Potent Inhibition of Tumor Necrosis Factor-α Production by Tetrafluorothalidomide and Tetrafluorophthalimides

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There has been a resurgence of interest in the teratogenic drug thalidomide (**1**) in recent years due, in large part, to its unique immunosuppressive and anti-inflammatory activities.[1] The molecular mechanisms underlying these activities remain unkown. Recently, Kaplan and co-workers reported that thalidomide specifically inhibits the production of tumor necrosis factor (TNF)-α from human monocytes upon stimulation with bacterial lipopolysaccharide (LPS), providing a possible explanation for its immunomodulatory properties.[2] As excessive production of TNF-α has been implicated in several human diseases including inflammation and septic shock,[3] we have been interested in finding more potent and less toxic analogs of thalidomide for inhibition of TNF-α production. Herein we report that substitution of four hydrogen atoms on the aromatic ring of thalidomide and other phthalimides with fluorine leads to an over 500-fold increase in potency for the inhibition of TNF-α production.

Tetrahalothalidomides **2**−**4** were synthesized for the following reasons. First, it has been hypothesized that the teratogenic[4] and antiangiogenic[5] effects of thalidomide are mediated by an arene oxide metabolite of the drug. We reasoned that epoxide formation would be prevented if the hydrogen atoms were substituted with electron acceptors such as halogen atoms. Second, it was recently reported by Hashimoto and co-workers[6] that tetrafluorophthalimides and tetrachlorophthalimides as well as thalidomide enhance TNF-α production in PMA-treated HL-60 cells. This is in contrast to the inhibitory activity of thalidomide for LPS-stimulated TNF-α production in human monocytes.[2] We thus synthesized (±)-tetrafluorothalidomide (**2**)[7] and examined its effect on the LPS-stimulated TNF-α production from the promonocytic cell line THP-1.[8,9] We found that tetrafluorothalidomide potently inhibits TNF-α production with an IC_{50} of ca. 400 nM (Figure 2). Compared with thalidomide, for which the IC_{50} is over 200 μM under the same conditions, this represents an increase in potency of over 500-fold. To assess whether other halogen atoms have the same effect as fluorine, we synthesized (±)-tetrachlorothalidomide (**3**) and (±)-tetrabromothalidomide (**4**).[7] Both analogs are much less active than tetrafluorothalidomide (**2**) in inhibiting TNF-α secretion (Figure 2). These observations suggest that the increase in potency with regard to TNF-α inhibition is unique to the fluorine substitution.

Several alkylated *N*-phenyltetrafluorophthalimide analogs (**5**, **6**, **8**, and **10**; Figure 1) previously reported[6] and three additional analogs (**7**, **9**, and **11**) were also found to inhibit, rather than enhance, TNF-α secretion in LPS-stimulated THP-1 cells (Figures 2 and 3). Among the

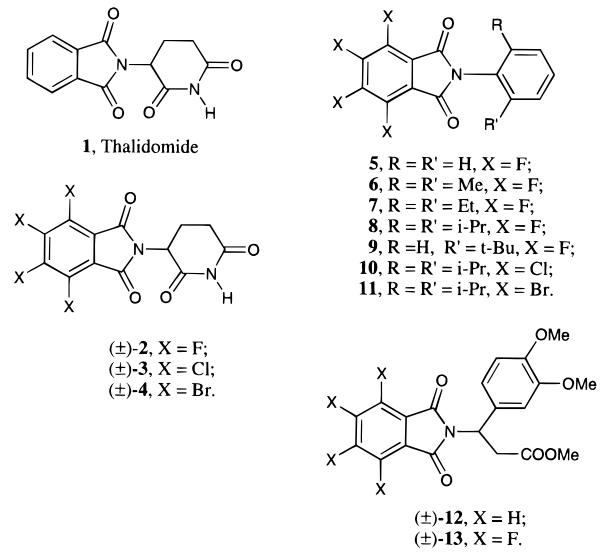

Figure 1. Structures of thalidomide and analogs.

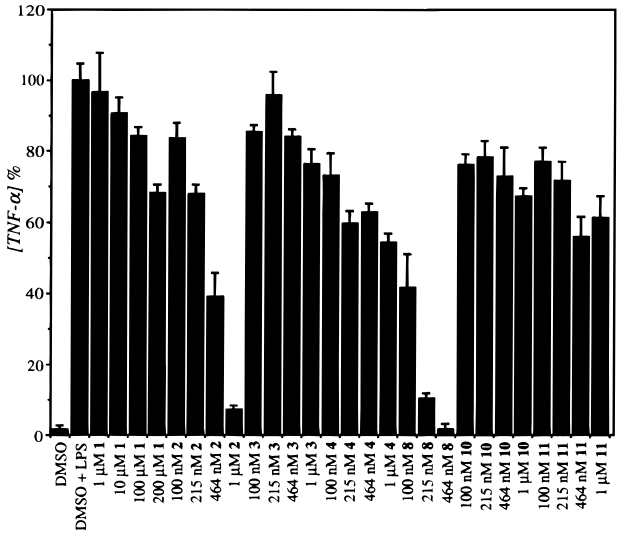

Figure 2. Inhibition of TNF-α production by thalidomide and tetrahalophthalimides.

tetrafluorophthalimides, a correlation was observed between the size of the substituents on the phenyl group and the potency of the compounds (Figure 3). Thus, these compounds decrease in potency in the following order: diisopropyl (**8**) > diethyl (**7**) > *tert*-butyl (**9**) > dimethyl (**6**) > dihydro (**5**). A possible explanation is that the phenyl ring has to be orthogonal to the phthalyl group for these compounds to be active; the bulky substituents on the phenyl ring are likely to interact with the two carbonyl oxygens on the phthalyl ring, preventing free rotation about the N−C(1) bond. Interestingly, the same rank order was observed for the TNF-α-enhancing effect in HL-60 cells.[6b,c] It may be predicted that the analogs that are potent in enhancing TNF-α production in HL-60 cells[6] will be potent as inhibitors of TNF-α production in THP-1 cells and primary monocytes. How the same compounds exhibit opposing effects on TNF-α production in two different cellular systems remains unknown. In addition, we observed a dramatic decrease in TNF-α inhibition when the hydrogens on the phthalyl group were replaced with either chlorine (**10**) or bromine compared to fluorine (**11**), supporting the notion that tetrafluorine substitu-

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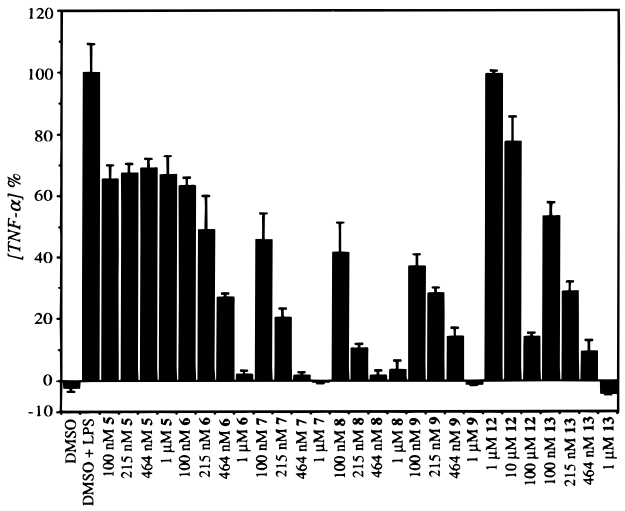

Figure 3. Inhibition of TNF-α production by tetrafluorophthalimides.

tion is key for the inhibition of TNF-α production by these analogs (Figure 2).

Recently, a novel thalidomide analog, methyl 3-phthalimido-3-(3,4-dimethoxyphenyl)propionate (**12**), was reported to inhibit TNF-α production with an IC_{50} more than 2 orders of magnitude lower than that of thalidomide,[12] making it the most potent thalidomide analog reported until that time. We synthesized both (±)-**12** and its tetrafluoro-substituted analog (±)-**13**. Compound **12** is indeed more potent than thalidomide, though we were unable to observe as significant an increase in potency as previously reported, possibly due to different assay conditions employed.[12] Gratifyingly, the tetrafluoro-substituted analog **13** is over 100-fold more potent than **12** (Figure 3).

In summary, we have synthesized a series of tetrafluorophthalimide derivatives which are potent inhibitors of LPS-induced TNF-α production. Although they exhibit higher cytotoxicity to cells than thalidomide,[8] inhibition of LPS-induced TNF-α production by these analogs occurs well under their cytotoxic concentrations and is therefore specific. As these tetrafluorophthalimide derivatives are much more potent than thalidomide (**1**), they may serve as new tools to dissect the lipopolysaccharide-mediated signaling pathway leading to TNF-α production. Given the roles of TNF-α in inflammation and septic shock, these compounds may serve as leads for novel therapeutic agents.

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(7) Tetrafluorothalidomide and other compounds were synthesized in a similar manner as previously prepared thalidomide derivatives (refs 6 and 10). All new compounds are characterized by ^{1}H-NMR and HR-MS or elemental analysis. As the two enatiomers of thalidomide are known to epimerize rapidly in aqueous media, particularly in the presence of human citric plasma (ref 11), all thalidomide derivatives **2**−**4** were prepared in racemic form.

(8) THP-1 cells were pretreated with 100 nM 1α,25-dihydroxyvitamin D_3 for 72 h and stimulated with 1 μg/mL LPS for 16 h as previously described (ref 9). Drug dissolved in DMSO or DMSO alone was added immediately prior to the addition of LPS with a final concentration of 0.2% DMSO in each well. Culture supernatants were harvested and analyzed for TNF-α by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies obtained from Pharmingen according to the supplier's instructions. All compounds had no significant effect on cell viability at the concentrations used as assayed by Trypan blue exclusion. We did, however, observe some cytoxocity of all compounds at higher concentrations. In comparison to thalidomide, which is not toxic to cells at 200 μM, tetrafluorothalidomide is more cytotoxic with an LD_{50} estimated at 20 μM.

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