Chemical, computational and functional insights into the chemical stability of the Hedgehog pathway inhibitor GANT61

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Figure S1. HPLC-UV chromatograms at different times confirm the disappearance of GANT61 and the formation of GANT61-D. a) GANT61 in CH$_3$CN; b–f) GANT61 in CH$_3$CN/100 mM NH$_4$OAc, pH=4.5; 95/5, v/v: b) t = 0 min; c) t = 15 min; d) t = 30 min; e) t = 75 min; f) t = 24 h.
Figure S2. HPLC-ELSD chromatograms at different times confirm the disappearance of GANT61 and the formation of GANT61-D. a) GANT61 in CH$_3$CN; b–f) GANT61 in CH$_3$CN/100 mM NH$_4$OAc, pH=4.5; 95/5, v/v: b) t = 0 min; c) t = 15 min; d) t = 30 min; e) t = 75 min; f) t = 24 h.
Figure S3. Magnification of GANT61-D binding modes to Gli1ZF. A) predicted binding mode of the neutral form of GANT61-D (cyan sticks); B) predicted binding mode of the mono-protonated form of GANT61-D (magenta sticks); C) predicted binding mode of the di-protonated form of GANT61-D (yellow sticks). Gli1ZF is showed as green cartoon in top panels and surface colored according with electrostatic potential in bottom panels. Residues within 4 Å from GANT61-D are shown as green sticks and are labeled.
$^1$H NMR of 2-(dimethylamino)benzaldehyde

400.13 MHz, CDCl$_3$
$^{13}$C NMR of 2-(dimethylamino)benzaldehyde

100.6 MHz, CDCl$_3$
$^1$H NMR of GANT61-D

400.13 MHz, CD$_3$OD
$^{13}$C NMR of GANT61-D

100.6 MHz, CD$_3$OD
$^1$H NMR of GANT61

400.13 MHz, DMSO-$d_6$
400.13 MHz, DMSO-$d_6$
$^{13}$C NMR of GANT61

100.6 MHz, DMSO-$d_6$
ESI MS of GANT61-D

GANT61_ACN_FM #231-277  RT: 0.81-0.98  
T: ITMS + p ESI Full ms (50.00-1500.00) 
NL: 6.54E3  
GANT61_ACN_FM #231-277  RT: 0.81-0.98  AV: 47  
T: ITMS + p ESI Full ms (50.00-1500.00)  
NL: 1.84E4  
c21h33 n4 +H:  
C21H33 N4  
p (gss , s /p:40) Chrg 1  
R: 3000 Res .Pwr . @FWHM  
[M+H]+
ESI MS of GANT61

GANT61 #199-215  RT: 0.71-0.75  AV: 13  N
T: ITMS + p ESI Full ms [50.00-1000.00]