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AMINO-SUBSTITUTED THALIDOMIDE ANALOGS: POTENT INHIBITORS OF TNF-α PRODUCTION

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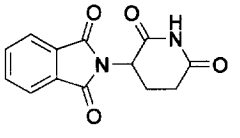
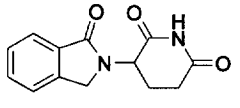
Abstract: Thalidomide, (**1**), is a known inhibitor of TNF-α release in LPS stimulated human PBMC. Herein we describe the TNF-α inhibitory activity of amino substituted analogs of thalidomide (**1**) and its isoindolin-1-one analog, EM-12 (**2**). The 4-amino substituted analogs were found to be potent inhibitors of TNF-α release in LPS stimulated human PBMC.

Introduction: Thalidomide (2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione), (**1**) was developed as a sedative without the side effects of barbiturates in the 1950's by Chemie Grunenthal.[1] Thalidomide quickly became a popular sedative in Europe and Australia and was subsequently used for the treatment of morning sickness in pregnant women. However, thalidomide was removed from the marketplace when its use was linked to birth defects. Thalidomide's teratogenic properties made the drug infamous and catalyzed the development of the current drug approval regulations. A serendipitous discovery in 1965 by Sheskin while treating erythema nodosum leprosum (ENL), an acute inflammatory condition associated with lepromatous leprosy led to the discovery that thalidomide possesses immunomodulatory properties.[2] Since this initial discovery, thalidomide has been found to afford clinical benefit in a variety of autoimmune and inflammatory disease states.[3]



Thalidomide (1)



EM-12 (2)

In 1991 it was reported that thalidomide was a selective inhibitor of tumor necrosis factor-α (TNF-α) over production in stimulated human monocytes.[4] TNF-α is a key cytokine in the inflammatory cascade and elevated TNF-α levels are associated with inflammatory diseases.[5] Recent successful clinical trials in rheumatoid arthritis and inflammatory bowel disease with TNF-α antibodies and soluble TNF-α receptors have validated the inhibition of TNF-α as a clinical treatment.[6]

The clinical activity of thalidomide and the importance of TNF-α inhibition led us to initiate a program to improve the TNF-α inhibitory activity of thalidomide by structural modification. We have previously

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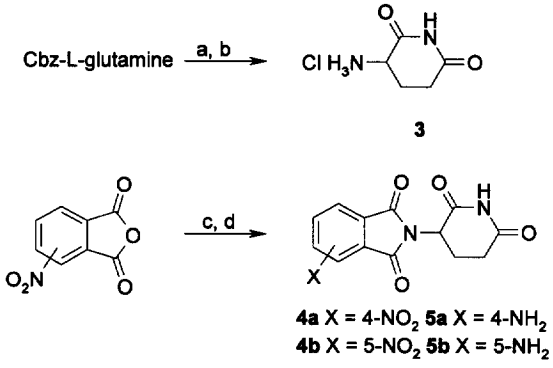
reported a series of thalidomide analogs derived from β-amino-β-arylpropanoic acid derivatives that are potent inhibitors of TNF-α.[7] Further studies revealed these compounds to be potent inhibitors of phosphodiesterase type 4 (PDE4).[8] The PDE4 inhibitory potency for most of these compounds has correlated with their TNF-α inhibitory activity. PDE4 is the major PDE isoenzyme present in monocytes and macrophages, key producers of TNF-α. PDE enzymes control the levels of cyclic adenosine monophosphate (cAMP) by hydrolysis of cAMP to 5'-AMP. Inhibition of PDE4 in stimulated monocytes has been demonstrated to elevate levels of cAMP and inhibit of TNF-α production.[9]

In further studies to improve the TNF-α inhibitory activity of thalidomide, we prepared a series of amino-phthaloyl substituted analogs of thalidomide (**1**) and its isoindoline-1-one analog, EM-12 (**2**). Some amino substituted thalidomide analogs have previously been reported but were not assayed for their TNF-α inhibitory activity.[10] EM-12 (**2**) has been reported to be a more potent teratogen than thalidomide in rabbits, rats, and monkeys.[11] When **2** was evaluated for TNF-α inhibitory activity in LPS stimulated human PBMC it was found to have similar activity to thalidomide. The isoindolinone replacement of the phthaloyl ring increases the stability of the molecule and may lead to increased bioavailability. Herein, we report the structure-activity relationships of amino substitution of the phthaloyl ring of thalidomide and isoindolinone ring of EM-12 on the TNF-α inhibitory activity in LPS stimulated human PBMC.

Scheme 1

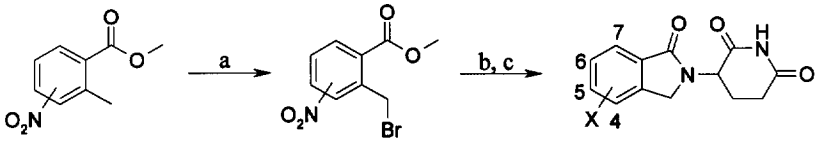


Reagents: (a) CDI, THF, reflux; (b) H_2, 10% Pd/C, EtOAc/4N HCl; (c) **3**, AcOH, reflux; (d) 10% Pd/C, acetone.

Chemistry. The amino substituted analogs of thalidomide were prepared as illustrated in Scheme 1.[12] The amino thalidomide analogs were prepared via the condensation of 3-aminopiperidine-2,6-dione hydrochloride, (**3**). Compound **3** was prepared in two steps from commercially available Cbz-L-glutamine. Treatment of Cbz-L-glutamine with carbonyl diimidazole (CDI) in refluxing THF afforded Cbz-aminoglutarimide. The Cbz protecting group was readily removed by hydrogenolysis under 50-60 psi of hydrogen in the presence of 10% Pd/C in a mixture of ethyl acetate and 4 N HCl. The hydrochloride (**3**) was used directly in the anhydride

condensation reaction without purification. Treatment of **3** with 3- or 4-nitrophthalic anhydrides in refluxing acetic acid afforded the 4- and 5-nitro substituted thalidomide analogs **4a** and **4b**, respectively, in good yields. The nitro groups of **4a** and **4b** were reduced by hydrogenation in a Parr shaker under 50-60 psi of hydrogen in the present of 10% Pd/C to afford the desired 4- and 5-amino substituted thalidomide analog **5a** and **5b**, respectively.[13] The amino substituted isoindolinone analogs were prepared as illustrated Scheme 2.[12] Treatment of **3** with the appropriately substituted nitro substituted methyl 2-(bromomethyl)benzoates, **6a-d** yielded the four isomeric nitro EM-12 analogs **7a-d**. The nitro groups were hydrogenated to the desired amino compound as described above to afford **8a-d**. The four isomeric nitro substituted methyl 2-(bromomethyl)benzoates (**6a-d**) were prepared by benzylic bromination of the corresponding commercially available nitro substituted methyl 2-methylbenzoates.

Scheme 2

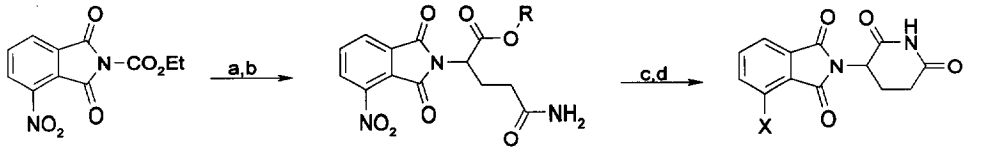


6a 3-NO_2 **7a** X = 4-NO_2 **8a** X= 4-NH_2
6b 4-NO_2 **7b** X = 5-NO_2 **8b** X= 5-NH_2
6c 5-NO_2 **7c** X = 6-NO_2 **8c** X= 6-NH_2
6d 6-NO_2 **7d** X = 7-NO_2 **8d** X= 7-NH_2

Reagents: (a) light, NBS, CCl_4, reflux; (b) **3**, Et_3N, DMF, 80 °C; (d) H_2, 10% Pd/C, MeOH

The R and S isomers of **5a** were prepared starting from the *S*- and *R*-isomers of glutamine *t*-butyl ester (Scheme 3). The nitro substituted Nef's reagent analog, **10** was prepared by treatment of 3-nitrophthalimide with ethyl chloroformate.[14] Nef's reagent is a reagent commonly used in the preparation of chiral N-phthaloyl protected amino acids. Treatment of **10** with the single isomers of t-butyl glutamine afforded the phthaloyl glutamine derivatives, **(*S*)-** and **(*R*)- 11**. The t-butyl group was removed using standard acidic conditions to afford **(*S*)-** and **(*R*)- 12**. To avoid racemization, the ring closure was accomplished using the method reported by Casini and Ferappi[15] for the synthesis of the single isomers of thalidomide to afford **(*R*)-** and **(*S*)-** of **4a**.[13] The nitro groups were reduced as described earlier in acetone to afford the single isomers of **5a**.

Scheme 3



10

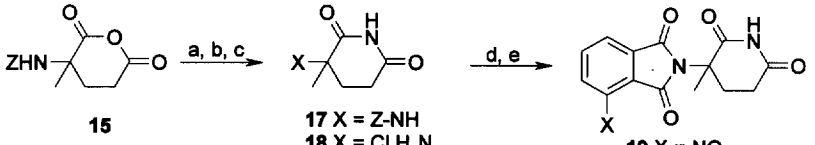
(S)- and (R)- **11** R = t-Butyl
(S)- and (R)- **12** R = H

(S)- and (R)- **4a** X = NO_2
(S)- and (R)- **5a** X = NH_2

Reagents: (a) Et_3N, (*R*) or (*S*) *t*-butyl glutamine HCl; (b) HCl, CH_2Cl_2; (c) $SOCl_2$, pyr/Et_3N; (d) H_2, 10% Pd/C, acetone.

The 4-amino-α-methyl analog (**14**) of thalidomide was prepared from α-methylglutamic acid (Scheme 4). By standard chemistry α-methylglutamic acid was converted to Cbz-α-methylglutamic acid anhydride (**15**). Treatment of the anhydride with ammonia afforded a mixture of α- and γ-amides, **16**. This mixture was cyclized with CDI to the Cbz-protected aminoglutarimide **17**. The Cbz-group was removed by hydrogenation under acidic conditions to afford aminoglutarimide hydrochloride **18**. Condensation with 3-nitrophthalic anhydride followed by reduction of the nitro group afforded **14.**

Scheme 4



Reagents: (a) NH_3, CH_2Cl_2; (b) CDI, THF; (c) H_2, 10% Pd/C, EtOH/4N HCl; (d) 3-NO_2-phthalic anydride, AcOH, reflux; (e) H_2, Pd/C, acetone.

Biological Assays. TNF-α inhibitory activity was measured in lipopolysacharide (LPS) stimulated PBMC as previously reported.[7] The human whole blood TNF-α inhibition assay was run in a similar fashion to the PBMC assay except heparinized fresh human whole blood was plated directly into microtiter plates. The assay was then continued as previously reported for the PBMC assay. The assay for PDE4 enzyme inhibition was run as previously described.[8]

Results and Discussion. Thalidomide has been reported to be a selective inhibitor of TNF-α in LPS stimulated human monocytes.[4] Thalidomide has a TNF-α IC_{50} of ~200 μM in LPS stimulated PBMC.[8] Previous research with thalidomide analogs suggested that phthaloyl substitution could lead to increases in activity.[8] Although the amino substitution had been previously described, these analogs had not been tested for their ability to inhibit TNF-α production (Table 1). The 5-amino analog, **5b**, was found to have a TNF-α IC_{50} of ~100 μM. No inhibitory activity was observed at the lower concentrations tested (less than or equal to 10 μM). The 4-amino analog, **5a**, was significantly more potent with an IC_{50} of 13 nM. Thus, this compound was ~15,000 times more potent than thalidomide as a TNF-α inhibitor in vitro. The novel isomeric amino-substituted EM-12 analogs were then prepared and tested. Unlike thalidomide where there are only two regio isomers, there are four possible regio isomers, **8a-d**. Only the 4-amino analog **8a** potently inhibited TNF-α production (IC_{50} less than 100 μM). Compound **8a** was found to have an IC_{50} of 100 nM (Table 1). This substitution correlates with the amino substitution on **5a** and demonstrated that the amino group needed to be opposite to the carbonyl of the isoindolinone for optimal activity. The *S*- and *R*-isomers of **5a** were prepared and evaluated. The *S*-isomer of **5a** was found to be the more active isomer with a TNF-α IC_{50} of 3.9 nM. The *R*-isomer was ~20-fold less active with a TNF IC_{50} of 94 nM. Although (***R***)-**5a**'s optical purity was greater than 95% ee, some activity was probably due to residual (*S*)-isomer in the sample.

The α-methyl analog of thalidomide, **13**, has also been reported to demonstrate similar TNF-α inhibitory activity to thalidomide.[16] This compound does not contain the racemizable chiral center found in thalidomide. The 4-amino analog **14** was a potent inhibitor of TNF-α with an IC_{50} of 44 nM. Work is in progress to prepare the single isomers of **14** and will be reported on in the future.

Compounds **5a**, **8a**, and **14** were evaluated for PDE4 inhibitory activity using PDE4 enzyme isolated from U937 cells.[8] All three compounds were inactive (<50% inhibition) at 100 μM, the highest concentration assayed. These results strongly suggested that these compounds do not act by PDE4 inhibition. The three active analogs, **5a**, **8a**, and **14** were evaluated for their ability to inhibit TNF-α levels LPS stimulated human whole blood to mimic their activity in vivo. The compounds had only modest declines in activity in this assay. (Table 1).

Table 1 TNF-α Inhibition in LPS Stimulated Human PBMC and Whole Blood

Compd	TNF-α Inhibit. At 100 μM	TNF-α IC_{50}	Whole Blood TNF-α IC_{50}
5a	95%	13 nM	25 nM
5b	55%	~100,000 nM	ND
8a	74%	100 nM	480 nM
8b	15%	ND	ND
8c	12%	ND	ND
8d	18%	ND	ND
14	98%	44 nM	216 nM
(*S*)-5a	99%	3.9 nM	14 nM
(*R*)-5a	85%	93 nM	73 nM

In summary, we have discovered three high potency inhibitors of TNF-α by 4-amino substitution of thalidomide, EM-12, and α-methylthalidomide. The (*S*)-4-amino substituted analog of **5a** was found to be ~50,000 times more potent than thalidomide at inhibiting TNF-α levels in LPS stimulated human PBMC. None the three compounds showed significant activity as a PDE4 inhibitor. A recent publication reported **14** to enhance TNF-α production in 12-O-tetradecanoyl-phorbol 13-acetate stimulated human leukemia HL-60 cells.[17] These discordant results are possibly related to our use of primary human cells stimulated with LPS in contrast to the other investigators use of the HL-60 cell line stimulated with TPA. Further, we have demonstrated that these compounds retain high activity in the milieu of whole human blood. We are presently investigating the structure-activity relationships of other substituted phthaloyl and isoindolinone analogs of thalidomide and EM-12 and will be publishing on the biological profiles of **5a**, **8a**, and **14**.[18]

References and Notes

1. Stirling, D. I. *Pharmaceutical News* **1996**, *3*, 17.
2. Sheskin, J. *Clinical Pharmacol. Ther.* **1965**, *6*, 303.
3. (a) Gutierrez-Rodriguez, O. *Arth. And Rheum.* **1984**, 27, 1118. (b) Peterson, D. L.; Georghiou, P. R.; Allworth, A. M.; Kemp, R. J. *Infection. Clin. Infect. Dis.* **1995**, *20*, 250. (c) Schuler, U.; Ehninger, G. *Drug Safety*, **1995**, *12*, 364. (d) Vogelsang, G. B.; Hess, A. D.; Gordon, G.; Brundrette, R.; Santos, G. W. *Transplantation Proc.* **1987**, *XIX*, 2658. (e) Klausner, J.; Makonkawkeyoon, S.; Akarasewi, P.; Nakata, K.; Kasinrerk, W.; Corral, L.; Dewar, R.; Lane, C.; Freedman, V.; Kaplan, G. *J. Acq. Immun. Def. Synd.* **1996**, *11*, 247. (f) Stirling, D.; Sherman, M.; Strauss, S. *J. Amer. Pharm.* Assoc. **1997**, NS37(3).
4. Sampaio, E. P.; Sarno, E. N.; Galilly, R.; Cohn, Z. A.; Kaplan, G. *J. Exp. Med.* **1991**, *173*, 699.
5. Eigler, A.; Sinha, B.; Hartmann, G.; Endres, S. *Immunology Today* **1997**, *18*, 487.
6. (a) van Dullemen, H; van Deventer, S; Hommes, D; Bijl, L; Jansen, J; Tytgat, G; Woody, J. *Gastroenterology* **1995**, *109*, 129. (b) Elliott, M.; Feldmann, M.; Maini, R. *Int. J. Immunopharmac.* **1995**, *17*, 141.
7. Muller, G. W.; Corral, L. G; Shire, M. G.; Wang, H.; Moreira, A.; Kaplan, G.; Stirling, D. I. *J. Med. Chem.* **1996**, *39*, 3238.
8. Muller, G.W.; Shire, M. G.; Wong, L. M.; Corral, L. G.; Patterson, R. T.; Chen, Y.; Stirling, D. I. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2669.
9. Torphy, T. J. *Am. J. Resp. Crit. Care* **1998**, *157*, 351.
10. He, W.; Engstrom, L.; Walker, S.; Vogelsang, G.; Leong, K. W. *Abstracts of Papers*, 206th American Chemical Society, Chicago, IL, Fall 1993; Med. Chem., paper 216.
11. Heger, W.; Schmahl, H.; Klug, S.; Felies, A.; Nau, H.; Merker, H.; Neubert, D. *Teratogenesis, Carcinogenesis, and Mutagenesis* **1994**, *14*, 115.
12. Muller, G. W.; Stirling, D. I.; Chen, R. S. C. United States Patent No. 5,635,517, **1997.** *Chem. Abstr.* **1997**, 127, 86110.
13. Chiral HPLC analyses were carried out at 240 nM using a Daicel chiralpak AD column (0.46 x 25 cm) eluting with 30/70 hexane/IPA at 0.75mL/min.
14. Siedlecka, R.; Skarzewski, J. *Pol. Synth. Commun.* **1997**, *27*, 2081.
15. Casini, G.; Ferappi, M. *Farmaco, Ed. Sci.* **1964**, 563.
16. Wnedt, S.; Finkam, M.; Wniter, W.; Ossig, J.; Raabe, G; Zwingenberger, K. *Chirality* **1996**, *8*, 390.
17. Miyachi, H.; Koiso, Y.; Shirali, R; Niwayama, S.; Liu, J. O.; Hashimoto, Y. *Chem. Pharm. Bull.* **1998**, *46*, 1165.
18. Corral, L. G.; Haslett, P. A.; Muller, G. W.; Chen, R.; Wong, M. L.; Ocampo, C.; Stirling D. I.; Kaplan, G. *J. Immun. accepted.*