

SAFit2: A Chemical Probe for FKBP5

Version 1.0 (29th July 2022)

Web link for more details: <https://www.sgc-ffm.uni-frankfurt.de/#!specificprobeoverview/SAFit2>

Overview

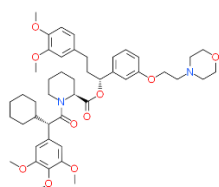
FKBP5 is a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes involving protein folding and trafficking. It acts as a peptidyl-prolyl cis/trans-isomerase (PPIase) that participate as cochaperone in the Hsp90 protein folding machinery and therefore plays an important role in stress endocrinology and glucocorticoid signalling.

Summary

Chemical Probe Name	SAFit2
Negative control compound	ddSAFit2
Target(s) (synonyms)	FKBP5 (FKBP prolyl isomerase 5, FKBP51)
Recommended <i>in vitro</i> assay concentration	Use at concentration up to 5 μ M for SAFit2 and ddSAFit2; use with control and pan-FKBP5 inhibitor compd 18-(S)Me (PMID: 34820091) for best interpretation of data
Suitability for <i>in vivo</i> use and recommended dose	Moderate brain-penetration potential; Tested in mice with 20 mg/kg (IP) twice daily for 5 days: shows reduced corticosterone at the peak, but not at the trough, of the circadian glucocorticoid rhythm
Publications	PMID: 25436518 , PMID: 26954324 , PMID: 33887102
<i>In vitro</i> assay(s) used to characterise	Fluorescence polarization (FP) assay
Cellular assay(s) for target-engagement	NanoBRET assay

Chemical Probe & Negative Control Structures and Use

SAFit2 Chemical Probe



SMILES:

COc1ccc(CC[C@H](c2cccc(c2)OCCN2CCOCC2)OC([C@@H]2CCCCN2C([C@@H])(C2CCCC2)c2cc(c(c(c2)OC)OC)=O)=O)cc1OC

InChiKey: ZDBWLRLGUBSLPG-FDHYQTMZSA-N

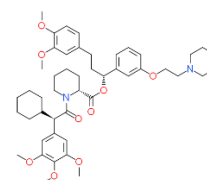
Molecular weight: 802.44 g/mol

Storage: As a dry powder or as DMSO stock solutions (10 mM) at -20 °C.

DMSO stocks beyond 3-6 months or 2 freeze/thaw cycles should be tested for activity before use

Dissolution: Soluble in DMSO up to 10 mM; use only 1 freeze/thaw cycle per aliquot

ddSAFit2 Negative Control



SMILES:

COc1ccc(CC[C@H](c2cccc(c2)OCCN2CCOCC2)OC([C@@H]2CCCCN2C([C@@H])(C2CCCC2)c2cc(c(c(c2)OC)OC)=O)=O)cc1OC

InChiKey: ZDBWLRLGUBSLPG-FFUXEPPCSA-N

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Chemical Probe Profile

In vitro Potency & Selectivity:

SAFit2 is a potent FKBP5 inhibitor with $K_d = 6 \pm 2$ nM (FP assay). It shows a good selectivity vs other family members in the FP assay (K_d): FKBP1B (38.9 ± 3.0 nM), FKBP1A (116.4 ± 7.1 nM), FKBP2 (1053 ± 93 nM), FKBP4 (> 50 μ M) and FKBP3 (> 10 μ M.) Off-targets in the GPCR panel (45 targets at 10 μ M) are (K_i [nM]) TMEM97 (226) and HRH4 (3.5).

Potency in Cells and Cellular Target Engagement:

In a NanoBRET assay for FKBP5 using FKBP12-NLuc $IC_{50} = 195.5 \pm 14$ nM and for FKBP51-NLuc $IC_{50} = 493 \pm 101$ nM.