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α-Fluoro-Substituted Thalidomide Analogues

Hon-Wah Man,* Laura G. Corral, David I. Stirling and George W. Muller*

Celgene Corporation, Warren, NJ 07059, USA

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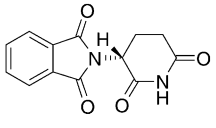
Abstract—Thalidomide, (**1**), has made a remarkable comeback from its days of a sedative with teratogenic properties due to its ability to selectively inhibit TNF-α, a key pro-inflammatory cytokine and its clinical benefit in the treatment of cancer. Thalidomide contains one chiral center and is known to be chirally unstable under in vitro and in vivo conditions. It has been hypothesized that different biological properties are associated with each isomer. Thus, chirally stable analogues of thalidomide, α-fluorothalidomide, (**3**) and α-fluoro-4-aminothalidomide (**4**) were prepared by electrophilic fluorination. Analogue **3** was found to be cytotoxic and did not inhibit TNF-α production in LPS stimulated hPBMC below toxic concentrations. On the other hand, **4** was non-cytotoxic at the tested concentrations and was found to be 830-fold more potent than thalidomide as TNF-α inhibitor.

Thalidomide (**1**) was initially marketed as a sedative in the late 1950s. Its apparent lack of the toxic effects normally associated with barbiturates made it a popular agent as a sleeping aid. This apparent lack of toxicity led to its use in the treatment of morning sickness in pregnant women in the United Kingdom, Europe, Canada, and Australia.[1] Its now well-known tragic teratogenic effects caused its withdrawal from the world market in the early 1960s. In 1965, the immunomodulatory activity of thalidomide was serendipitously discovered. Sheskin and co-workers in 1965 reported that thalidomide had a prompt and dramatic beneficial effect in the treatment of erythema nodosum leprosum (ENL), an acute inflammatory state occurring in lepromatous leprosy.[2] The report of thalidomide's selective inhibition of TNF-α in 1991[3] led to the renewal of interest in the clinical development of thalidomide and discovery of novel analogues with improved activities and decreased side effects. In 1998, thalidomide received FDA approval for the treatment of ENL. Recent clinical trials have demonstrated its clinical utility in treatment of various hematologic malignancies[4] and solid tumors,[5] as well as variety of inflammatory and autoimmune diseases.[6] It is now commonly used in 2nd and 3rd line treatment of multiple myeloma. These reports along with earlier reports have validated thalidomide as a multi-dimensional clinically effective therapeutic agent.

The discovery of thalidomide activity as a selective inhibitor of tumor necrosis factor-α (TNF-α) production in lipopolysaccharide (LPS) stimulated human monocytes provided a rationale for the mechanism of action for its anti-inflammatory effects.[7,8] TNF-α is a key pro-inflammatory cytokine in the immune system's inflammatory cascade. Elevated level of TNF-α has been correlated with a number of inflammatory and autoimmune diseases, such as rheumatoid arthritis, Crohn's disease, aphthous ulcers, cachexia, graft versus host disease, asthma, ARDS and AIDS.[9] The importance of TNF-α in the inflammatory cascade has resulted in a large pharmaceutical research effort to discover TNF-α inhibitors. The clinical efficacy of the anti-TNF biologicals such as Remicade[10] and Enbrel[11] in the treatment of rheumatoid arthritis and Crohn's disease has validated clinically anti-TNF-α treatment as a viable therapy.



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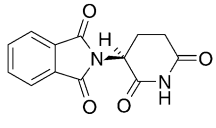

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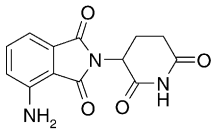

Actimid (2)

*Corresponding author. Tel.: +1-732-271-4124; fax: +1-732-271-4184; e-mail: hwman@celgene.com

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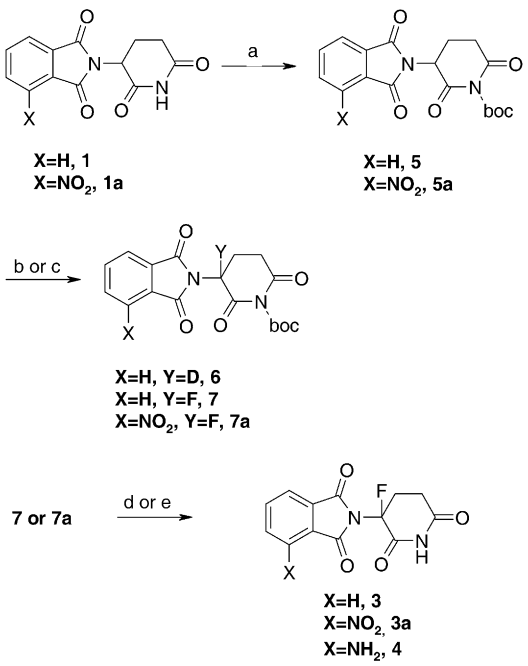
Thalidomide is a chiral molecule that has always been used clinically as a racemate. Literature dogma has implied that (*S*)-thalidomide ((*S*)-1) is the enantiomer responsible for the teratogenic activity and that the *R*-isomer is devoid of teratogenic activity.[12] The report of this selectivity was in mice, a non-sensitive species to thalidomide's teratogenic effects. A later report in rabbits, a sensitive species, demonstrated effects from both isomers. Moreover, both *R* and *S* isomers have been reported to have similar activity in the inhibition of TNF-α in vitro.[13] Furthermore, the enantiomers of thalidomide have been shown to be chirally unstable in vitro and in vivo.[14] Therefore, the benefit of administrating a single enantiomer of thalidomide instead of the racemate in order to avoid the undesirable teratogenic effect is probably moot. One way to prevent racemization is to replace the hydrogen by other groups at the asymmetric center of thalidomide. Wnendt et al. evaluated the single isomers of α-methyl thalidomide for the inhibition of TNF-α production.[15] In that report, the *S*-isomer of α-methylthalidomide was found to be more active than the *R*-isomer. However, these results may have been biased by the steric bulk of the α-methyl group. The recent report by Takeuchi et al.[16] on α-fluorothalidomide has prompted us to report our results[17] in this area. Similarly to Takeuchi we were drawn to replacing the chiral acidic hydrogen with fluorine. Fluorine is commonly used as an isosteric replacement for hydrogen and would be expected to act similarly due to their similar size, but would result in a chirally stable molecule without an acidic hydrogen. We recently reported on the 4-amino analogue (Actimid™, **2**) of thalidomide that is 19,000-fold more potent as a TNF-α inhibitor than thalidomide in LPS stimulated human PBMC.[18] Actimid is presently in Phase I/II clinical trials for the treatment of multiple myeloma.[19] Herein, we report a synthesis of α-fluorothalidomide (**3**) and the α-fluoro analogue (**4**) of **2** and their effects on TNF-α production in LPS stimulated human PBMC.

The use of the obvious starting material (α-fluoroglutamine) to prepare α-fluorothalidomide (**3**) was unsuccessful due to propensity of α-fluoro-α-amino acids to readily decompose via loss of hydrogen fluoride.[20] Several other synthetic routes were investigated without success. The synthesis of **3** was accomplished by a route involving electrophilic fluorination of *N*-BOC protected thalidomide (**5**) (Scheme 1).

The imide NH of thalidomide (**1**) was BOC protected to yield **5** in a 90% yield. Deprotonation of **5** with lithium hexamethyldisiliane (LHMDS) at −78 °C, followed by quenching of the resulting enolate with D-acetic acid, afforded α-deuterated **6** with 30–40% deuterium corporation in 46% yield. Although the yield was moderate, deuterium was selectively encorporated at the 3′-position. No deuterium incorporation was observed at the 5′-positions by ^{1}H and ^{13}C NMR. When the enolate was quenched with *N*-fluorobenzene sulfonimide[21] under the similar conditions, the fluoride (**7**) was isolated in 20% yield.[22] When ethyl triflate was used as electrophile, only *O*-alkylation product was observed (not shown). It appears that the low yield is caused by



Scheme 1. (a) $(BOC)_2O$, DMAP, dioxane; (b) Y = D: LHMDS, CH_3COOD, THF; (c) Y = F: LHMDS, $FN(SO_2Ph)_2$, THF; (d) X=H: CF_3COOH, CH_2Cl_2; (e) $X=NO_2$: (i) H_2, Pd/C, EtOAc; (ii) CF_3COOH, CH_2Cl_2.

the competition between the oxygen and carbon centers of the enolate with the electrophiles. The hard electrophile ethyl triflate reacts preferentially with the less hindered and harder oxygen. On the other hand, the smaller and softer *N*-fluorobenzene sulfonimide or D-acetic acid increases the rate of addition to the softer carbon. Therefore, a moderate amount of carbon-substituted product was obtained. After the fluoride was installed, the BOC group of **7** was removed with trifluoroacetic acid in methylene chloride to give the target compound, α-fluorothalidomide (**3**) in 93% yield.[23] The 4-amino analogue **4** was prepared starting from 4-nitrothalidomide (**1a**)[18] via a similar sequence that was completed by hydrogenation of the nitro group of **7a**.[24]

α-Fluorothalidomide (**3**) was evaluated for TNF-α inhibition in LPS stimulated human PBMC[25] and did not inhibit TNF-α at concentration up to 10 μM. TNF-α production however was almost completely suppressed at 100 μM. α-Fluorothalidomide (**3**) was found to be cytotoxic at this concentration (60% cell death at 100 μM) thus making the results at 100 μM hard to inter-

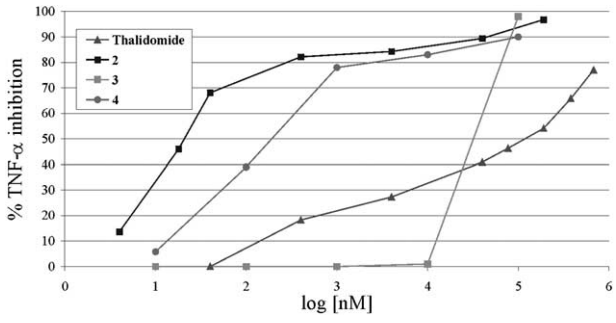


Figure 1. TNF-α inhibition in LPS stimulated human PBMC.

pret.[26] It is unclear why the α-fluorine causes this cytotoxicity, since α-fluoro-4-amino analogue (**4**) was not cytotoxic at concentrations up to 100 μM. Furthermore, α-fluoro-4-amino analogue **4** is a potent TNF-α inhibitor with IC_{50} of 230 nM, which is 830-fold more active than thalidomide. Similarly to thalidomide, **4** has a very flat dose response curve as shown in Figure 1.[13]

In summary, α-fluorothalidomide (**3**) was synthesized by a three steps procedure involving an electrophilic fluorination of *N*-BOC-thalidomide (**5**). Disappointingly in our hands α-fluorothalidomide (**3**) was found to be quite cytotoxic at 100 μM in our assay and to exhibit no TNF-α inhibitory activity at a non-toxic 10 μM concentration. Although Takeuchi and co-workers reported ~80% inhibition of TNF-α at 360 μM for the *S*-isomer in LPS stimulated human PBMC, they made no mention of cytotoxicity.[16] Interestingly, the α-fluoro analogue (**4**) of Actimid **2** was found to be a potent inhibitor of TNF-α with an IC_{50} at 230 nM and was non-toxic at all concentrations tested. However, analogue **4** is 23-fold less potent then the corresponding des-fluoro analogue (**2**). Therefore, although the α-fluoro analogue demonstrates sub-μM potency against TNF-α, it is considerably less active than the parent analogue. Research is continuing to investigate the properties of these α-fluoro analogues that are structural modification of thalidomide prepared to improve thalidomide pharmacological profile and probe the SAR surrounding thalidomide and its activity as an anti-inflammatory agent.

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22. Compound **7**: mp, 156.0–157.0 °C; ^{1}H NMR ($CDCl_3$, 250 MHz); δ 1.62 (s, 9H), 2.41–2.72 (m, 2H), 2.87–3.03 (m, 1H), 3.52–3.65 (m, 1H), 7.81–7.97 (m, 4H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 26.80 ($^2J_{C-F}$ = 27 Hz), 27.39, 28.98 ($^3J_{C-F}$ = 7.5 Hz), 67.15, 93.50 (J_{C-F} = 218 Hz), 124.31, 130.84, 135.29, 147.17, 161.80 ($^2J_{C-F}$ = 28 Hz), 165.93, 167.57; Anal calcd for $C_{18}H_{17}N_2O_6F$: C, 57.45; H, 4.55; N, 7.44; F, 5.05. Found: C, 57.78; H, 4.62; N, 7.23; F, 4.94.
23. Compound **3**: mp, 238–240 °C; ^{1}H NMR (DMSO-d_6, 250 MHz); δ 2.44–2.61 (m, 2H), 2.84–2.99 (m, 1H), 3.24–3.31 (m, 1H), 7.93 (brs, 4H), 11.49 (s, 1H); ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 26.91 ($^2J_{C-F}$ = 27 Hz), 28.41 ($^3J_{C-F}$ = 8 Hz), 93.57 (J_{C-F} = 211 Hz), 123.75, 130.91, 135.29, 164.29 ($^3J_{C-F}$ = 6 Hz), 164.70 ($^3J_{C-F}$ = 6 Hz), 166.21 ($^4J_{C-F}$ = 1 Hz), 171.58 ($^3J_{C-F}$ = 6 Hz); Anal calcd for $C_{13}H_9N_2O_4F$: C, 56.53; H, 3.28; N, 10.14; F, 6.88. Found: C, 56.59; H, 3.43; N, 10.01; F, 6.98.
24. Compound **4**; mp, 248.0–250.0 °C; ^{1}H NMR (DMSO-d_6, 250 MHz); δ 2.45–2.62 (m, 2H), 2.73–2.88 (m, 1H), 3.26–3.31 (m, 1H), 6.67 (br s, 2H), 6.99–7.05 (m, 2H), 7.49 (dd, *J* = 7.0, 8.4 Hz, 1H), 11.42 (br s, 1H); ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 27.40 (J_{C-F} = 27 Hz), 28.61 (J_{C-F} = 8 Hz), 93.40 (J_{C-F} = 210 Hz), 107.96, 111.24, 121.01, 131.42, 135.99, 147.32, 164.77 (J_{C-F} = 26 Hz), 166.41 (J_{C-F} = 1.4 Hz), 167.36, 171.61; Anal calcd for $C_{13}H_{10}N_3O_4F$ + 0.1 EtOAc: C, 53.64; H, 3.63; N, 14.00; F, 6.33. Found: C, 53.61; H, 3.47; N, 13.84; F, 6.30. (HNMR showed the sample contained ~10% of EtOAc).
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26. Toxicity of the drugs was assessed by the Trypan blue exclusion method.