability of compounds to stimulate dedifferentiation of lineage committed myoblasts; dedifferentiated cells were then stimulated to differentiate into specific cellular subtypes. Reversine, a small molecule that is a 2,6-disubstituted purine analog was identified in such a screen.<sup>66</sup> It was shown to act via MEK signaling and non-muscle myosin II heavy chain, and has dedifferentiation activity on cell types including dermal fibroblasts with high efficiency *in vitro* and *in vivo*.<sup>67</sup>

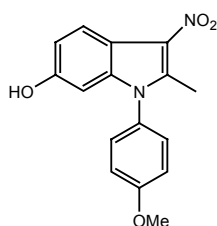
iPS cell generation has also been improved through the application of small molecules that target histone modifications, such as valproic acid and trichostatin A, both of which inhibit histone deacetylases (HDACs). Valproic acid and trichostatin A both enhance the yield of iPS cells through the traditional four-factor dedifferentiation method, but valproic acid has also been shown to enhance three-factor reprogramming (minus c-Myc)<sup>68</sup> and two-factor reprogramming (without c-Myc and Klf4).<sup>69</sup> DNA and histone methylation are other targets for small molecules in somatic cell reprogramming. Application of the DNA methyltransferase inhibitor, 5-azacytidine, can result in a yield 100-fold higher than the four-factor method alone.<sup>70</sup> BIX 01294 is a small molecule with a similar mechanism in that it enhances iPS cell conversion through inhibiting G9a histone methyltransferase, particularly when used in combination with Bay K 8644.<sup>71</sup> This combination of small molecules can be used to reduce the number of transcription factors used in reprogramming to just two (Oct4 and Sox2). Moreover, combinations of small molecules have also been shown to induce iPS cell reprogramming in the absence of all of the transcription factors from the four factor-method.<sup>72</sup>

Another target for small molecules in the reprogramming of somatic cells is the TGF- $\beta$  pathway, as inhibitors of the pathway have been used in the generation of both mouse and human iPS cells. A 83-01 is an inhibitor of TGF- $\beta$ R1, ALK4 and ALK7 activation, whilst RepSox is a selective inhibitor of TGF- $\beta$ R1. Both of these compounds have roles in the generation of iPS cells.<sup>73</sup>

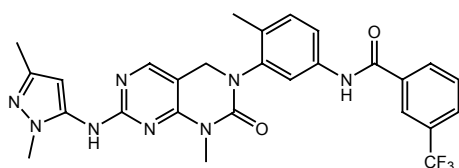
### Box 6 | Selected Small Molecules for ES Self-Renewal



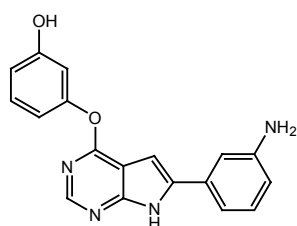
**BIO (3194)**  
Potent GSK-3 inhibitor



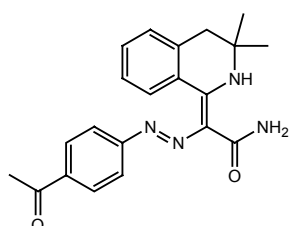
**ID 8 (3853)**  
Sustains self-renewal  
and pluripotency of ESCs



**Pluripotin (4433)**  
ERK1 and RASGAP inhibitor



**TWS 119 (3835)**  
Potent GSK-3 $\beta$  inhibitor

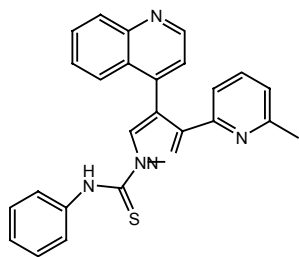


**IQ 1 (4713)**  
Wnt pathway inhibitor

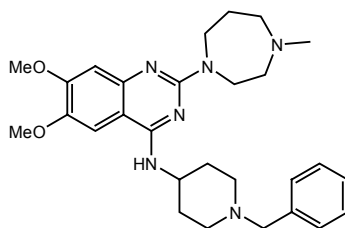
## Conclusion

Small molecules have significant roles in stem cell research as their application can regulate stem cell proliferation, or they can be utilized to guide stem cell fate down specific differentiation lineages. Their role in stem cell biology is determined by their action on specific key signaling pathways; therefore, high-throughput screening and studying the mechanism of such molecules can provide insight into the mechanisms of key signaling pathways. As stem cell research becomes more popular and better understood, the demand for small molecules that can modulate cell fate grows, because investigators require reliable molecular tools to reproducibly control stem cell fate and proliferation. For stem cell therapy to become widely available, it

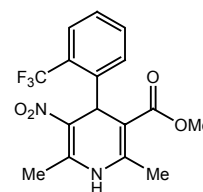
## Box 7 | Selected Small Molecules that Target the FGF and Notch Signaling Pathways



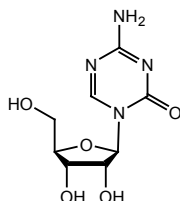
**A 83-01 (2939)**  
Potent inhibitor of TGF- $\beta$ RI, ALK4 and ALK7



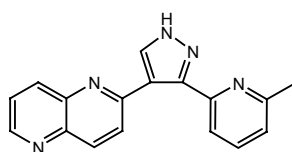
**BIX 01294 (3364)**  
GLP/G9a histone methyltransferase inhibitor



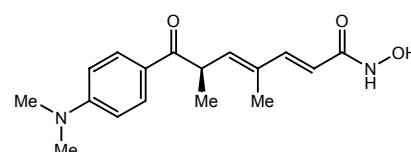
**(±)-Bay K 8644 (1544)**  
Ca<sup>2+</sup>-channel activator (L-type)



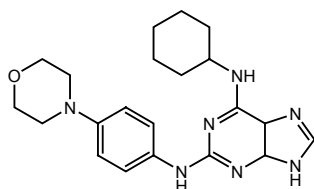
**5-Azacytidine (3842)**  
DNA methyltransferase inhibitor



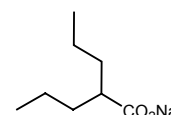
**RepSox (3742)**  
Potent and selective TGF- $\beta$ RI inhibitor



**Trichostatin A (1406)**  
Potent and selective HDAC inhibitor



**Reversine**  
MEK inhibitor



**Valproic acid, sodium salt (2815)**  
HDAC inhibitor

is important that large populations of pluripotent stem cells can be maintained in culture and that guided differentiation of such cells is an efficient and reproducible process.

Small molecules play an important role in the *in vitro* culture of such cells and have the potential to enable guided differentiation for cell replacement therapies. Moreover, small molecules that modulate integral stem cell pathways may play a prominent role in the modulation of endogenous stem cell and progenitor cell

populations. Small molecules may also play a role in encouraging self-repair through promoting the proliferation and differentiation of host cells using pharmacological cues. Therefore, the development and characterization of small molecules that interact with key signaling pathways that control self-renewal and differentiation is integral to the advance and optimization of stem cell research, and may have applications in drug discovery and regenerative medicine.

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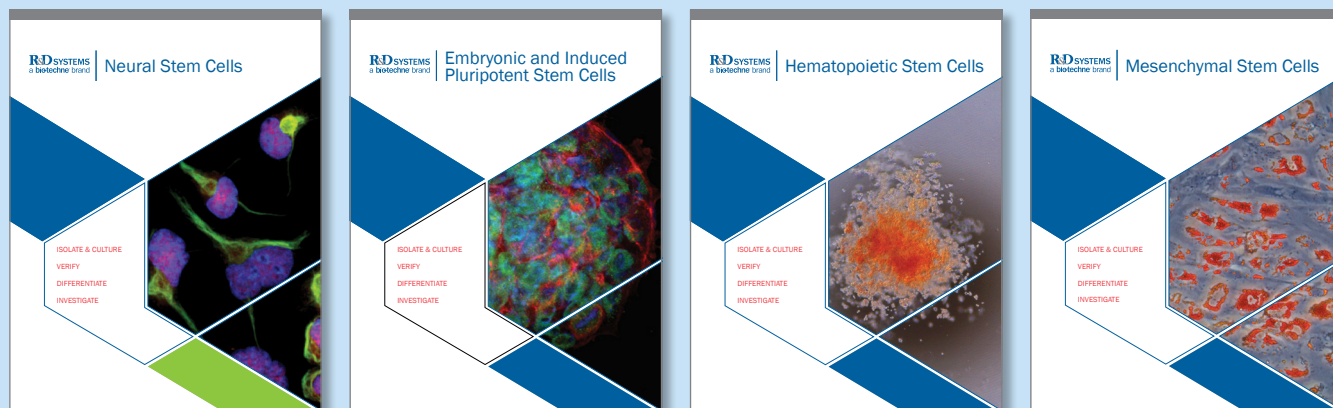
## Stem Cell Compounds Available from Tocris

Catalog No.	Product Name	Primary Action
<b>Stem Cell Reprogramming and Differentiation</b>		
4055	L-Ascorbic acid	Enhances the generation of iPSCs; increases reprogramming efficiency
3842	5-Azacytidine	DNA methyltransferase inhibitor; improves reprogramming efficiency
1544	(±)-Bay K 8644	Ca <sup>2+</sup> -channel activator (L-type); enhances iPSC conversion
3364	BIX 01294	GLP/G9a HMTase inhibitor; potentiates induction of iPSCs
4423	CHIR 99021	Highly selective GSK-3 inhibitor; enables reprogramming of mouse embryonic fibroblasts into iPSCs
4489	DBZ	Notch pathway inhibitor; stimulates formation of iPSCs from keratinocytes
4703	3-Deazaneplanocin A	Histone methyltransferase inhibitor; enhances Oct4 expression in chemically-induced pluripotent stem cells
1398	Kenpaullone	CDK inhibitor; generates iPSCs from somatic cells
4887	OAC-1	Oct4 activator; enhances iPSC reprogramming efficiency
4192	PD 0325901	Potent inhibitor of MEK1/2; enhances generation of iPSCs
2653	Pifithrin-μ	Inhibitor of p53-mitochondrial binding; enhances reprogramming efficiency
3742	RepSox	Selective TGF-βRI inhibitor; enhances reprogramming efficiency
3295	RG 108	Non-nucleoside DNA methyltransferase inhibitor; enhances efficiency of iPSC generation
3845	Thiazovivin	Improves the efficiency of fibroblast reprogramming and induction of iPSCs
3852	Tranylcypromine	Irreversible inhibitor of LSD1; enables reprogramming of mouse embryonic fibroblasts into iPSCs
1406	Trichostatin A	Potent HDAC inhibitor; induces accelerated dedifferentiation of primordial germ cells
0761	TTNPB	Retinoic acid analog; enhances efficiency of reprogramming in iPSCs
2815	Valproic acid, sodium salt	HDAC inhibitor; enables induction of pluripotent stem cells from somatic cells
<b>Stem Cell Differentiation</b>		
2840	AICAR	AMPK activator; promotes osteogenic differentiation of bone marrow-derived MSCs
3842	5-Azacytidine	DNA methyltransferase inhibitor; induces differentiation of MSCs into cardiomyocytes
3851	Cardiogenol C	Induces cardiomyocyte differentiation in ESCs
4475	Cardionogen 1	Inhibitor of Wnt/β-catenin signaling; induces ES cell cardiac differentiation
5233	CCG 1423	Induces intermediate mesoderm differentiation from ESCs; Rho/SRF pathway inhibitor
5329	CKI 7	CK1 inhibitor; induces retinal cell differentiation from human ESCs and iPSCs
1623	Cyclopamine	Smo inhibitor; induces differentiation of hESCs into hormone expressing endocrine cells
2634	DAPT	γ-secretase inhibitor; induces neuronal differentiation
1126	Dexamethasone	Anti-inflammatory glucocorticoid; induces differentiation of human MSCs
4126	DMH-1	Selective ALK2 inhibitor; promotes iPSC neurogenesis in combination with SB 431542 (Cat. No. 1614)
3176	DMSO, sterile filtered	Improves responsiveness of hESCs and hiPSCs to differentiation signals
3093	Dorsomorphin	ALK2, ALK3 and ALK6 inhibitor; promotes cardiomyocyte differentiation in mouse ESCs
1041	1-EBIO	K <sub>Ca</sub> activator; promotes ESC differentiation into cardiomyocytes
4011	EC 23	Photostable synthetic retinoid; induces differentiation of stem cells
1261	EHNA	Adenosine deaminase inhibitor; suppresses spontaneous differentiation of human ESCs
5254	FH 1	Enhances iPSC-derived hepatocyte differentiation and maturation
0927	Fluoxetine	5-HT reuptake inhibitor; induces differentiation of neuronal precursors
1099	Forskolin	Adenylyl cyclase activator; induces neuronal differentiation
4918	GSA 10	Smo receptor agonist; induces differentiation of mesenchymal progenitor cells into osteoblasts
2845	IBMX	PDE inhibitor; facilitates differentiation of neural progenitor cells
4015	IDE 1	Induces definitive endoderm formation in mouse and human ESCs
4016	IDE 2	Induces definitive endoderm formation in mouse and human ESCs
5513	Isotretinoin	Inducer of neuronal differentiation; endogenous agonist for retinoic acid receptors
4439	ISX 9	Induces neuronal differentiation of SVZ progenitors; also induces cardiomyogenic differentiation
5068	ITD 1	Selective inhibitor of TGF-β signaling; induces cardiomyocyte differentiation in ESCs
5214	IWP 4	Potent inhibitor of Wnt/β-catenin signaling; induces cardiomyocyte differentiation of human ESCs and iPSCs
4513	Kartogenin	Potently induces chondrogenesis in MSCs
4888	KHS 101	Selective inducer of neuronal differentiation in hippocampal neural progenitors
4731	KY 02111	Inhibits canonical Wnt signaling; promotes differentiation of human ESCs and iPSCs into cardiomyocytes

Catalog No.	Product Name	Primary Action
2864	Metformin	Activator of LKB1/AMPK; enhances neurogenesis
3656	Neurodazine	Induces neurogenesis in mature skeletal muscle cells
5186	Neuropathiazol	Selective inducer of neuronal differentiation in hippocampal neural progenitors
3854	1-Oleoyl lysophosphatidic acid sodium salt	LPA <sub>1</sub> and LPA <sub>2</sub> agonist; inhibits differentiation of neural stem cells into neurons
4076	P7C3	Neuroprotective compound; enhances neurogenesis <i>in vivo</i>
3044	PD 173074	Selective FGFR1 and -3 inhibitor; inhibits proliferation and differentiation of oligodendrocyte progenitors
0238	O-Phospho-L-serine	Group III mGlu agonist; enhances neuronal differentiation of progenitor cells
4847	PluriSn 1	Inhibitor of SCD1; selectively eliminates undifferentiated hPSCs from culture
4551	Purmorphamine	Smo receptor agonist; induces differentiation of mesenchymal progenitor cells into osteoblasts
0695	Retinoic acid	Endogenous retinoic acid receptor agonist; promotes ESC differentiation
5325	Rosiglitazone	Promotes differentiation of adipocytes; also potent and selective PPAR $\gamma$ agonist
4366	SAG	Enhances neuronal differentiation of iPSCs into dopaminergic neurons; Smo agonist
4923	Shz 1	Activator of early cardiac genes in pluripotent stem cells; induces differentiation in M-PBMCs
3850	Sodium butyrate	HDAC inhibitor; directs differentiation of mESCs into hepatocytes
1496	SP 600125	JNK inhibitor; prevents BMP9-induced osteogenic differentiation of MSCs
3741	Stauprimide	Inhibits NME2 nuclear translocation; primes ESCs for differentiation
4749	Strontium chloride	Calcium sensing receptor (CaSR) agonist; induces osteogenic differentiation of MSCs
3300	SU 5402	Potent FGFR and VEGFR inhibitor; attenuates integrin $\beta_4$ -induced differentiation of neural stem cells
5383	TA 01	Induces cardiomyocyte differentiation from hESCs
5384	TA 02	Induces cardiomyocyte differentiation from hESCs
3877	TCS 2210	Inducer of neuronal differentiation in MSCs
3070	Thioridazine	Selective inducer of cancer SC differentiation; anticancer agent
3835	TWS 119	GSK-3 $\beta$ inhibitor; induces neuronal differentiation in ESCs
3115	WHI-P 154	JAK3 inhibitor; also induces differentiation of neural progenitor cells
5148	Wnt-C59	Wnt signaling inhibitor; induces differentiation of iPSCs to cardiomyocytes
2293	Zebularine	DNA methyltransferase inhibitor; induces cardiomyocyte differentiation in MSCs
<b>Stem Cell Proliferation, Expansion and Self-Renewal</b>		
3336	A 769662	Potent AMPK activator; inhibits MSC proliferation
2939	A 83-01	Selective inhibitor of TGF- $\beta$ RI, ALK4 and ALK7; maintains self-renewal of human iPSCs
4095	Amiodarone	Selectively inhibits proliferation of NSCs in hESC-derived cell populations
3194	BIO	Potent, selective GSK-3 inhibitor; maintains self-renewal and pluripotency of ESCs
4027	16,16-Dimethyl Prostaglandin E <sub>2</sub>	Synthetic prostaglandin E <sub>2</sub> (Cat. No. 2296) derivative; regulates HSC development
4827	Garcinol	Promotes expansion of human HSCs; PCAF/p300 HAT inhibitor
3849	Gatifloxacin	Antibiotic; activates short-term renewal of ESCs
5283	HLM 006474	E2F transcription factor inhibitor; attenuates hESC proliferation
3853	ID 8	Sustains self-renewal and pluripotency of ESCs
4997	INDY	Dyrk1A/B inhibitor; impairs self-renewal of NSCs
4713	IQ 1	Enables Wnt/ $\beta$ -catenin-driven expansion of ESCs; prevents spontaneous differentiation
3533	IWP 2	PORCN inhibitor; suppresses self-renewal in R1 ESCs
1130	LY 294002	Prototypical PI 3-kinase inhibitor; suppresses proliferation of mESCs
3044	PD 173074	Selective FGFR1 and -3 inhibitor; inhibits proliferation and differentiation of oligodendrocyte progenitors
1213	PD 98059	MEK inhibitor; enhances ESC self-renewal
4433	Pluripotin	Dual ERK1/RasGAP inhibitor; maintains ESC self-renewal
2296	Prostaglandin E <sub>2</sub>	Induces hematopoietic stem cell proliferation
4978	Pyrintegrin	Enhances survival of human ESCs following enzymatic dissociation
1202	SB 203580	Selective inhibitor of p38 MAPK; stimulates neural stem cell proliferation
1616	SB 216763	GSK-3 inhibitor; maintains mouse ESCs in a pluripotent state
1614	SB 431542	Induces proliferation, differentiation and sheet formation of ESC-derived endothelial cells
3848	Sinomenine	Stimulates short-term renewal of human ESCs <i>in vitro</i>
3847	Theanine	Promotes hESC self-renewal

Catalog No.	Product Name	Primary Action
3114	Troglitazone	Selective PPAR $\gamma$ agonist; inhibits growth of hematopoietic cell lines
5413	WH-4-023	Supports self-renewal of naive hESCs
1254	Y-27632	Selective p160ROCK inhibitor; increases survival rate of human ESCs undergoing cryopreservation
<b>Stem Cell Signaling</b>		
1515	17-AAG	Selective Hsp90 inhibitor; protects neuroprogenitor cells against stress-induced apoptosis
3299	AMD 3100	Highly selective CXCR4 antagonist; mobilizes hematopoietic stem cells <i>in vivo</i>
5051	BIO 5192	Integrin $\alpha_4\beta_1$ inhibitor; mobilizes HSCs and progenitors
3874	BIO-acetoxime	Selective GSK-3 $\alpha/\beta$ inhibitor; inhibits CD8 <sup>+</sup> T cell effector differentiation
5050	CASIN	Cdc42 GTPase inhibitor; functionally rejuvenates aged hematopoietic SCs
4529	Ciliobrevin A	Hedgehog (Hh) pathway antagonist; inhibits ciliogenesis
1769	Flurbiprofen	Cyclooxygenase inhibitor; regulates prostate stem cell antigen
3889	GANT 58	GLI1 antagonist; inhibits Hh signaling
3191	GANT 61	GLI antagonist; inhibits Hedgehog (Hh) signaling
4474	20(S)-Hydroxycholesterol	Allosteric activator of Hedgehog (Hh) signaling; induces Smo accumulation
3532	endo-IWR 1	Wnt/ $\beta$ -catenin signaling inhibitor; axin stabilizer
3341	JK 184	Downstream Hh signaling pathway inhibitor; inhibits alcohol dehydrogenase 7
2197	L-755,507	Enhances CRISPR-mediated homology-directed repair (HDR) efficiency in human iPSCs
4079	Niclosamide	STAT3 and mTORC1 signaling inhibitor; antineoplastic against AML stem cells
1267	Pifithrin- $\alpha$	p53 inhibitor; suppresses self-renewal of embryonic stem cells
3324	QS 11	ARFGAP1 inhibitor; modulates Wnt signaling pathway
3667	SR 3677	Potent, selective Rho-kinase (ROCK) inhibitor
4855	WIKI4	Tankyrase inhibitor; inhibits Wnt signaling
3748	XAV 939	Tankyrase inhibitor; promotes cardiomyogenesis
<b>Tocriscreen Collections</b>		
5060	Tocriscreen Stem Cell Toolbox	80 Stem Cell modulators supplied pre-dissolved in DMSO (250 $\mu$ L 10 mM solutions)

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