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Design, synthesis and biological evaluation of new thalidomide analogues as TNF-α and IL-6 production inhibitors

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1*H*-Pyrrolo-[2,3-*b*]pyridine

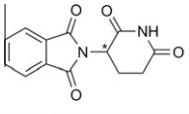
ABSTRACT

Several thalidomide analogues were synthesized and compared to thalidomide and its more active analogue, lenalidomide, for their ability to inhibit the production of the pro-inflammatory cytokine tumour necrosis factor (TNF)-α and interleukin (IL)-6 by LPS-activated peripheral blood mononuclear cells (PBMCs). Among these compounds, two analogues containing sulfonyl group displayed interesting downregulation of TNF-α and IL-6 production.

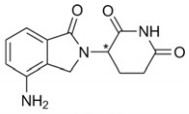
Thalidomide (2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione) is a synthetic glutamic acid derivative that was developed and synthesized by Chemie Grunenthal in 1956.[1–3] It was initially marketed as a sedative, its rapid speed of onset, lack of hangover effect, and apparent safety after overdose making it an attractive alternative to barbiturates. In addition, it was a powerful antiemetic, and was widely taken by pregnant women for the treatment of morning sickness and as an aid to help them sleep. Nearly four years later, multiple birth defects including phocomelia (absence or hypoplasia of arms), absence of ears, deafness, defects of the femur and tibia, as well as malformations of the heart and the bowel were observed and associated with use of thalidomide in early pregnancy. Abnormalities of the alimentary tract, urinary tract and other defects of internal organs were also reported.[4,5] This resulted in the withdrawal of thalidomide from the world market.

In recent years, scientific interest in thalidomide has been renewed, because of its effectiveness in various diseases including erythema nodosum leprosum, arthritis, tuberculosis and conditions associated with human immunodeficiency virus infections.[6] In 1998, thalidomide was reintroduced onto the market, and has undergone clinical investigation for various immunomodulatory and anti-inflammatory applications.[7,8] Thalidomide selectively and partially inhibits the production of TNF-α in human monocytes in a dose-dependent fashion, with no effect on total protein synthesis or on the production of other cytokines.[9] It is the consequence of the half-life reduction of TNF-α mRNA from approximately 30–17 min by thalidomide.[10] Additional studies have shown that thalidomide also reduces the synthesis other cytokines, including IL-6.[11]

A few years later, immunomodulatory thalidomide analogues (IMiDs) were designed to optimise some properties, including anticancer properties, while abolishing or reducing the toxicity associated with thalidomide. Extensive preclinical testing of this class of compound, including its pharmacology, pharmacokinetics and toxicity, has led to the selection of lenalidomide for clinical development in the oncology setting.[12,13]



Thalidomide



Lenalidomide

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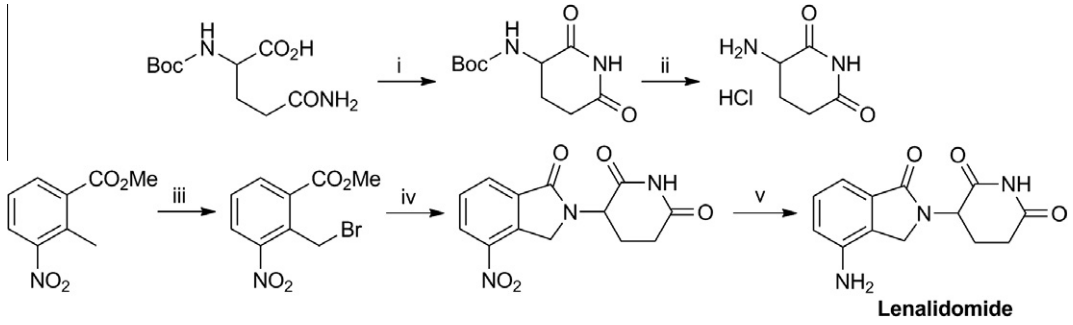
In a previous study, Lima et al. demonstrated that a derivative of sulfonyl-isoindoline was active in modulating TNF-α release. They have shown that compounds including the sulfonamide moiety connected to the phthalimide ring of thalidomide are better inhibitors of TNF-α.[14,15] Taking these results into account, we designed other molecules with structural similarities. The first group include derivatives containing a tricyclic structure derived from an imidazo-indole-dione, which has a more rigid structure than thalidomide, albeit still including the carbonyl-amine-carbonyl sequence, present in the phthalimide moiety. The second group is composed of 7-azaindole derivatives, reported to display some of the biological activity associated with sulfonamide.

Lenalidomide was synthesized following the synthetic route of Muller et al.,[16] shown in Scheme 1. It was obtained from the *N*α-(*tert*-butoxycarbonyl)-L-glutamine and the methyl-2-methyl-3-nitrobenzoate in five steps with an overall yield of 18%.

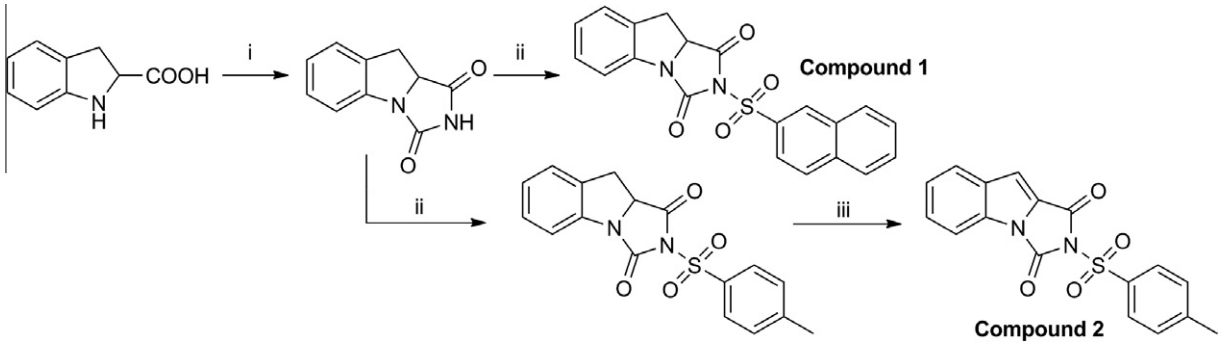
We then developed a series of tricyclic molecules (Group I: compounds **1**–**4**). Compounds **1** and **2** could be easily obtained, with correct yields, by a synthetic sequence shown in Scheme 2. The condensation of potassium isocyanate with indoline-2-carboxylic acid at reflux led to the formation of 9,9a-dihydro-1*H*-imidazo[1,5-*a*]indole-1,3(2*H*)-dione. This derivative was then functionalised with arylsulfonyl-methylimidazolium triflate, prepared in situ, to furnish compound **1**, and the precursor of compound **2**, which after reduction gives the desired product with a good yield.

An analogue of compounds **1** and **2** was developed from the 7-azaindole skeleton, as shown in Scheme 3. The first step was the sulfonylation at position 1 in order to steer the introduction of a carboxylic acid group to position 2 in a second step. The aim was the esterification of the acid group. A classical reaction with sulfuric acid in ethanol at reflux led to the formation of the ethylic ester, and at the same time to desulfonylation, with very good yield. To complete the synthetic pathway to compound **3**, a cyclisation with phenylisocyanate was performed, which furnished the derivative with correct yield.

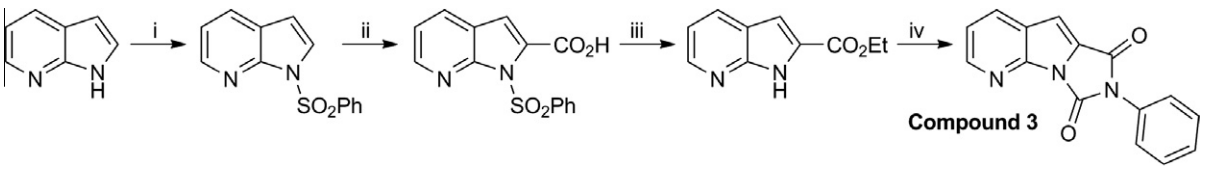
In order to determine the importance of the ring size, another tricyclic compound was considered with an isoquinoline structure. The synthetic pathway of compound **4** is depicted in Scheme 4. The condensation of formaldehyde with phenylalanine at reflux gave 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid with a correct yield. The carboxylic acid was then esterified, and the amino group sulfonylated, followed by a cyclisation at room temperature. After an N-alkylation with a good yield, the ethyl-4-mercaptobenzoate was introduced. The synthetic route to the compound **4** was accomplished by the oxidation of the sulfur with *m*CPBA.



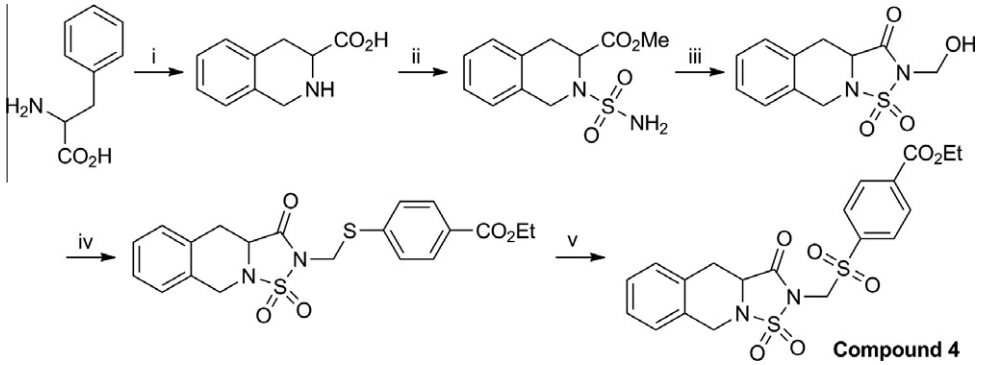
Scheme 1. Reagents and conditions: (i) CDI, DMAP, toluene, reflux, 12 h; 65%; (ii) HCl 1 N, THF, rt, 12 h; quant.; (iii) NBS, AIBN, CCl_4, reflux, 24 h; 49%; (iv) 3-aminopiperidine-2,6-dione, TEA, MeCN, 55 °C, 18 h; 57%; (v) H_2, Pd/C, MeOH, DMF; quant.



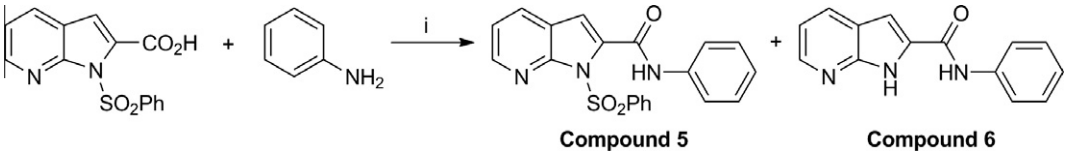
Scheme 2. Reagents and conditions: (i) KNCO, HCl, H_2O, reflux, 3 h; 49%; (ii) arylsulfonyl-methylimidazolium triflate, Et_3N, THF, rt, 12 h; 63%; (iii) NBS, AIBN, CCl_4, reflux, 1 h; 85%.



Scheme 3. Reagents and conditions: (i) NaOH, Bu_4NBr, $ClSO_2Ph$, DCM, 0 °C to rt, 2 h; 90%; (ii) (a) LDA, THF, −35 °C, 30 min; (b) CO_2, −35 °C to rt, 12 h; 65%; (iii) H_2SO_4, EtOH, reflux, 12 h; 80%; (iv) PhNCO, $NaOCH_3$, toluene; 45%.



Scheme 4. Reagents and conditions: (i) HCHO, HCl, H_2O, reflux, 3 h; 62%; (ii) (a) H_2SO_4, MeOH, reflux, 12 h; 95%; (b) HCO_2H, $ClSO_2NCO$, TEA, DCM, 0 °C, 4 h; 69%; (iii) (a) NaH, THF, rt, 2 h; 59%; (b) HCHO, H_2O, reflux, 1 h; 70%; (iv) (a) $SOCl_2$, Et_2O, rt, 48 h; (b) $SHPhCO_2Et$, DBU, CH_3CN, rt, 12 h; 67%; (v) *m*CPBA, DCM, rt, 3 h; 91%.



Scheme 5. Reagents and conditions: (i) PyBOP, DIEA, DMF, rt, 24 h; compound **5**, 34%; compound **6**, 34%.

Finally, a last series of molecules (Group II: compounds **5**–**9**) was prepared from the 1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylic acid through a peptide coupling with PyBOP and various amines including a sulfonamide group. The synthesis schemes are shown in Scheme 5 and Scheme 6. Only the aniline coupling led to the formation of the both products sulfonylated and desulfonylated. The other amines furnished the desulfonylated compounds.

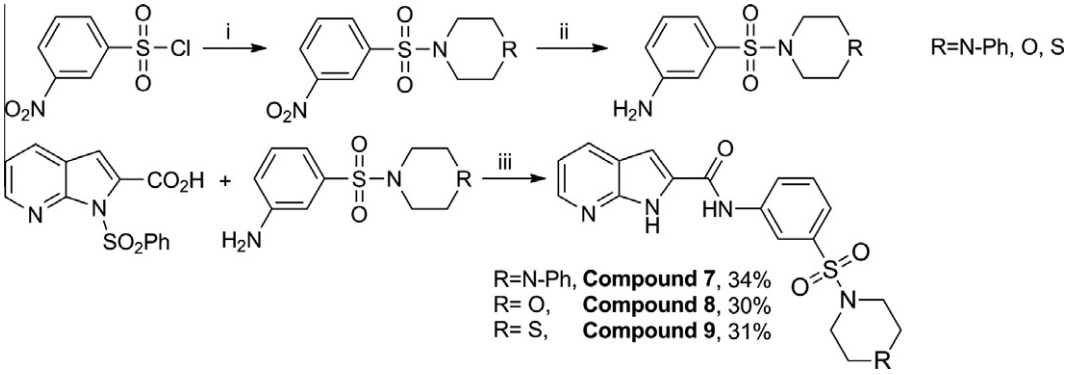
Thalidomide and the compounds **1**–**9** were screened at concentrations of 100, 10 and 1 μM for their ability to regulate TNF-α and IL-6 production.[17] Cytokines concentrations measured in 48 h LPS-stimulated PBMCs supernatants, are summarized in Figure 1a and b We noticed that none of these products at the concentration tested in vitro displayed cytotoxic activity for PBMCs, as assayed by propidium iodure labelling and flow cytometry detection.

We confirmed that thalidomide inhibits TNF-α production, but IL-6 production is unchanged. But, as reported in the literature, lenalidomide inhibits TNF-α and IL-6 2–3 times more effectively than thalidomide.

In the Group I, we were interested in the rigidity of molecules with a tricyclic structure, such as an imidazo-indole-dione or imidazo-pyrrolopyridine-dione. Compounds **1** and **2** are effective inhibitors of TNF-α secretion, but do not affect IL-6 production. Compound **3**, compared to compounds **1** and **2,** was less inhibitor of TNF-α and displayed a similar IL-6 regulating activity. Finally compound **4** at 100 μM inhibits significantly TNF-α and IL-6 secretion, but is devoid of effect at 10 and 1 μM. One of the structural differences between compound **3** and compounds **1**, **2** and **4** is the absence of the sulfonyl group. The results obtained for this set of molecules teach us that the presence of a sulfonyl group is important for regulating the activity of TNF-α.

The compounds in the Group II are less rigid. They are built around a common core, 7-azaindole, and they have for most of them a sulfonyl group (compounds **5**, **7**, **8** and **9**). Compound **5** induced a dose-dependent inhibition of TNF-α production and a best inhibition of IL-6 compared to thalidomide. Compounds **6**–**9** displayed a weak inhibitory effect on TNF-α and IL-6 production. We also noted that the presence of the thiomorpholine, which emerged as very important in the Lima's studies, was not relevant to the downregulation of TNF-α production in our study.

In conclusion, nine thalidomide analogues were synthesized and evaluated for their ability to inhibit TNF-α and IL-6 production by LPS-activated PBMCs. Our results show the importance of these new compounds as potential candidates to inhibit the production



Scheme 6. Reagents and conditions: (i) Functionalized piperazines, DCM, rt, 30 min; 93–95%, (ii) $SnCl_2$, $2H_2O$, DCM, MeOH, rt, 12 h; 94–97%; (iii) PyBOP, DIEA, DMF, functionalised piperazines, rt, 24 h; 30–34%.

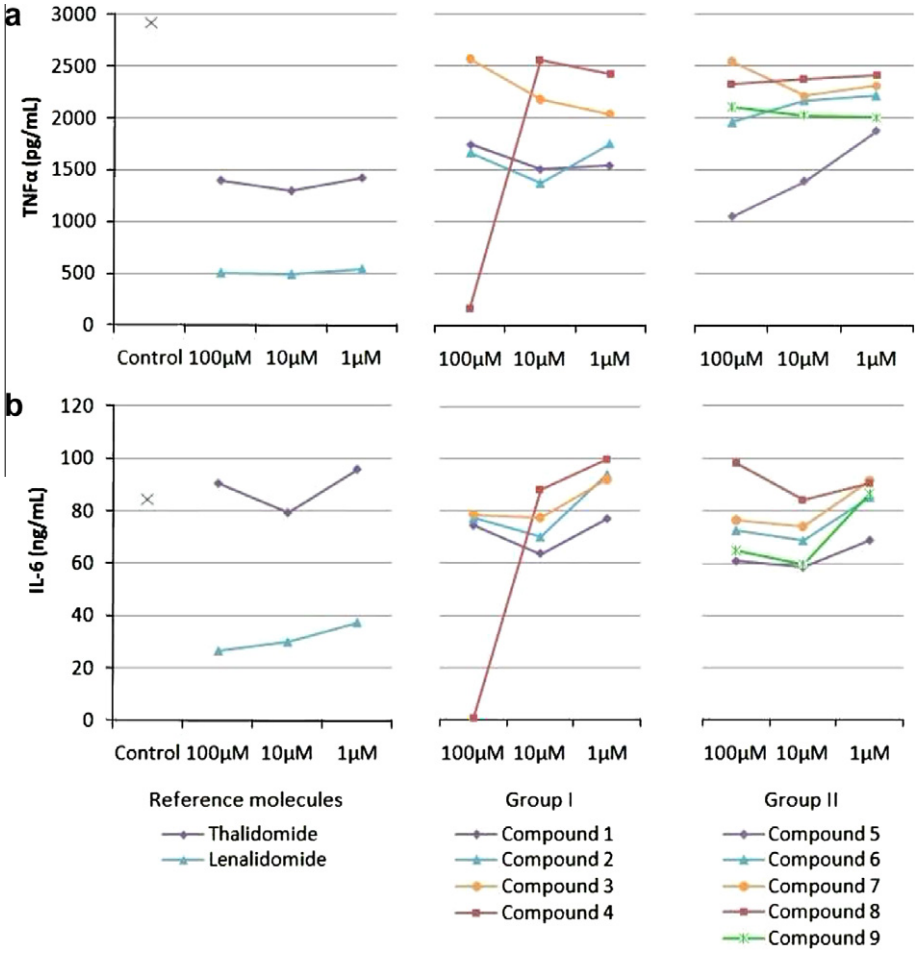


Figure 1. Effects of thalidomide, lenalidomide and compounds **1**–**4** (Group I) and **5**–**9** (Group II) on TNF-α (Fig.1a) and IL-6 (Fig. 1b) secretion. Normal PBMCs from three normal donors were cultured for 48 h with 1 μg/ml LPS, in the absence (control) or presence of 100, 10, or 1 μM of thalidomide, leanlidomide or compounds **1**–**9**. Cytokines were assayed in culture supernatants by ELISA. Each dot represents the mean of three independents experiments. Not indicated for comprehension, variability is in all cases <20%.

of the pro-inflammatory cytokines TNF-α and IL-6. The activity results indicate that compounds **4** and **5** show promising activity against TNF-α. The tricyclic structure and the sulfonyl group can be considered to be scaffolds for the future design and development of anti-TNF-α and IL-6 agents. The importance of these new compounds cannot be ignored, as they are intermediates in the lead optimization process.

Acknowledgements

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17. *Cell culture and reagents*. Normal human peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll Hypaque (Biochrom AG, Berlin, Germany) centrifugation. For all experiments, PBMCs were grown in culture medium consisting of RPMI 1640 (Invitrogen Life Technologies, Cergy Pontoise, France) supplemented with Glutamax I, 10% heat inactivated foetal calf serum (Sigma Aldrich, Saint Quentin Fallavier, France), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen Life Technologies). One million PBMCs were cultured for 48 h in 1 ml of medium with 1 μg/ml *E. coli* LPS (Sigma Aldrich), in the absence or presence of 100 μM, 10 μM, 1 μM thalidomide, lenalidomide, compounds **1**–**9**. The use of PBMCs for research studies was approved by the Ethics Committee of the Poitiers Hospital University Center. *ELISA for cytokine measurements*. Cytokines were measured by ELISA in 48-h culture supernatants. Commercial kits were used to measure IL-6 (Eli-pair, Diaclone, Besançon, France) and TNF-α (Matched Antibodies pairs for ELISA, R&D systems). ELISA was performed according to manufacturers' instructions. All assays were performed in duplicate, in 96-well, flat-bottomed Maxisorp microtiter plates (Nunc, Rochester, NY).