SUPPLEMENTARY MATERIAL

Preclinical Development of Novel Rac1-GEF Signaling Inhibitors using a Rational Design Approach in Highly Aggressive Breast Cancer Cell Lines

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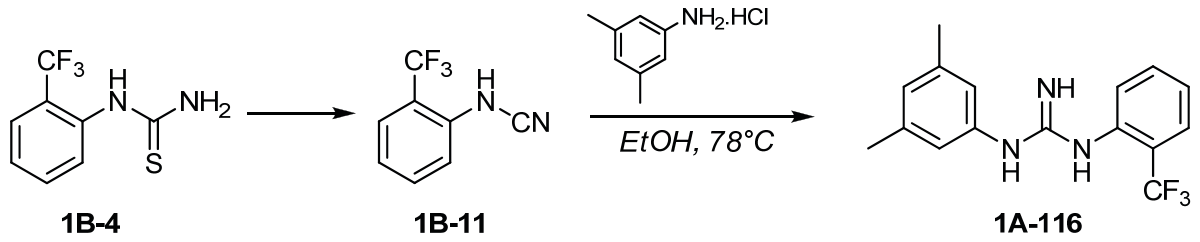
Synthesis of 1-(3,5-dimethylphenyl)-3-(2-(trifluoromethyl)phenyl)guanidine (1A-116)

Substituted thiourea 1B-4 was synthesized by reaction of benzoyl chloride and ammonium thiocyanate with 2-trifluoromethylaniline hydrochloride by following standard literature procedures (Synthesis of some new 2,4-disubstituted thiazoles as possible antibacterial and anti-inflammatory agents. Shivarama Holla, B., Malini, K. V., Sooryanarayana Rao, B., Sarojini, B. K., Suchetha Kumari, N. Eur. J. Med. Chem., 2003, 313-318). 1B-4 was transformed into the corresponding reactive cyanamide 1B-11 (Acylcyanamides, acylurée, ary allophanates déthyl diversement subdtitués; action sur le systeme nerveux central. Foussard-Blapin, S.; Uchida-Ernouf, G.; Anatol, J.; Berecoechea, J.Eur. J. Med. Chem (1979), 14(3), 215-218) by treatment with iodine in ethyl acetate. This unstable intermediate was immediately subjected to condensation conditions with one equivalent of 2,4-dimethylaniline hydrochloride to yield the desired compound 1A-116 in good yield (Scheme **1**).



Scheme 1. Synthesis of 1A-116.

Experimental Procedure

General Procedures. All reagents were commercially available and were obtained from Sigma-Aldrich. Melting points were determined with an Electrothermal IA9000 Series apparatus and are uncorrected. Column chromatography was performed on a Teledyne Isco CombiFlash Companion instrument under gradient elution conditions with RediSep disposable flash columns. 1H and 13C NMR spectra were recorded on a Bruker AVANCE DPX-400 instrument at 400 and 100 MHz, respectively. Spectra are referenced to the solvent in which they were run (7.26 ppm for CDCl3). Electron impact mass spectra were recorded on a Shimadzu QP2010 instrument at 70 eV. Elemental analyses were performed by UMYMFOR (CONICET) Buenos Aires, Argentina.

A solution of 3,5-dimethylaniline hydrochloride (1.35 g, 8.60 mmol) and N-(2-(trifluoromethyl)phenyl)cyanamide (1.61 g, 8.60 mmol) in absolute ethanol (27 mL) was stirred at reflux for 24 h. PH was adjusted to 10 by addition of 0.5 M NaOH and water was added to maximize precipitation of the reaction product. The obtained suspension was stirred at 0 °C for one hour; the solid was filtered and dried under vacuum at 50° C to yield 1.98 g of the desired compound 1A-116 as a yellowish solid almost pure by 1H-NMR. The solid was purified by flash column chromatography (dichloromethane:triethyl amine 99:1) to yield 1.36 g (52% yield) of the desired product 1A-116 as a white solid: mp = 127 °C. IR (cm-1) 1648, 1560, 1316; 1H NMR (400 MHz, CDCl3) δ 7,63 (d, J = 6,5 Hz, 1 H), 7,44 (m, 1 H), 7,08 (m, 2 H), 6,84 (s, 2 H), 6,77 (s, 1 H), 4,27 (sa, 2 H), 2,28 (s, 6 H); 13C NMR (100 MHz, CDCl3) δ 149.43, 147.59, 139.12, 132.79, 126.90 (q, J = 5 Hz), 125.97, 125.31, 124.39 (q, J = 272 Hz), 124.04 (q, J = 29 Hz), 122.12, 120.36, 21,28. MS (m/z (RI)) 307 (23), 238 (5.7), 121 (100). Elemental Anal. Calcd. for C16H16F3N3: %C, 62.53, %H, 5.25, %N, 13.67. Found: %C, 62.35, %H, 5.25, %N, 13.26.