Supplementary Information

Small Molecule Inhibition of the TNF Family Cytokine CD40 Ligand Through a Subunit Fracture Mechanism

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Contents:

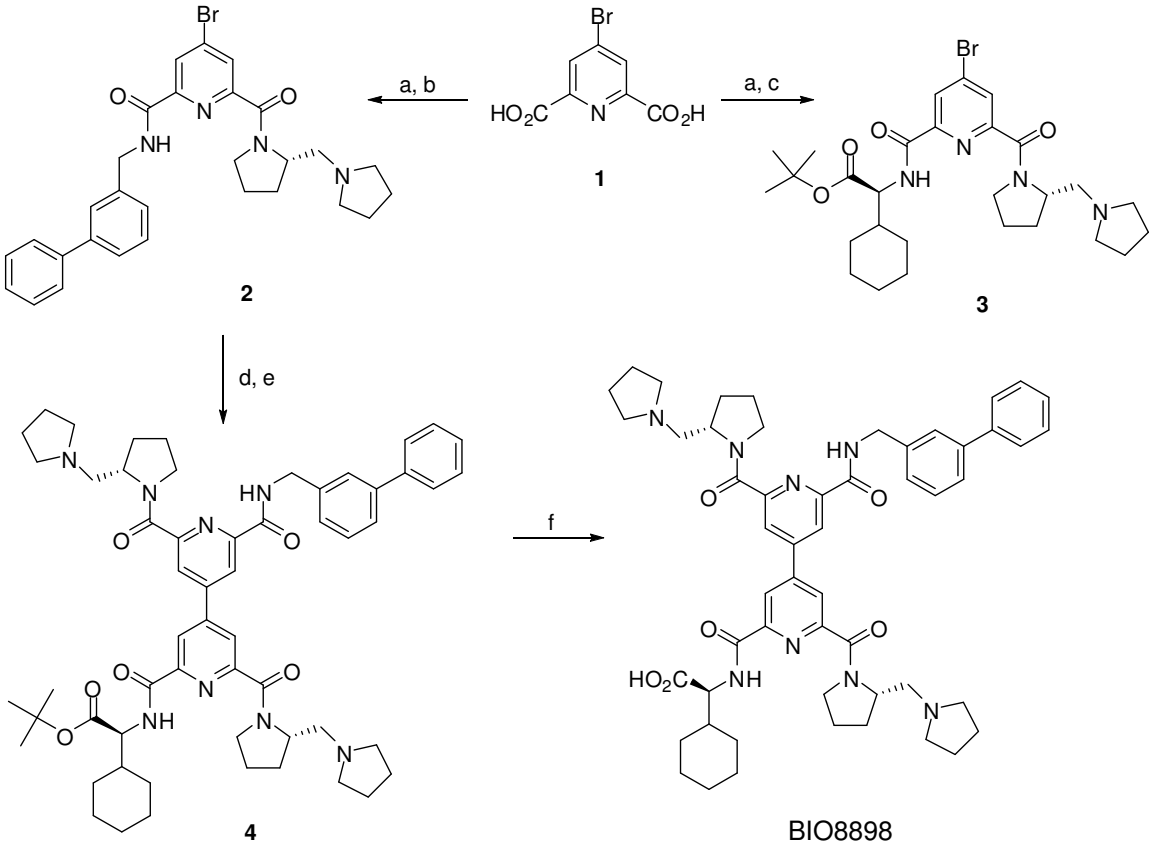
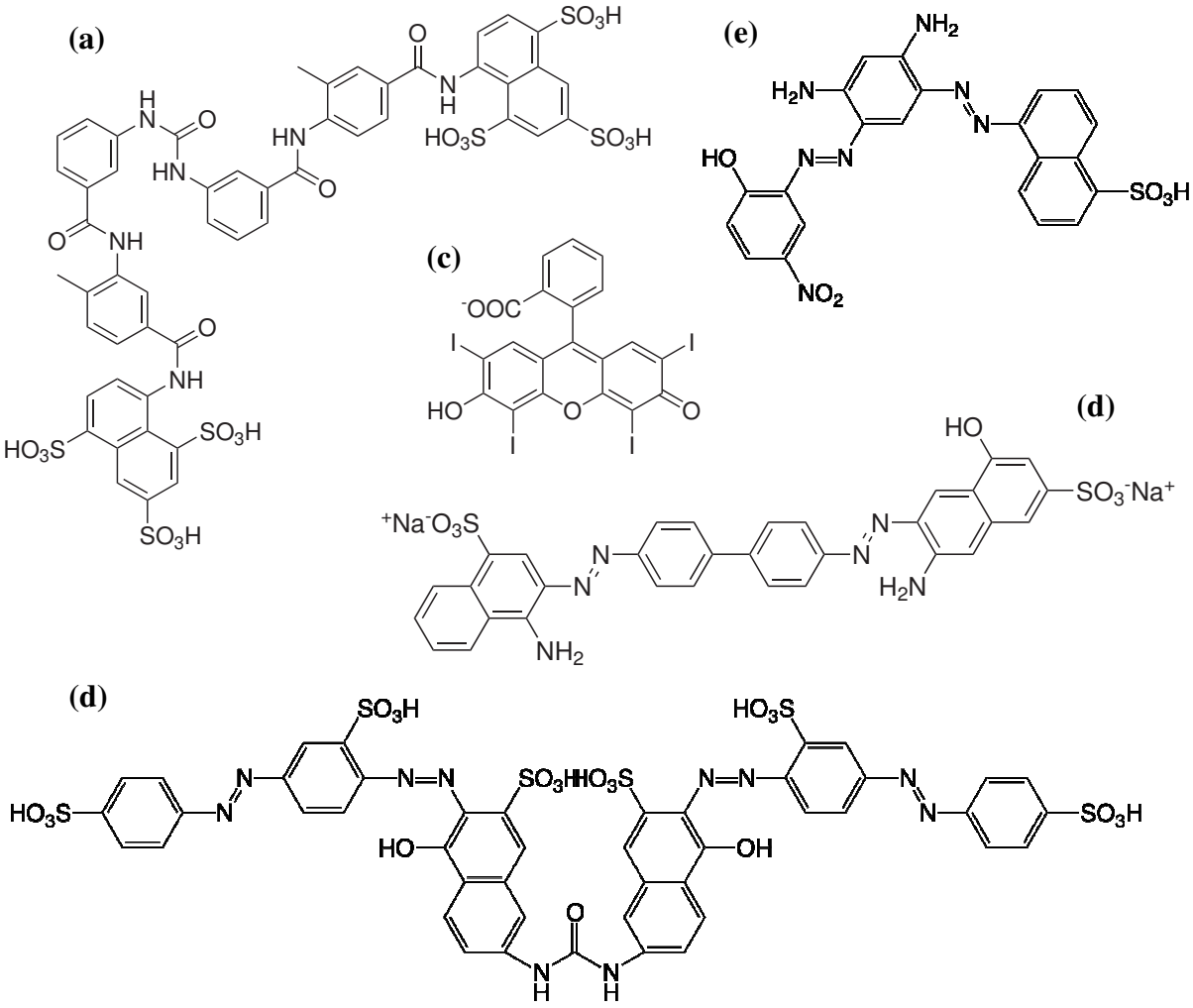


Figure S1. Synthesis of BIO8898 (For additional details, see Materials and Methods)

(a). Oxalyl chloride; (b). Et_3N, CH_2Cl_2, (*S*)-1,2'-methylenedipyrrolidine, biphenyl-4-ylmethanamine; (c). Et_3N, CH_2Cl_2, (*S*)-1,2'-methylenedipyrrolidine, (*S*)-tert-butyl 2-amino-2-cyclohexylacetate (HCl salt); (d). bis(pinacolato)diboron, [1, 1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (complex with CH_2Cl_2), KOAc, DMF, 80 ^oC; (e). aq. Na_2CO_3, 80 ^oC; (f). TFA, CH_2Cl_2.

Figure S2. Examples of other compounds that have been reported to inhibit CD40L or other TNF family cytokines. (a) Suramin[1] (b) DR13[2] (c) DR80[2] (d) erythosine[2,3] and (e) MB1[4]. (For additional examples, see reference 2.)



[1]Margolles-Clark, E., Jacques-Silva, M. C., Ganesan, L., Umland, O., Kenyon, N. S., Ricordi, C., Berggren, P. O., and Buchwald, P. (2009) Suramin inhibits the CD40-CD154 costimulatory interaction: a possible mechanism for immunosuppressive effects, *Biochem Pharmacol 77*, 1236-1245.

[2]Margolles-Clark, E., Umland, O., Kenyon, N. S., Ricordi, C., and Buchwald, P. (2009) Small-molecule costimulatory blockade: organic dye inhibitors of the CD40-CD154 interaction, *J Mol Med 87*, 1133-1143.

[3]Ganesan, L., Margolles-Clark, E., Song, Y., and Buchwald, P. (2011) The food colorant erythrosine is a promiscuous protein-protein interaction inhibitor, *Biochem Pharmacol 81*, 810-818.

[4]Margolles-Clark, E., Kenyon, N. S., Ricordi, C., and Buchwald, P. (2010) Effective and specific inhibition of the CD40-CD154 costimulatory interaction by a naphthalenesulphonic acid derivative, *Chem Biol Drug Des 76*, 305-313.

Figure S3. Top view of a superposition of subunit B of CD40L (grey ribbons) with the same subunit in the CD40L/BIO8898 complex structure (colors, according to subunit) showing how the binding of one BIO8898 per trimer distorts the structure of the protein at all three receptor binding sites.