Supporting information

Discovery and Development of Potent LFA-1/ICAM-1 Antagonist SAR 1118 as an Ophthalmic Solution for Treating Dry Eye

Min Zhong,*[,†,§] Thomas R. Gadek,*[,‡] Minna Bui,[†] Wang Shen,[†] John Burnier,[‡] Kenneth J. Barr,[†] Emily J. Hanan,[†] Johan D. Oslob,[†] Chul H. Yu,[†] Jiang Zhu,[†] Michelle R. Arkin,[†] Marc J. Evanchik,[†] W. Mike Flanagan,[†] Ute Hoch,[†] Jennifer Hyde,[†] Saileta Prabhu,[†] Jeffrey A. Silverman,[†] and Jasmin Wright[†]

[†]Sunesis Pharmaceuticals, Inc., 395 Oyster Point Boulevard, Suite 400, South San Francisco, California 94080, United States
[‡]SARcode Bioscience, Inc., 1000 Marina Boulevard, Suite 250, Brisbane, California 94005, United States

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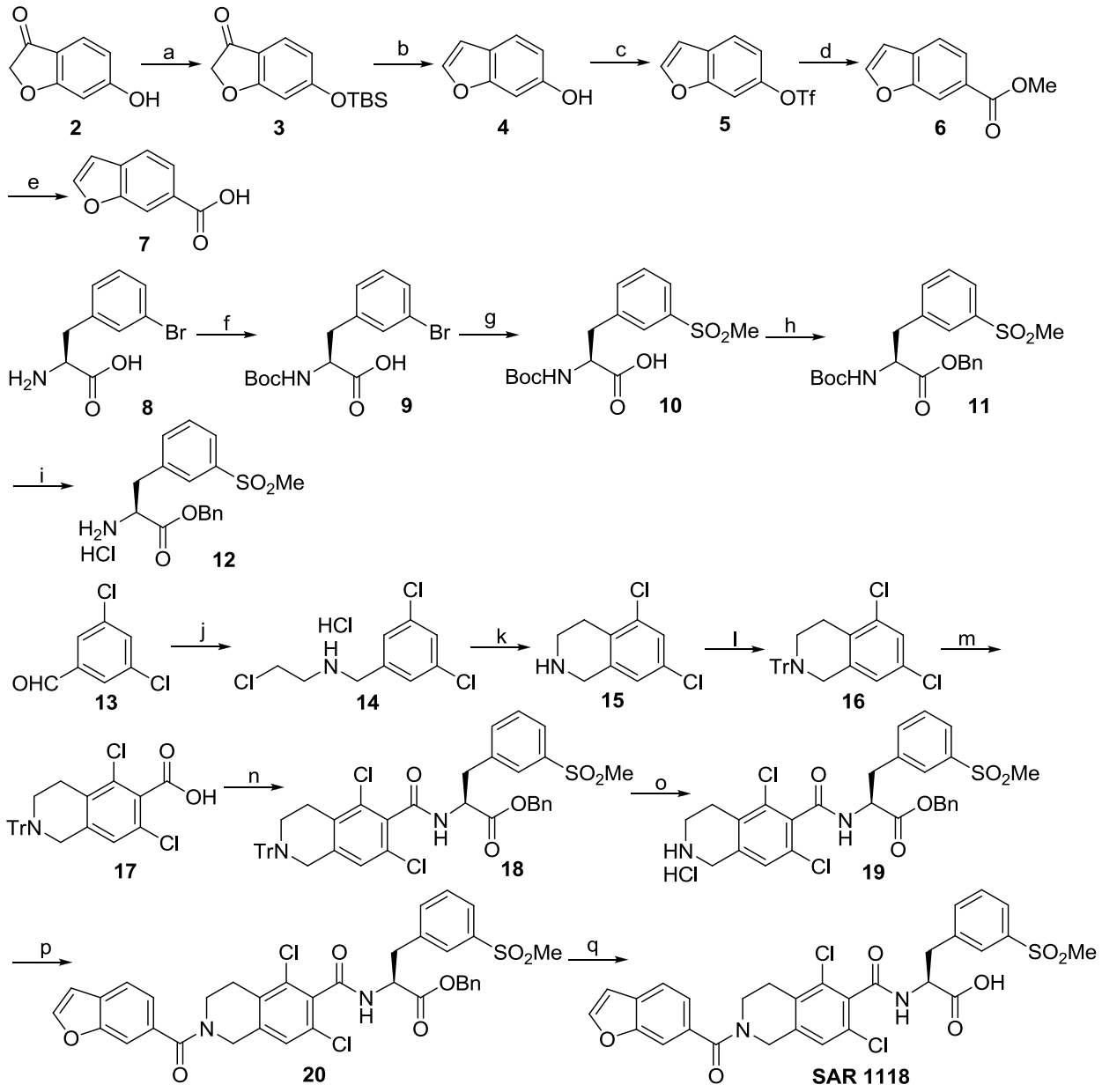
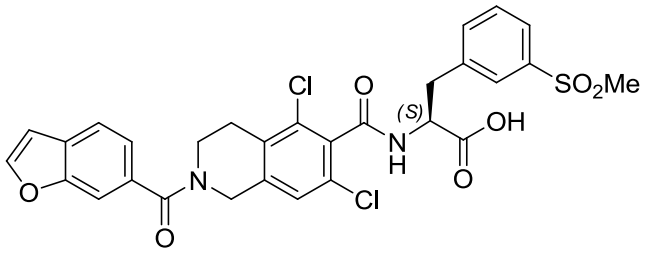
Scheme 1. Process chemistry route to SAR 1118[a,l]



[a]Reagents and conditions: (a) TBDMSCl, Et_3N, acetone, rt, 79% yield; (b) $NaBH_4$, MeOH, rt, overnight; acetone, rt, 2 hr; 4 N aq. HCl, THF, Ar, dark, rt, overnight, 95% yield; (c) Tf_2NPh, DCM, rt, 72 hr, 69% yield; (d) $Pd(OAc)_2$, dppp, DMF/MeOH, CO (8 bar), 70 °C, 6 hr, 91% yield; (e) LiOH, MeOH/H_2O, rt, overnight; 1 N aq. HCl, 97% yield; (f) Boc_2O, $NaHCO_3$, dioxane/H_2O, rt, overnight, 98% yield; (g) NaOS(O)Me, CuI, Cs_2CO_3, L-Proline, DMSO, 100 °C, 9 hr, 96% yield; (h) BnOH, EDCI, DMAP, DMF, rt, overnight, > 98% yield; (i) 4 N HCl in dioxane, DCM, 0 °C, overnight, 94% yield; (j) 1-chloro-2-aminoethane, $NaCNBH_3$, MeOH, rt, overnight, 35% yield; (k) $AlCl_3$, 185 °C, 91% yield; (l) TrCl, DIEA, DCM, rt, 4 hr, 89% yield; (m) *n*-BuLi, TMEDA, THF, -78 °C to rt; CO, -78 °C to rt, 75% yield; (n) **12**, HATU, TEA, DMF, DMF, rt, 18 hr, 70% yield; (o) 4 N HCl in dioxane, rt, 2 hr, 95% yield; (p) acid chloride of **7**, DIEA, DCM, 0 °C, 1 hr, > 98% yield; (q) 10% Pd/C, HCOOH/Et_3N (5/2 (*v*/*v*)), MeOH/THF (5/1 (*v*/*v*)), rt, 4 hr, 95% yield.

General information of SAR 1118



SAR1118

Chemical Formula: $C_{29}H_{24}Cl_2N_2O_7S$
Molecular Weight: 615.48

SAR 1118 is a white to off-white solid crystallized from methylethylketone. m.p. 154.4 ^{o}C; $[\alpha]_D^{25}$ = -5.0^{o} (c = 1% (*w/w*) in MeOH); solubility 90 μg/mL in water at 25 ^{o}C (parent); FT-IR (KBr): ν_{max} 3427, 3302, 3030, 2923, 1727, 1659, 1294, 1140, 826, 764 cm^{-1}; ESI-MS: *m/z* 615.1 $[M+1]^+$, 637.0 $[M+Na]^+$; ^{1}H NMR (300 MHz, d^6-DMSO): δ 12.90 (bs, 1H), 9.05 (d, *J* = 6.0 Hz, 1H), 8.13 (d, *J* = 1.9 Hz, 1H), 7.73 (m, 4H), 7.57 (m, 1H), 7.41 (bs, 1H), 7.05 (d, *J* = 1.9 Hz, 1H), 4.78 (bm, 3H), 3.63 (bm, 3H), 3.30 (m, 1H), 3.16 (s, 3H), 3.02 (m, 1H), 2.77 (m, 2H) ppm; ^{13}C NMR (75.5 MHz, d^6-DMSO): δ 172.1, 169.6, 163.6, 153.7, 147.8, 140.6, 125.7, 106.9, 53.1, 43.6, 36.4, 26.0 ppm; Elemental analysis: calcd. for $C_{29}H_{24}Cl_2N_2O_7S$: C 56.6%, H 3.9%, N 4.6%, S 5.2%, Cl 11.5%; found C 55.1%, H 4.0%, N 4.4%, S 5.2%, Cl 11.2%.

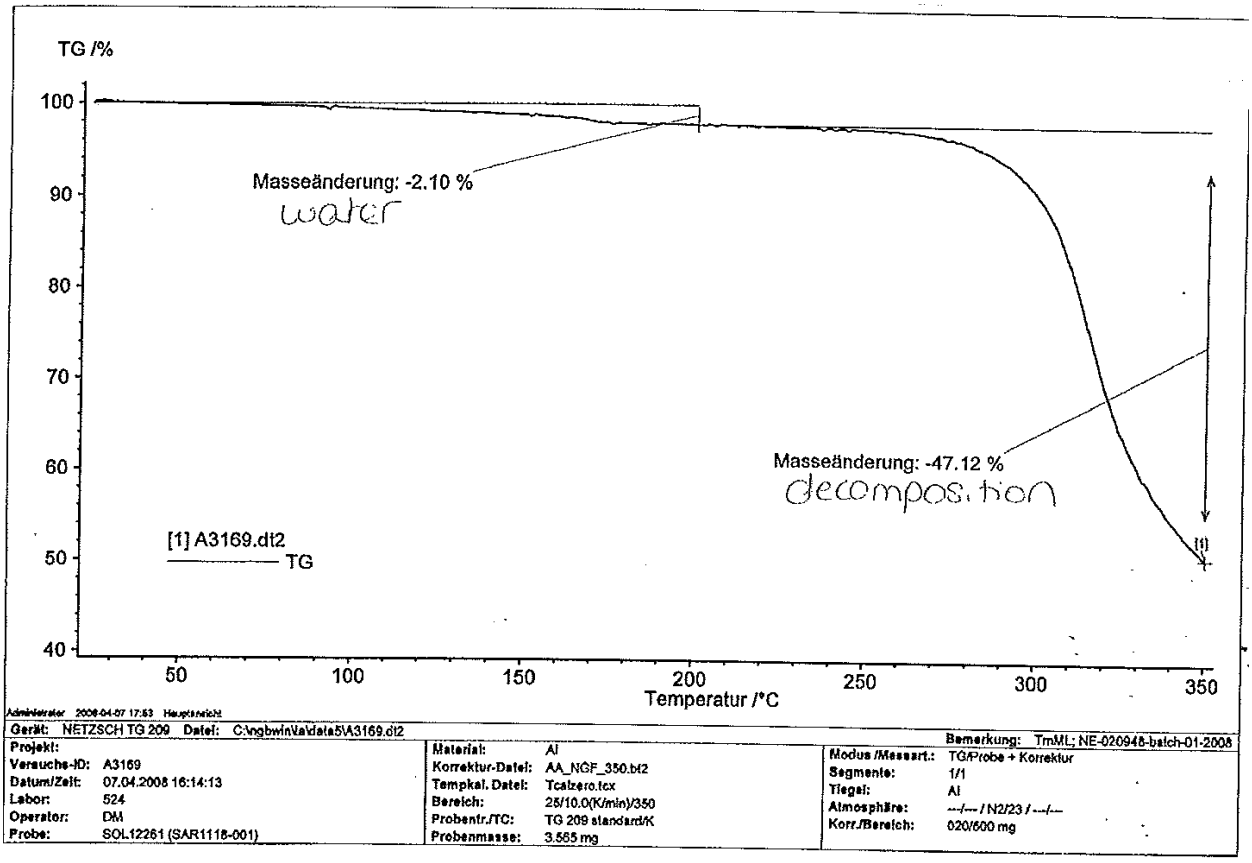


Fig. 1. TGA analysis spectrum of SAR 1118 recorded on Netzsch Thermo-Microbalance TG 209 analyzer

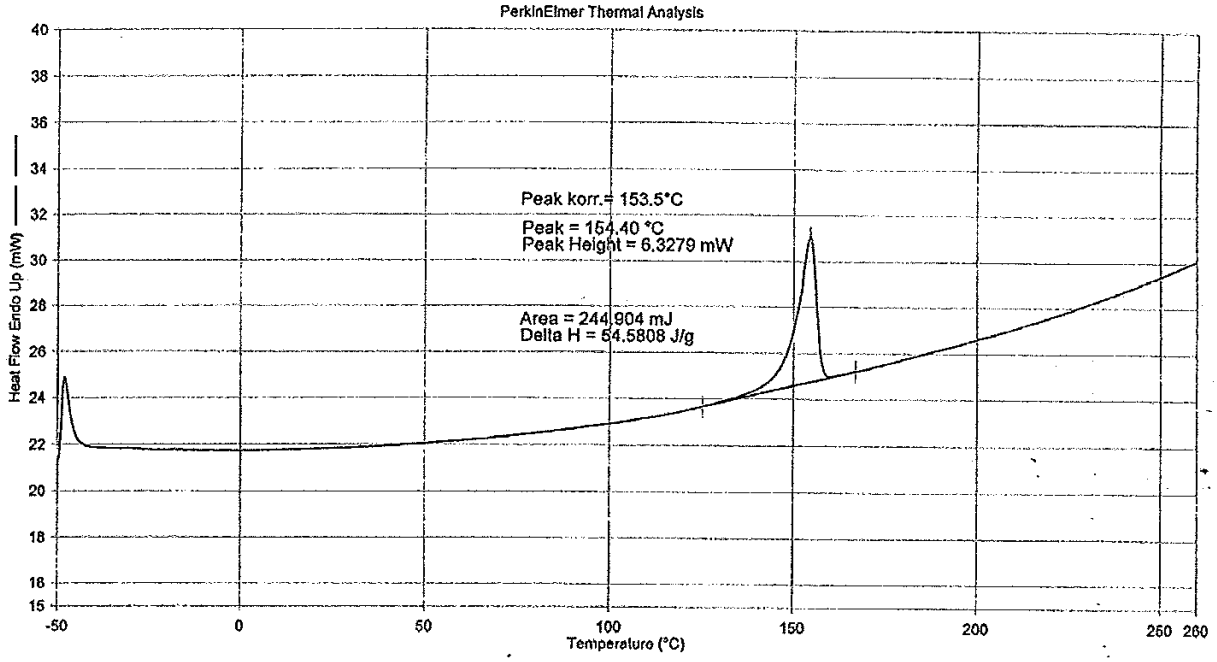


Fig. 2. DSC analysis spectrum of SAR 1118 recorded on Perkin Elmer DSC 7

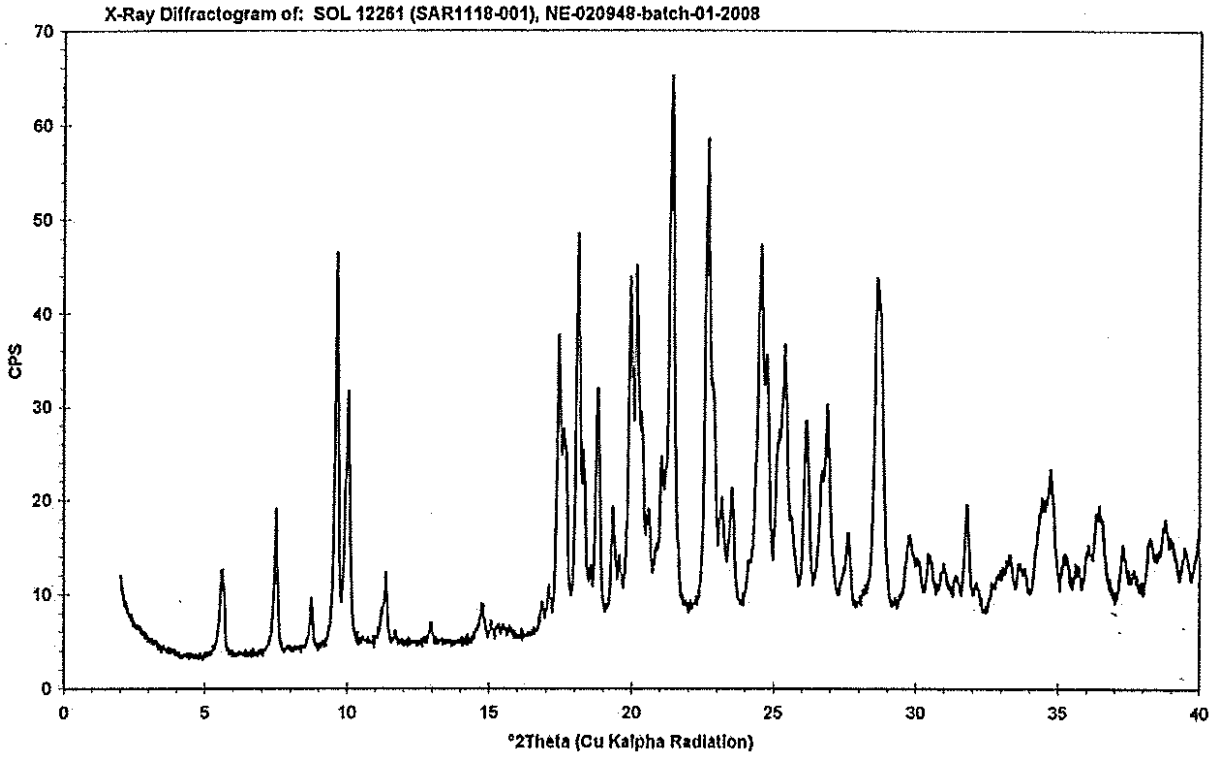


Fig. 3. XRPD analysis spectrum of SAR 1118 recorded on Bruker D8 Advance X-ray diffractormeter

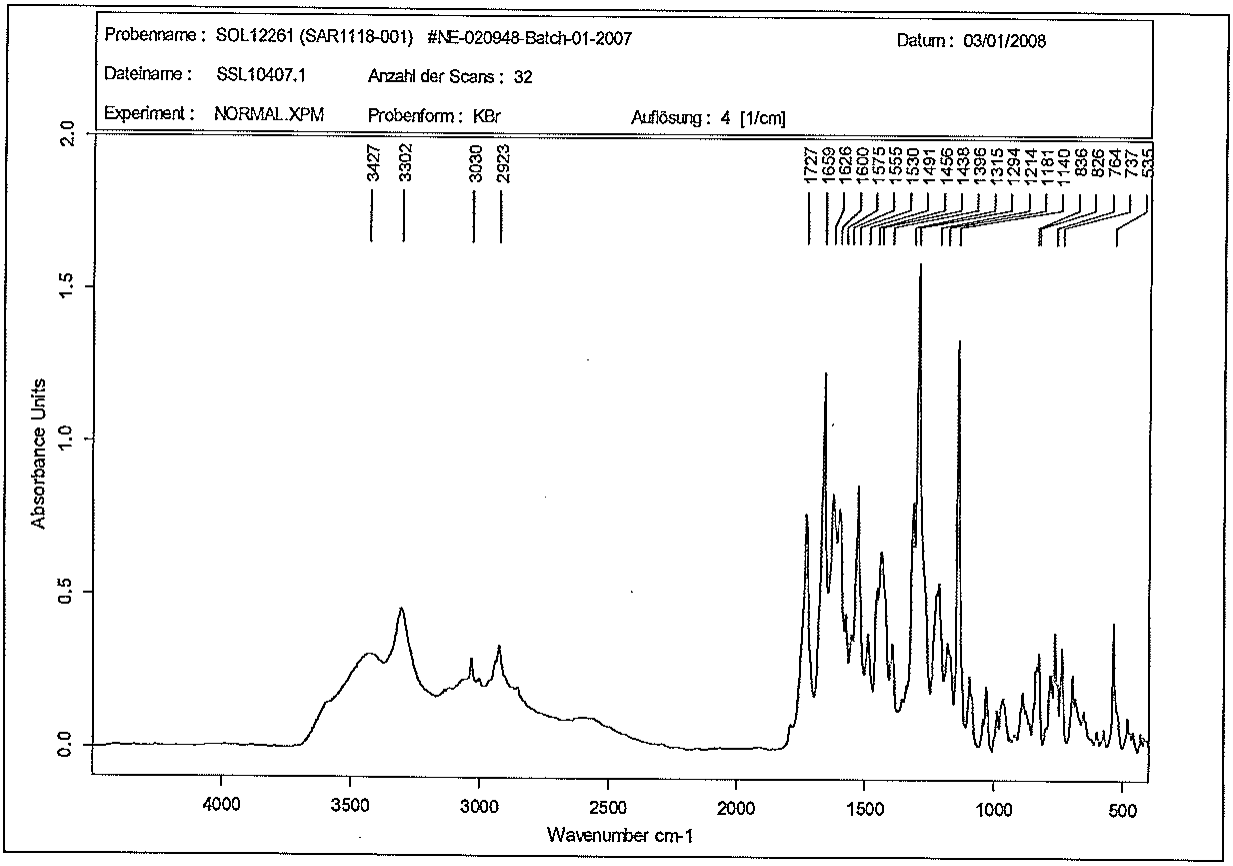


Fig. 4. FT-IR spectrum of SAR 1118 recorded on Bruker FT-IR spectrometer IFS48

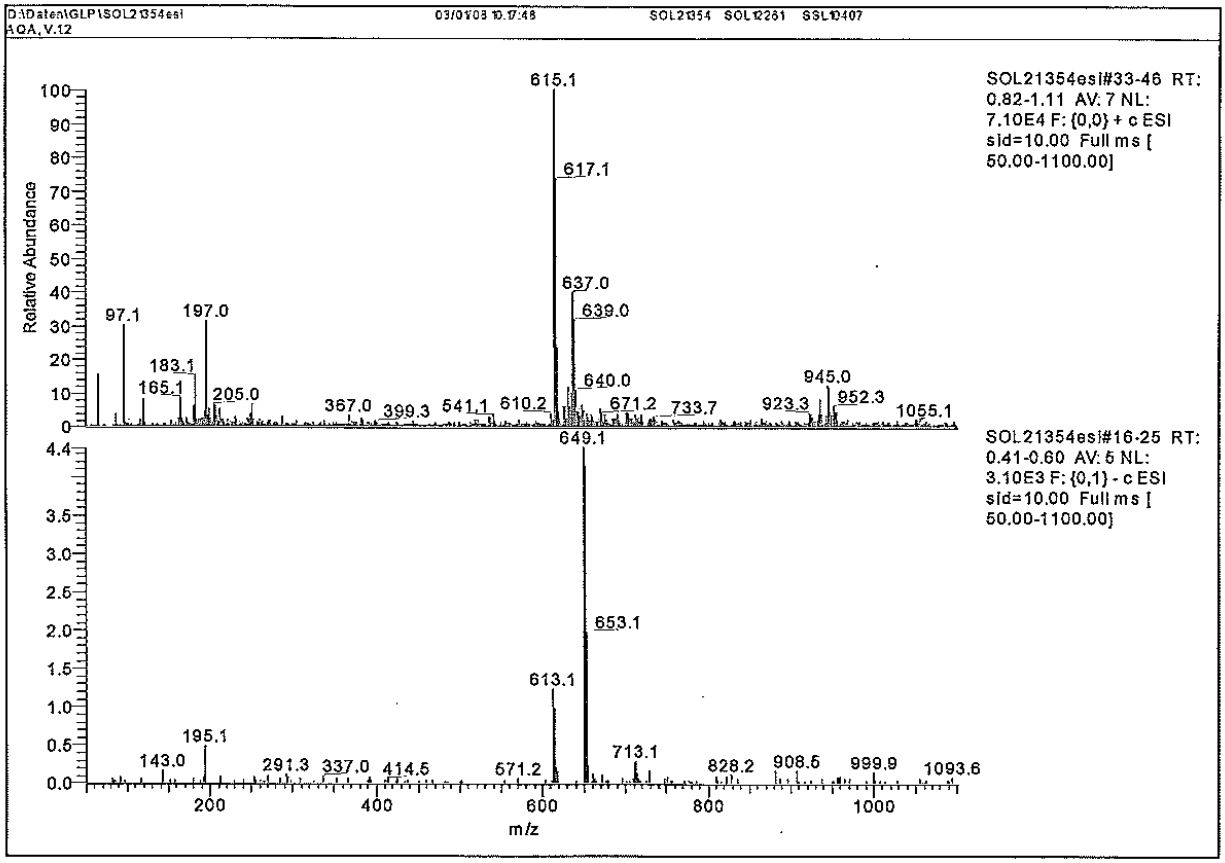


Fig. 5. ESI-MS spectrum of SAR 1118 recorded on ThermoQuest Mass spectrometer Finnigan AQA

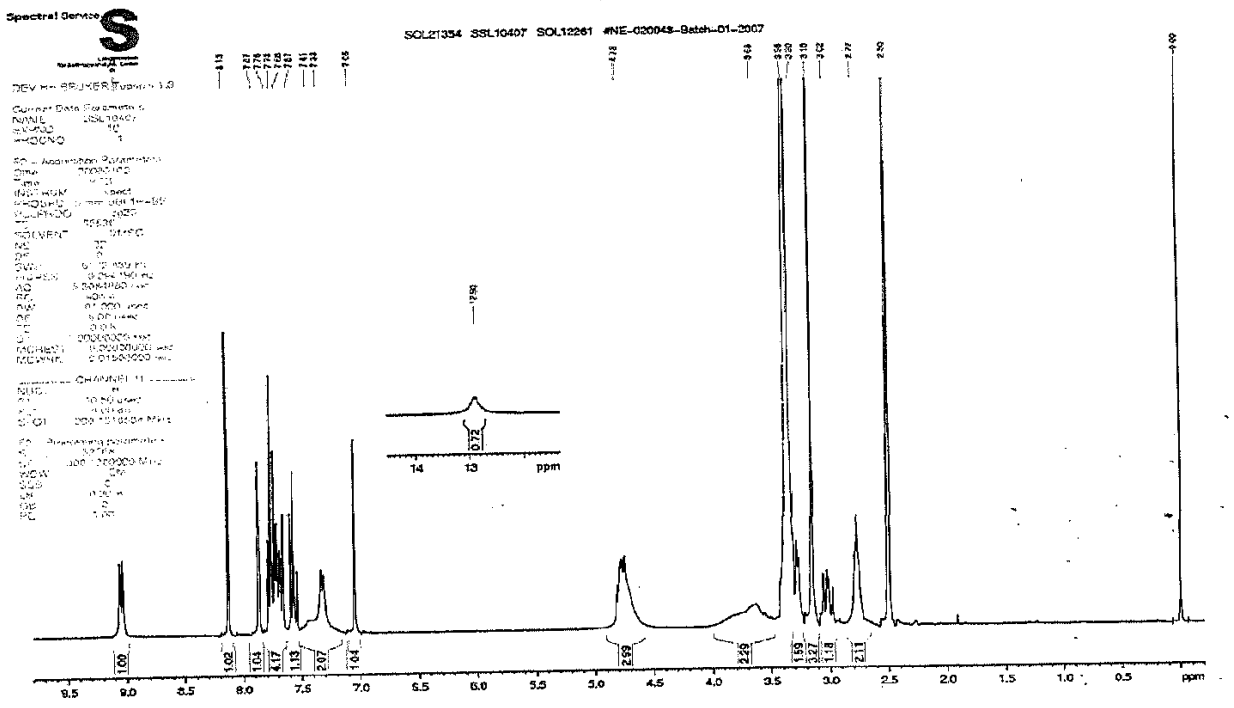


Fig. 6. ^{1}H NMR of SAR 1118 recorded on Bruker NMR spectrometer Avance 300

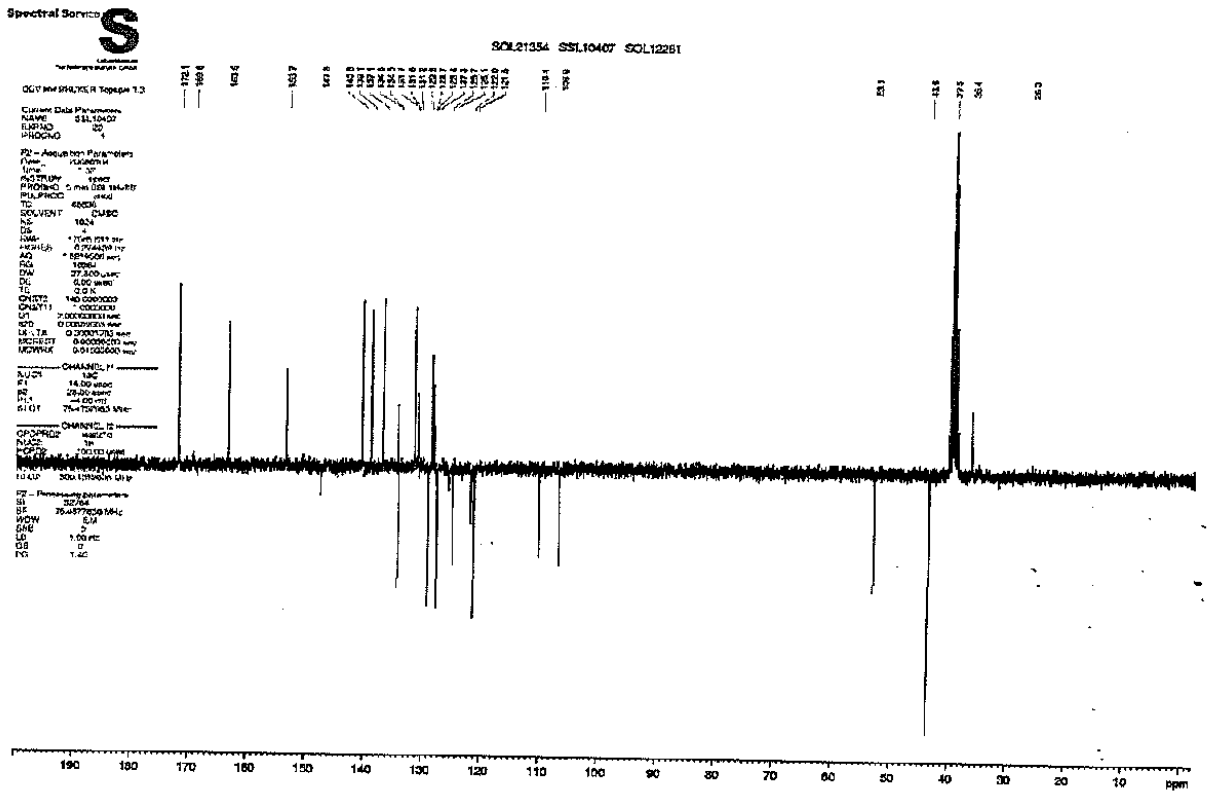


Fig. 7. ^{13}C NMR spectrum of SAR 1118 recorded on Bruker NMR spectrometer Avance 300

Detailed information of the HuT 78 T-cell adhesion assay (cell attachment assay)[2]

The HuT 78 T-cell adhesion assay is performed using a human T-lymphoid cell line HuT 78 (ATCC TIB-161). Goat anti-HuIgG(Fc) is diluted to 2 μg/mL in PBS and 96-well plates are coated with 50 μL/well at 37 ^{o}C for 1 hr. Plates are washed with PBS and blocked for 1 hr at rt with 1% BSA in PBS. 5 domain ICAM-Ig is diluted to 100 ng/mL in PBS and 50 μL/well was added to the plates O/N at 4 ^{o}C. HuT 78 cells are centrifuges at 100 g and the cell pellet is treated with 5 mM EDTA for 5 min at 37 ^{o}C in a 5% CO_2 incubator. Cells are washed in a mixture of 0.14 M NaCl, 0.02 M Hepes, 0.2% glucose and 0.1 mM $MnCl_2$ (assay buffer) and centrifuged. The cells are re-suspended in assay buffer to 3.0 x 106 c/mL. Compounds are diluted in assay buffer to a 2x final concentration and pre-incubated with HuT 78 cells for 30 min at rt. 100 μL/well of cells and compounds are added to the plates and incubated at rt for 1 hr. 100 μL/well PBS is added and the plates are sealed and centrifuged at 100 g for 5 min. Unattached cells are flicked out of the plate and excess PBS is blotted on a paper towel. 60 μL/well *p*-nitrophenyl *n*-acetyl-β-D-glucosaminide (0.257 g to 100 mL citrate buffer) is added to the plate and incubated for 1.5 hr at 37 ^{o}C. The enzymatic reaction is stopped with the addition of 90 μL/well 50 mM glycine/5 mM EDTA and read on a plate reader at 405 nM. HuT 78 cell adhesion to 5 domain ICAM-Ig is measured using the *p*-nitrophenyl *n*-acetyl-β-D-glucosaminide method.[3]

Detailed information of the Jurkat T-cell adhesion assay (cell attachment assay)[4]

To evaluate the ability of SAR 1118 to inhibit the attachment of Jurkat cells to intercellular adhesion molecule (ICAM)-1 *in vitro*, 100 mM stock solutions of SAR 1118 and a positive control (Compound A shown below)[5-6] were prepared in an aqueous solution of dimethylsulfoxide (1:1 (*v/v*)), respectively, and then diluted by adding assay medium to achieve and maintain the desired concentration throughout the assay. The Jurkat cells were labeled with an 8 μM solution of 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein in growth medium at rt for 15 min. Labeled cells were incubated in 70 μL of assay medium in each well of a 96-well plate at 500,000 cells per well with 70 μL of SAR 1118 or the positive control in assay medium at 37 °C for 30 min. A 100 μL aliquot of this fluorescence-labeled Jurkat cell suspension was allowed to settle in the presence of SAR 1118 or the positive control in wells of a 96-well plate coated with recombinant human ICAM-1 expressed as an Fc chimera (R&D Systems, Minneapolis, MN) at 37 °C for 1 hr. A dose titration of each test compound was run (10^{-11} –10^{-6} M) in two separate lanes on a single plate, and the assay was replicated on a second, separate plate (**Figure 8**). Non-adherent cells were removed by washing and centrifugation at 100 g for 1 min. Adherent cells were quantitated as the intensity of adherent fluorescence, and the IC_{50} values were calculated by using a standard four-parameter logistic nonlinear regression analysis of the data (GraphPad Software Inc., San Diego, CA). The reported IC_{50} is the average of data for two plates.

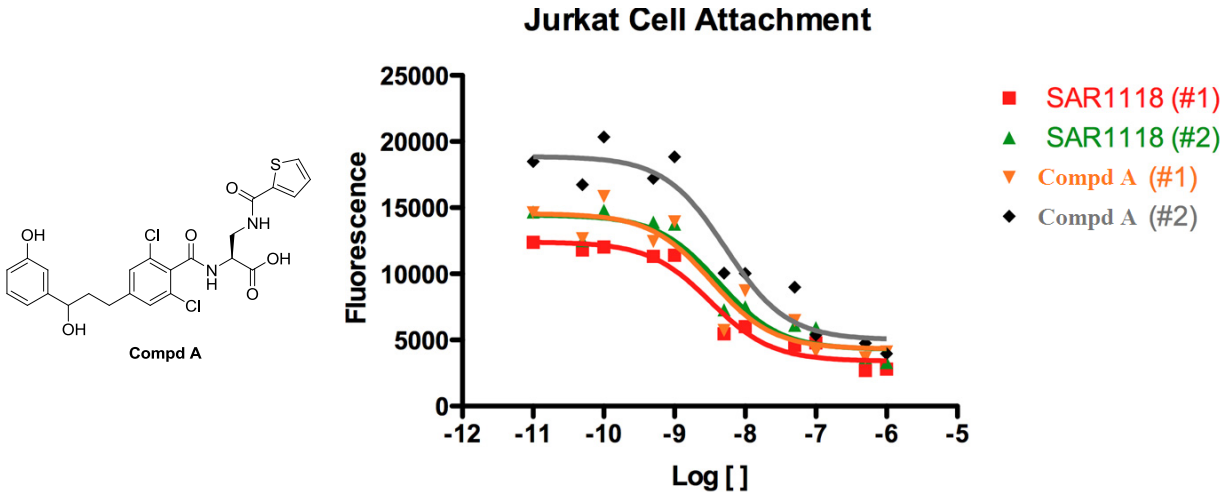


Fig. 8. Titration curves of SAR 1118 and Compound A in the Jurkat T-cell adhesion assay

References

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