Recent Advances in the Chemistry of Phthalimide Analogues and their Therapeutic Potential

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Abstract: Phthalimide analogues have been extensively used in medicinal chemistry owing to their wide range of applications as anti-convulsant, anti-inflammatory, analgesic, hypolipidimic and immunomodulatory activities. Number of anti-inflammatory phthalimide analogues have been synthesized as tumor necrosis factor-α (TNF-α) inhibitors. TNF-α plays a critical role in certain physiological immune systems and its over-production causes severe damage to the host. It promotes the inflammatory response leading to many of the clinical problems associated with auto-immune disorders like rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, psoriasis and refractory asthma. One of the phthalimide derivatives, LASSBio-468, was recently demonstrated to inhibit TNF-α production induced by lipopolysaccharide (LPS), *in vivo*. Its potential against chronic inflammatory diseases was also witnessed. Another derivative, DIMP, showed good anti-androgenic activity. These analogues have also been employed for the synthesis of several kinds of important therapeutic synthones. However extensive research on the chemistry and biological activities of phthalimide analogues has been carried out and number of reports appeared, a compilation focusing on chemistry and biological activity is still needed. This review, concisely describes the chemical and therapeutic aspects of phthalimide derivatives.

Keywords: Phthalimide, polyamide, TNF-α, anti-inflammatory, anti-angiogenesis, anti-cancerous, peripheral analgesic, structure activity relationship.

1. INTRODUCTION

Phthalimide (**1**) (Fig. (**1**)) (1,3 isoindolinedione) is a white solid aromatic imide in which two carbonyl groups bound to an amine functional moiety. It is a very important starting synthone for organic synthetic chemists for preparing diverse biologically active molecules. Its alkali metal salt is generally used for Gabriel synthesis of amine [1]. Number of phthalimide derivatives has been synthesized with interesting biological activities [2, 3]. Among these activities, inhibition of tumor necrosis factor-α (TNF-α) production is an important biological activity shown by various phthalimides.

TNF is a large family of cell surface and secreted cytokines, mediating host defence and immune regulation. Among the members of this family, TNF-α which is produced mainly by macrophages and T cells in response to various stimuli, possess the widest range of activities. It is biologically active both as a trans membrane protein and as a homotrimeric secreteing molecule. These extend beyond the well-characterized pleiotropic pro-inflammatory properties to include diverse signals for cellular differentiation, proliferation and death [4-6]. An important cytokine produced by activated monocytes/ macrophages, was originally identified as an endotoxin-induced serum factor that causes hemorrhagic necrosis of transplanted solid tumors [7]. TNF-α has attracted attention as a potential target for antitumor drugs because of its cytotoxicity selectively towards various tumor cells [8]. It has numerous effects on mammalian cells which are initiated by binding to high-affinity receptors [9]. Though, TNF-α plays a critical role in certain physiological immune systems, it causes severe damage to the host when produced in excess. Therefore, TNF-α can be regarded to show both favorable and unfavorable effects. The favorable effects include direct tumor-killing effect [9], stimulation of the host's immune system [10] and action as a growth factor for normal B-cells [11]. The unfavorable effects include induction of tissue inflammation [12], tumor-promoting action [13], stimulation of human immunodeficiency virus (HIV) replication [14] and induction of insulin resistance [15]. Due to this pleiotropy of TNF-α, the drugs which act as their production enhancer or inhibitor could be employed as biological response modifiers (BRMs) under various circumstances [16]. Moreover, tissue or cell-type-specific regulation of TNF-α production would be useful, since it is rapidly cleared from the circulation. Thalidomide [*N*-phthalimido glutarimide (**2**) (Fig. (**1**))] is a very effective lead structural moiety for this purpose which was initially developed as a hypnotic agent but had to be withdrawn from the market because of its teratogenicity. In the recent years due to its potential for the treatment of acquired immunodeficiency syndrome (AIDS) [14], graft-*versus*-host disease (GVHD) [17], leprosy [18], and other related diseases [19], it has been further used as an important therapeutic agent. The therapeutic effect of thalidomide has been attributed to its ability to specifically inhibit TNF-α production [20]. Thalidomide (**2**) enhances TPA-induced TNF-α production by human leukemia HL-60 cells, while it inhibits TPA-induced TNF-α production by

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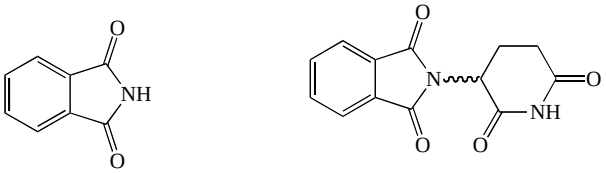
another human leukemia cell line THP-1. However, it inhibits its TNF-α production by both human promyelocytic leukemia cells (HL-60) and THP-1 cells when the cells are stimulated with okadaic acid (OA) [21].



Phthalimide (**1**) Thalidomide (**2**)

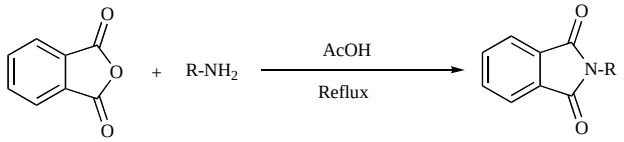
Fig. (1). Structure of phthalimide and thalidomide.

On the basis of these findings, number of scientific groups are engaged in structural modification of thalidomide with the aim of creating superior regulators of TNF-α production. For the evaluation of the compounds obtained, TNF-α production- enhancing activity was assayed using HL-60 stimulated with TPA, and TNF-α production-inhibiting activity was assayed using HL-60 stimulated with OA [22]. Number of reports has been published on synthesis of phthalimide derivatives and their biological activities; however to the best of our knowledge a review article focusing on chemistry and biological activities is still needed. Hence, the present review concisely describes the chemistry, medicinal potential and structure activity relationship of phthalimide derivatives.

2. SYNTHESIS OF PHTHALIMIDE DERIVATIVES

2.1. Conventional Synthesis

Apart from conventional synthesis of phthalimides a number of methods have been extensively used for the synthesis of phthalimides. Miyachi *et al.* in 1996 and Shibata *et al.* in 1996 synthesized *N*-substituted phthalimide derivatives with *n*-butyl, *tert*-butyl, hexyl, adamantly and different substituted phenyl groups (Fig. (**2**)) [23, 24]. All the prepared compounds were tested for TNF-α production enhancing activity and their effects were compared with thalidomide. All compound showed the activity except N-n-butyl phthalimide. Among all the tested compounds *N*-adamantyl phthalimide showed the most potent, TNF-α production-enhancing activity. These compounds were prepared in good yield by condensation of appropriate amine with phthalic anhydride (**3**) (Scheme **1**).

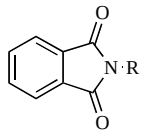


Phthalic anhydride (**3**)

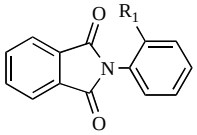
R = Different alkyl or aryl groups

Scheme 1.

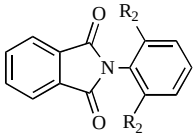
Aromatic polyimides and heterocycles are known as high performance polymer material for their excellent electrical



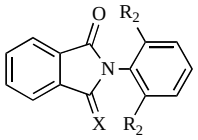
R = n-C_4H_9 (**4**) , C_6H_6 $C(CH_3)_3$ (**5**), adamantyl (**6**)



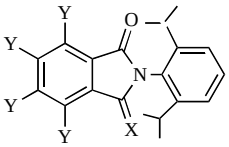
R_1 = H (**7**), CH_3 (**8**), C_2H_5 (**9**), $CH(CH_3)_2$ (**10**), $C(CH_3)_3$ (**11**), adamantyl (**12**), OCH_3 (**13**), SCH_3 (**14**), $(CH_2)_2CH_3$ (**15**), $(CH_2)_3CH_3$ (**16**), $(CH_2)_4CH_3$ (**17**), $(CH_2)_5CH_3$ (**18**), $(CH_2)_6CH_3$ (**19**), $(CH_2)_2C_6H_5$ (**20**), $(CH_2)C_6H_{11}$ (**21**), $N(CH_3)_3$ (**22**), NO_2 (**23**)



R_2 = H (**24**), CH_3 (**25**), $CH(CH_3)_2$ (**26**)



X	**R_2**	
H_2	H	**27**
H_2	CH_3	**28**
O	H	**29**
O	CH_3	**30**

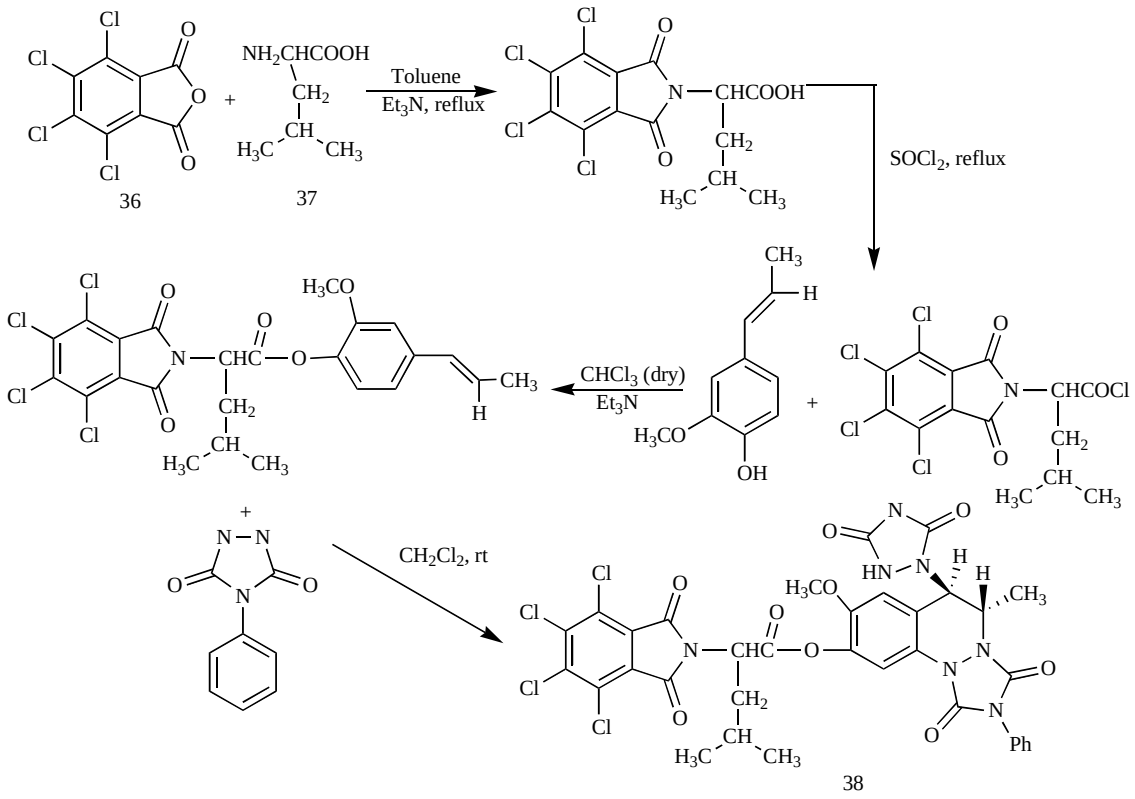


X	**Y**	
H_2	H	**31**
O	H	**32**
S	H	**33**
O	F	**34**
S	F	**35**

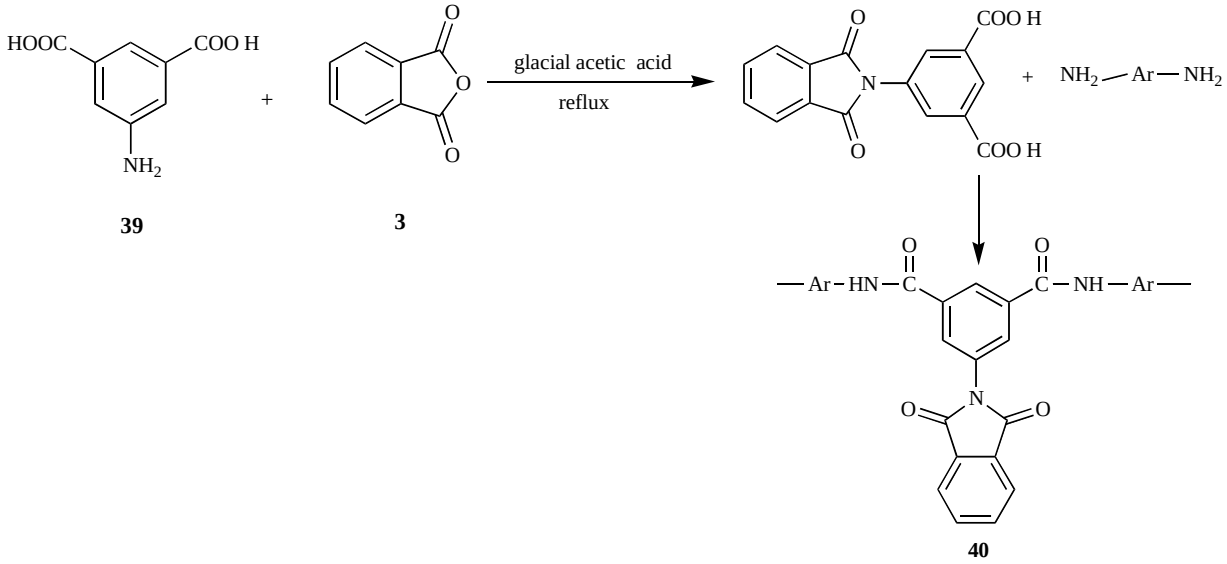
Fig. (2). Phthalimide derivatives.

and mechanical properties. Mallakpour *et al.* [25] reported synthesis of phthalimide based polymer (**38**). Tetra-chloro-phthalic anhydride (**36**) was reacted with L-leucine (**37**) in refluxing toluene in the presence of triethylamine and the resulting imide acid was obtained in quantitative yield (Scheme **2**).

Phthalimide based high performance, processable and high temperature resistance polyamides were synthesized [26]. Due to thermal stability of phthalimide group, its incorporation into polymer backbone remarkably enhanced the thermal stability of polymer. (Scheme **3**) [27].



Scheme 2.



Scheme 3.

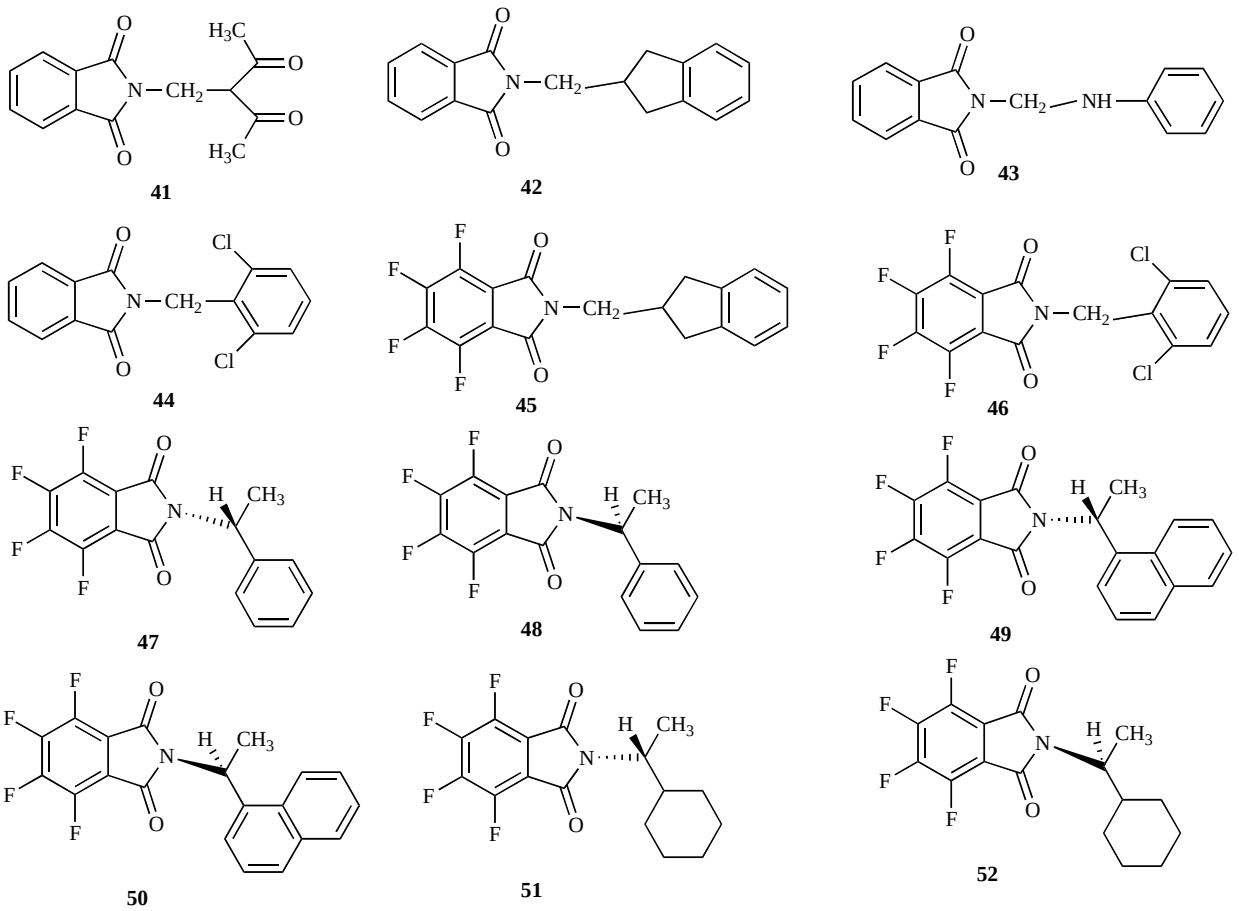


Fig. (3). Compounds with anti-androgenic activity.

Miyachi *et al.* synthesized phthalimide derivatives (Fig. (**3**)) acting as androgen antagonist [28]. Androgen antagonists are the compounds which antagonize the biological responses induced by endogenous, by inhibiting competitively their binding to the nuclear androgen receptor. The growth of several kinds of tumors, especially prostate tumors, is stimulated by androgen antagonists, therefore these phthalimides could be effective for treatment of these androgen-dependent tumors [29]. Androgen-antagonistic activities of the compounds were evaluated by well established methods such as Chloramphenicol acetyl transferase (CAT) assay [30]. These compounds were synthesized by using same process of condensation between phthalic anhydride or tatraflurophthalic anhydride and appropriate primary amine.

Phthalimide derivatives with peripheral analgesics activity were synthesized by Antunes *et al.* (Scheme **4**) [31]. Seven derivatives were synthesized and their analgesic properties were evaluated. All of them showed significant analgesic and anti-inflammatory properties.

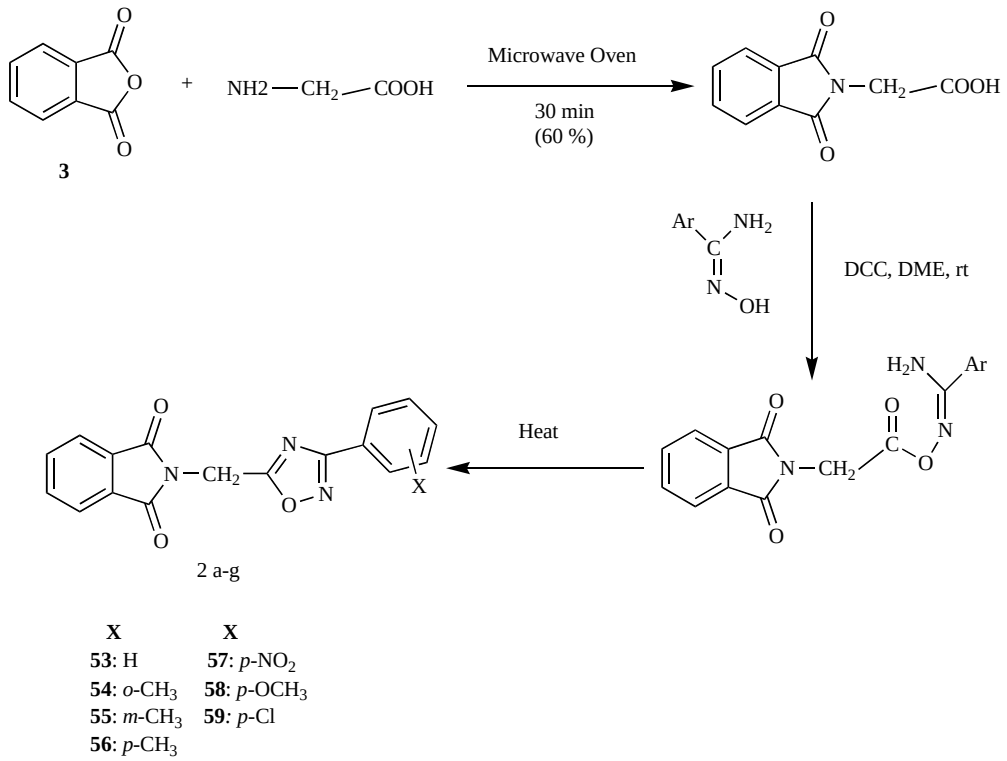
Heravi *et al.* reported synthesis of *N*-substituted phthalimides catalyzed by 1,4-diazabicyclo[2,2,2] octane [DABCO] in solvent less system (Scheme **5**) [32]. Unlike conventional method, this solvent free approach required only few minutes.

Ragavendran *et al.* designed (Fig. (**4**)) and synthesized two series of pharmacophoric hybrids of phthalimide-gamma amino butyric acid (GABA)-anilides/hydrozones and evaluated their anticonvulsant and neurotoxic properties [33]. Three and four steps processes were used for the synthesis of anilides and hydrazones respectively (Scheme **6**).

The anticonvulsant activity of the compounds (**60-85**) (Tables **1** and **2)** was determined using four seizured animal models which included maximal electroshock seizure (MES), subcutaneous pentylenetetrazole (scPTZ), subcutaneous strychnine (scSTY) and intraperitoneal picrotoxin (ipPIC)-induced seizure threshold tests. The acute neurological toxicity was determined in the rotarod test.

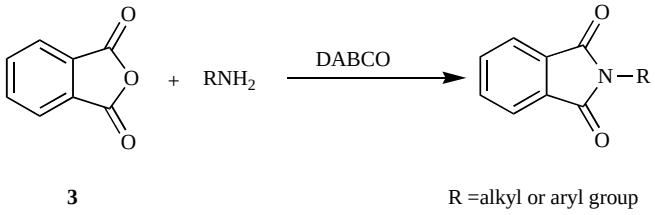
The most active compound of this study was found to be 4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-*N*-(2,6-dimethylphenyl)-butanamide (**63**) as it is effective at both the time-points observed except in the scPTZ model. Of the two series of pharmacophoric hybrids, the phthalimide-GABA-anilides were found to be more effective than the corresponding phthalimide-GABA-hydrazone derivatives.

Dubreuil *et al.* developed a green process for the synthesis of *N*-substituted phthalimide derivatives (Scheme **7**) [34]. They carried out rapid and clean synthesis of phthalimide derivatives at high-temperature and high-pressure H_2O/EtOH mixture achieved by autoclave. Results obtained with different amine are given in Table **3**.

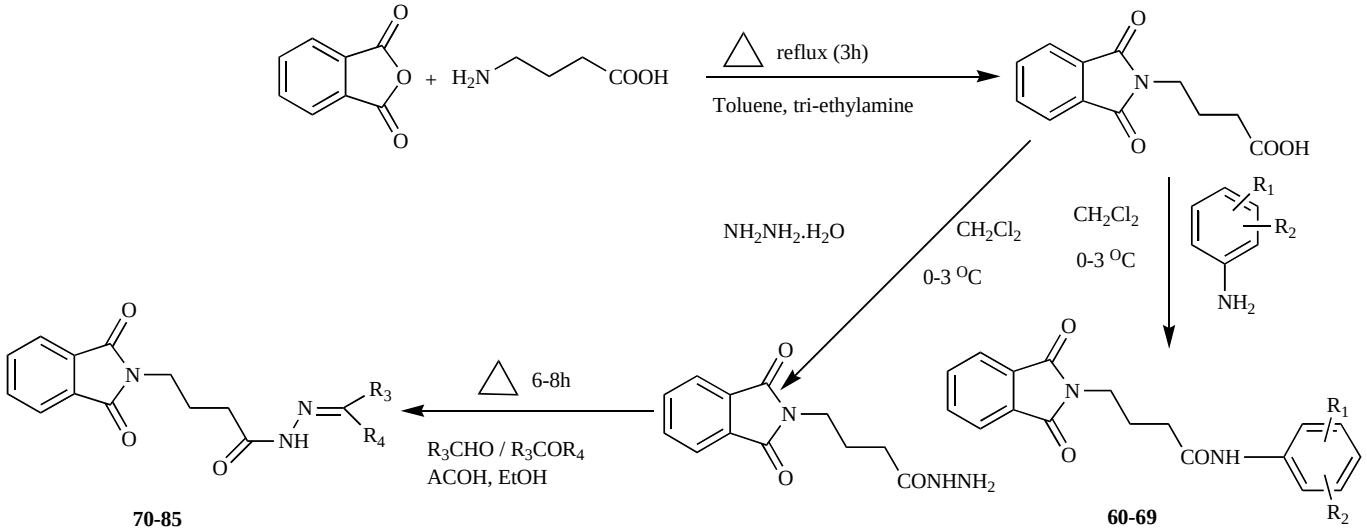


X	X
53: H	**57**: *p*-NO_2
54: *o*-CH_3	**58**: *p*-OCH_3
55: *m*-CH_3	**59**: *p*-Cl
56: *p*-CH_3	

Scheme 4.



Scheme 5.



Scheme 6.

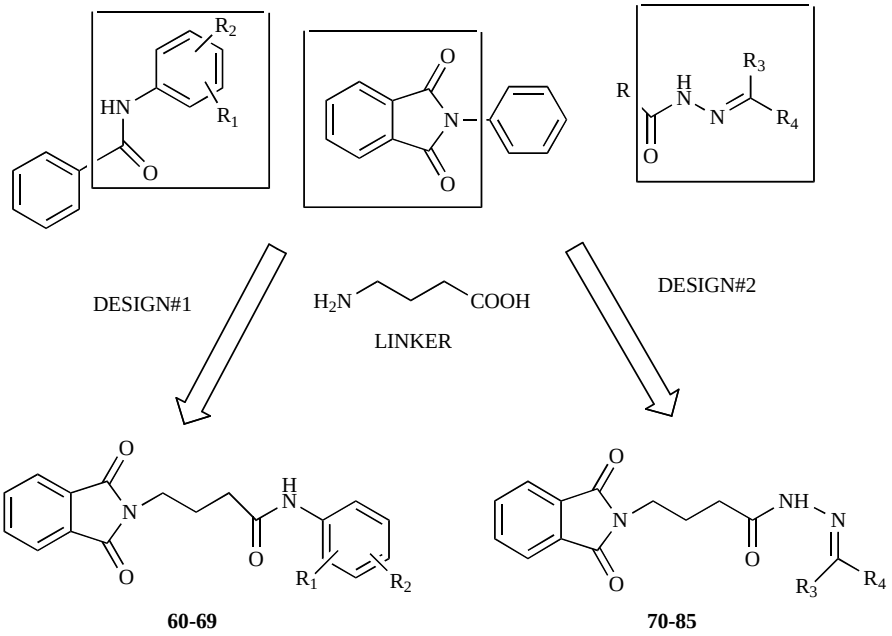


Fig. (**4**). Anticonvulsant compounds designed by pharmacophore combination.

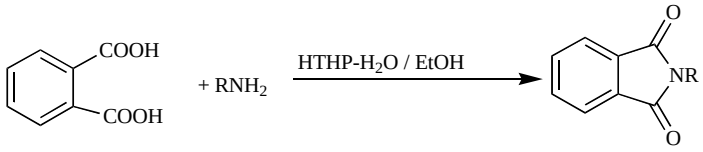
Table 1. (1,3-dihydro-2-isoindol-2-yl)-*N*-(Substituted Phenyl) Butanamides

Compound	R_1	R_2
60	H	4-Cl
61	H	2-CF_3
62	H	3-F
63	2-CH_3	6- CH_3
64	2-CH_3	5- CH_3
65	4-Br	3- CH_3
66	2-CH_3	4- CH_3
67	H	4- CH_3
68	H	2-Br
69	3-Cl	2- CH_3

A palladium-catalyzed one-step process was developed for the synthesis of phthalimide derivative by carbonylative cyclization of *o*-halobenzoates and primary amines (Scheme **8**) [35]. This methodology provides a good one-step approach towards this important class of heterocycles and tolerates a variety of functional groups, including methoxy, alcohol, ketone and nitro groups as shown in Table **4**. However, in present method cyclic phthalimide derivatives were not obtained when aryl amines with either strong electronegative group (**142, 143**) or more hindered amine was used (**144-148**).

Table 2 *N*-aryl/alkylidene-4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl) butanoyl hydrazides

Compound	R_3	R_4
70	H	C_6H_5
71	H	3-NO_2-C_6H_4
72	H	4-NO_2-C_6H_4
73	H	2-OH-C_6H_4
74	H	4-OH, 3-OCH_3-C_6H_3
75	Br	C_6H_5
76	CH_3	4-NO_2-C_6H_4
77	CH_3	2-OH-C_6H_4
78	CH_3	4-OH-C_6H_4
79	CH_3	3-NH_2-C_6H_4
80	CH_3	4-CH_3-C_6H_4
81	C_2H_5	CH_3
82	C_6H_5	C_6H_5
83	H	Cyclopentylene
84	H	Cyclohexylene
85	H	5-Chloro isatinyl



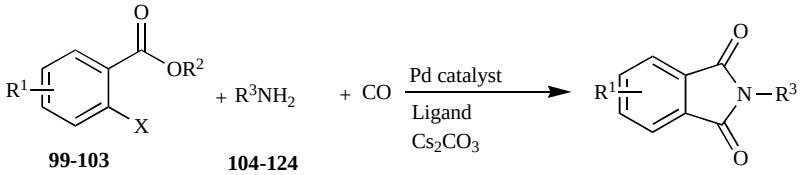
86 **87-98**

Scheme 7.

Table 3. Preparation of Different Amines at Different H_2O/EtOH Ratio

Compound	Amine	H_2O/EtOH v/v ratio	Yield (%)
87	4-hydroxyaniline	1/1	78
88	4-bromoaniline	1/0	51
89	2-(aminomethyl)pyridine	1/1	70
90	*p*-phenylene diamine	1/1	83
91	4-nitroaniline	1/1	—[a]
92	4-aminopyridine	1/1	—[a]
93	4-aminobenzoic acid	1/1	64
94	L-phenylalanine	1/1	86
95	glycine	1/1	76
96	n-butyl amine	1/0	62
97	allylamine	1/0	35
98	-	1/1	95

[a] A complex mixture of products was obtained resulting from the decomposition of starting materials.



99-103 **104-124** **125-148**

Scheme 8.

Table 4. Synthesis of Isoindole-1,3-diones by the Aminocarbonylative Cylization of *o*-halo Benzoate Ester

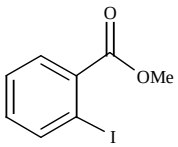
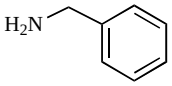
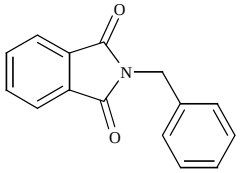
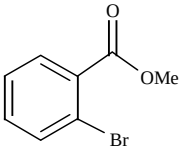
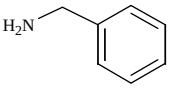
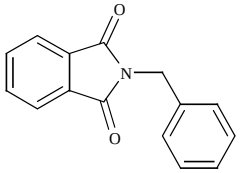
Compound	o-halo ester	Amine	Product
125	 99	 104	
125	 100	 104	

Table 4. contd….

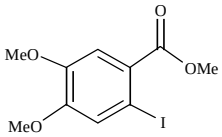
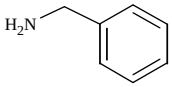
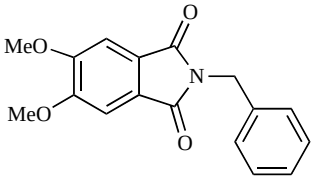
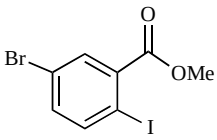
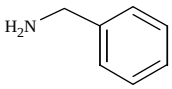
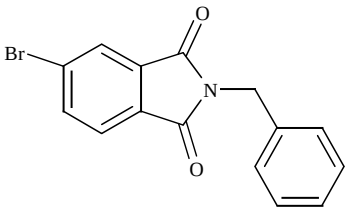
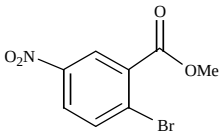
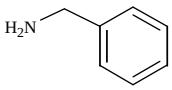
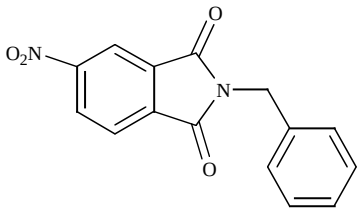
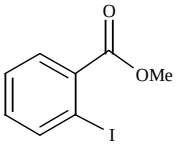
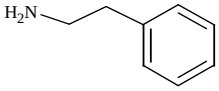
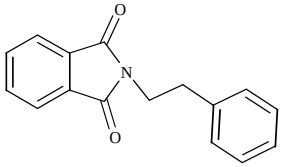
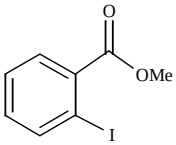
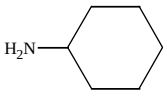
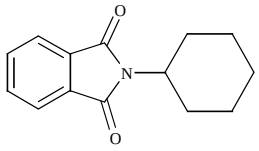
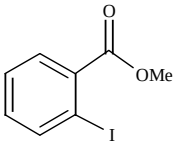
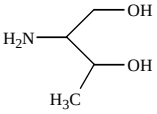
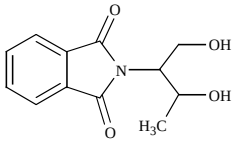
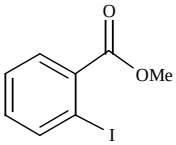
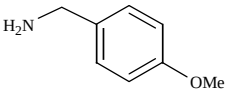
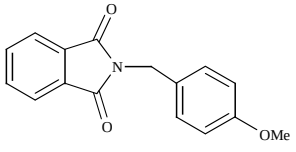
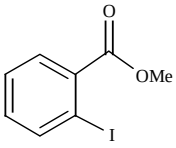
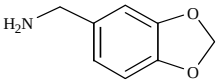
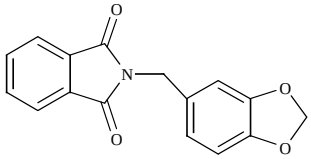
Compound	o-halo ester	Amine	Product
126	 101	 104	
127	 102	 104	
128	 103	 104	
129	 99	 105	
130	 99	 106	
131	 99	 107	
132	 99	 108	
133	 99	 109	

Table 4. contd….

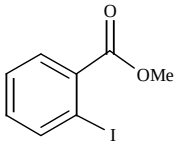
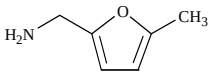
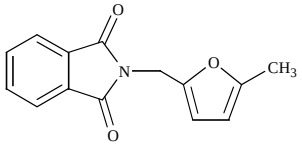
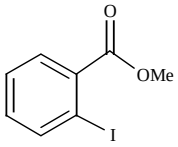
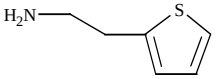
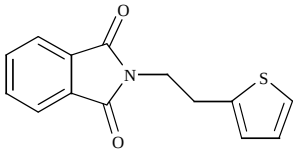
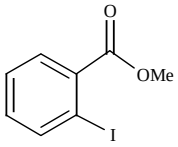
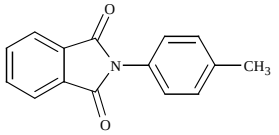
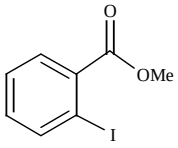
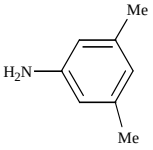
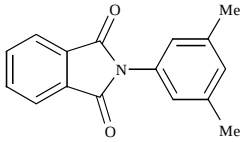
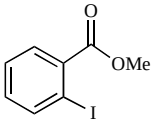
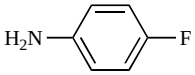
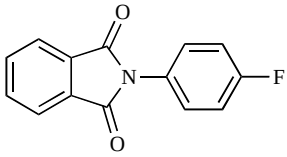
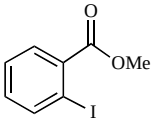
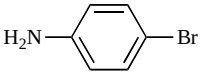
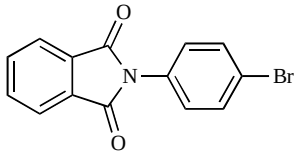
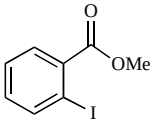
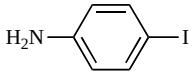
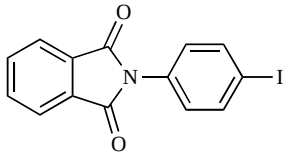
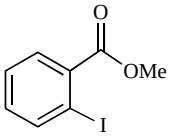
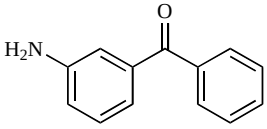
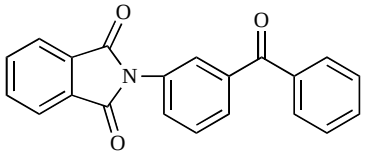
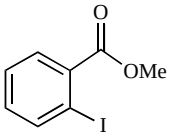
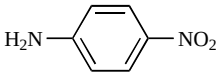
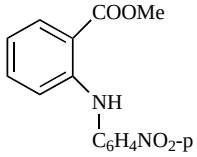
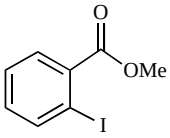
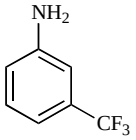
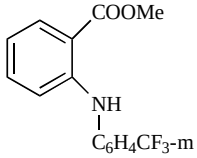
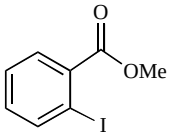
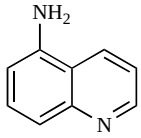
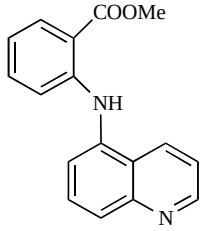
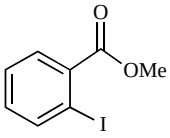
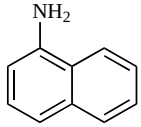
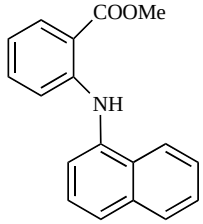
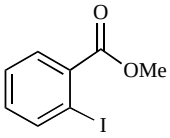
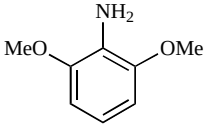
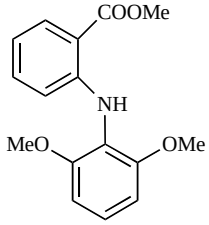
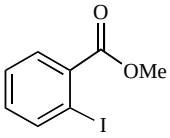
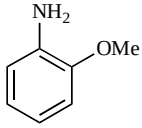
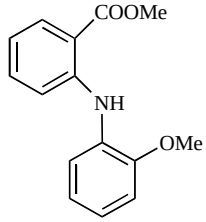
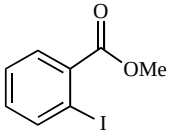
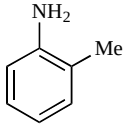
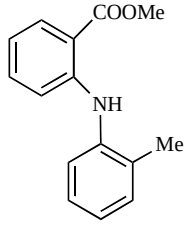
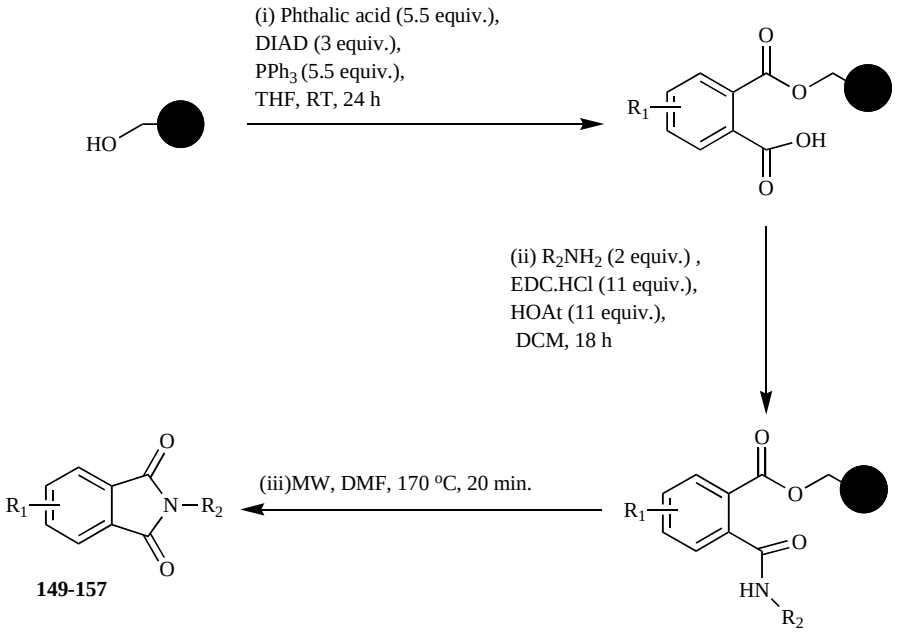
Compound	o-halo ester	Amine	Product
134	 99	 110	
135	 99	 111	
136	 99	 112	
137	 99	 113	
138	 99	 114	
139	 99	 115	
140	 99	 116	
141	 99	 117	

Table 4. contd….

Compound	o-halo ester	Amine	Product
142	 99	 118	
143	 99	 119	
144	 99	 120	
145	 99	 121	
146	 99	 122	
147	 99	 123	
148	 99	 124	



Scheme 9.

2.2. Microwave Assisted Solid Phase Synthesis of Phthalimide Derivatives

Over the years, microwave-assisted organic synthesis (MAOS) became a commonly applied mainstream tool for the synthesis of heterocyclic compounds. The broad range of emerging applications in this field is mainly due to the significant contribution of MAOS to the development of ecofriendly processes. Various transformations have been developed for the synthesis of *N*-heterocycles under microwave conditions, including fast and selective processes. In most of the transformations, microwave conditions dramatically enhanced reaction rates as well as provided improved yields. A traceless solid-phase synthesis of substituted phthalimide was carried out in 2003 [36]. The target compounds were obtained within minutes by a microwave-assisted cyclative cleavage in good yields and excellent purities (Scheme **9**). Conditions were optimized for cyclative cleavage by using different solvents (dioxane, AcOH and DMF) and it was observed that aprotic solvent reaction condition were more favorable to cyclative cleavage than protic one. The use of DMF as solvent at 170 ^{O}C gave best yield.

In a second group of attempts, the influence of the substitution of the amino part of the template on the ring closure was studied Table **5**.

Various phthalimide derivatives characterized by different substitution patterns on the aromatic ring and various amines were synthesized as shown in Table **6**. As already discussed for modification of the amino part of the template, the introduction of substituents on the aromatic ring was well tolerated. The desired phthalimides (**149-157**) were generally obtained in good yields.

Table 5. Influence of the Amine on Ring Closure (R_1 = H)

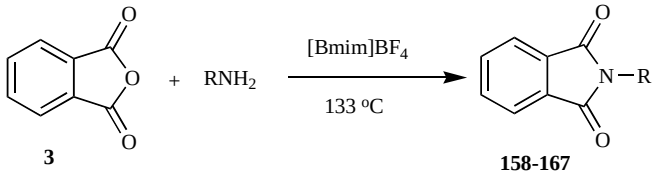
Entry	R_2	Yield (%)
a	C_3H_6Ph	68
b	$CH(CH_3)C_2H_4Ph$	83
c	C_8H_{17}	56
d	4- CH_3OBn	24
e	4-ClBn	12
f	C_5H_9	35

Table 6. Synthesis of Substituted Phthalimides

Compound	R_1	R_2
149	H	C_3H_6Ph
150	4-F	C_3H_6Ph
151	4-F	C_5H_9
152	5-Br	C_3H_6Ph
153	4,5,6,7-F	C_3H_6Ph
154	5-CH_3	C_3H_6Ph
155	5-CH_3	C_5H_9
156	5,6-C_4H_4	C_3H_6Ph
157	5,6-C_4H_4	C_5H_9

2.3. Synthesis by using Ionic Liquids

Synthesis in ionic liquids has attracted great attention in last few years. Phthalimide derivatives were also synthesized using ionic liquids. In 2003, Zhou *et al.* synthesized phthalimide derivatives by using [Bmim]PF_6 [37] in long period. Later on Le *et al.* synthesized these derivatives by using [Bmim]PF_6 and [Bmim]BF_4 (Scheme **10**) [38]. Some examples of phthalimides synthesis in ionic liquid have been shown in Table **7**.



Scheme 10.

Table 7. The Reaction of Phthalic Anhydride with Amine in Ionic Liquid [Bmim][PF_6]

Compound	Amine (RNH_2)
158	*p*-$ClC_6H_5NH_2$
159	*p*-$CH_3C_6H_5NH_2$
160	*p*-$NO_2C_6H_5NH_2$
161	*m*-$HOC_6H_5NH_2$
162	4,5-dimethoxyaniline
163	$C_6H_5CH_2NH_2$
164	NH_2 CH_2COOH
165	L-phenylalanine
166	*n*-$C_4H_9NH_2$
167	$NH_2CH_2CH_2OH$

3. MECHANISM OF ACTION OF PHTHALIMIDE DERIVATIVES

Initially thalidomide (**2**) was developed as a "safe" sedative agent without any toxicity and addictive potential of barbiturates. But after few years it was withdrawn from the market due to its teratogenic effect [39]. However, even after the initial impact of the thalidomide disaster, the drug has shown potential for the treatment of many diseases. The beneficial pharmacological effects elicited by thalidomide include (a) anti-cachexia activity (b) anti-tumor-promoting activity, (c) anti-angiogenic activity, (d) anti-cell invasion activity, (e) anti-viral activity, and (f) hypoglycemic effect. Thalidomide has been reported to regulate production of various cytokines, including TNF-α, interleukins (ILs) 2, 4, 5, 6, 10 and 12, and interferon-γ [40]. Regulation of these cytokines affects the function and population of T-cells. Thalidomide potentially activates cluster of differentiation (CD8+) T-cells and increases the Th2 cell population in the Th1/Th2 cell balance [40]. Though thalidomide affects production of various cytokines as mentioned above, the prevailing hypothesis is that all of the beneficial effects of thalidomide are associated with the regulation of TNF-α production [41-43]. This stimulated the scientific community to reinvestigate the development of TNF-α production regulators based on thalidomide.

3.1. Phthalimide Derivatives as TNF-α Inhibitor

The molecular mode of action of phthalimide derivatives on TNF expression is thought to be involved the inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) signaling pathway, specifically inhibiting the activity of the IκB kinase, IKKα [44] (Fig. (**5**)).

Lima L.M. *et al.* [45] synthesized (Fig. (**6**)) (Scheme **11**) and screened the phthalimide analogues (**168a-e**) for their ability to inhibit the acute inflammatory responses, measured by LPS-induced TNF-α inhibition and neutrophil infiltration into mice lungs. They found that the most active compound was **168e** (LASSBio 468). To correlate the anti-inflammatory activity of compound **168e** (LASSBio 468) with a possible effect on TNF-α production, the cytokine levels were also evaluated which revealed its ability to inhibit TNF-α levels in bronchoalveolar lavage fluid (BALF) of mice lungs treated with LPS. It is well-known that elevation of intracellular levels of cyclic 3',5'-adenosine monophosphate (cAMP) in leukocytes is accompanied by significant inhibition of the production of TNF-α and is associated with inhibition of phosphodiesterase (PDE-4) activity [46-48]. Therefore, the new thalidomide analogues **168e**, **169e** and **170** were also evaluated, *in vitro*, as PDE-4 and PDE-3 inhibitors. The results obtained with PDE's from bovine aorta assay [49], indicated that none of the compounds showed a significative inhibitory effect on PDE-4 and PDE-3 activity. For instance, **204e** (LASSBio 468) presented only 40% of PDE-4 inhibition at 300 mM concentration and was ineffective on PDE-3. Compound **170** (LASSBio 596) and **169e** (LASSBio 595) presented the same poor profile of bioactivity. The study clearly indicated the absence of direct correlation between these two mechanisms.

Further in 2005 Alexandre-Moreir *et al.* [50] studied whether LASSBio-468 would affect chronic inflammation process associated with the production of this pro-inflammatory cytokine. The treatment with LASSBio-468 before a lethal dose injection of LPS in animals greatly inhibited endotoxic shock. This effect seems to be mediated by specific down regulation of TNF-α and nitric oxide production, regulated mainly at the ribonuclic acid (RNA) level. Thus it was concluded that the ability of this compound in modulating TNF-α production is mainly related with messenger ribonucleic acid (mRNA) expression. Also, LASSBio-468 partially inhibits glaucomatous reaction mainly due to the diminished macrophagic activation.

To effectively measure inhibition of NFκB pathway signalling by each analogue, a TNF transcriptional reporter cell line was used by Kroeger, K.M. *et al.* and Karimi, M. *et al.*. The green fluorescent protein (GFP) reporter gene, under the control of the NFκB- responsive human TNF promoter, was inserted into the genome of the human T cell line, Jurkat to generate a transcriptional reporter line, FRT-Jurkat TNF,

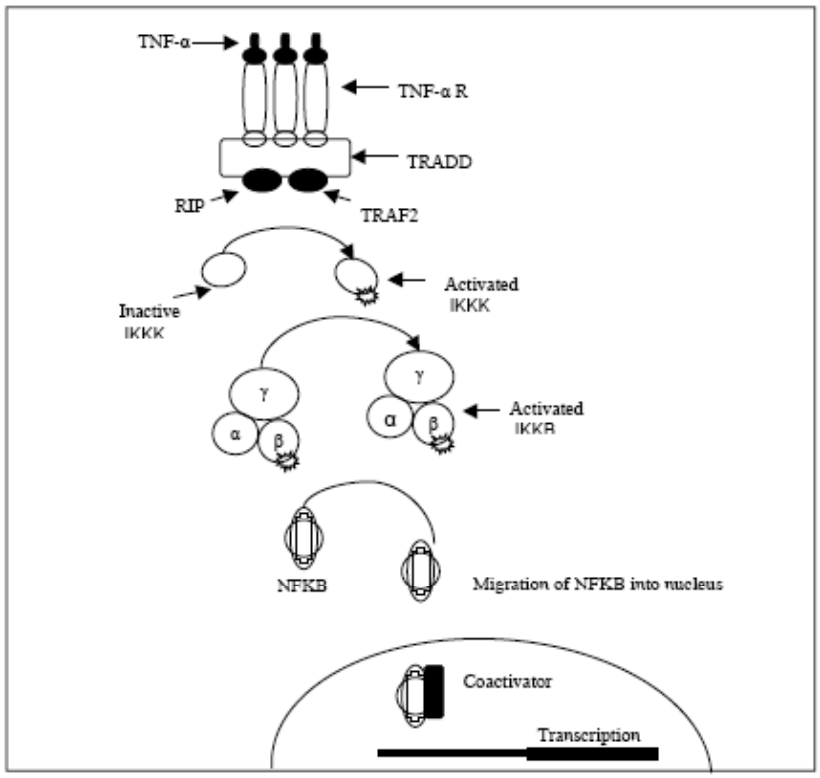
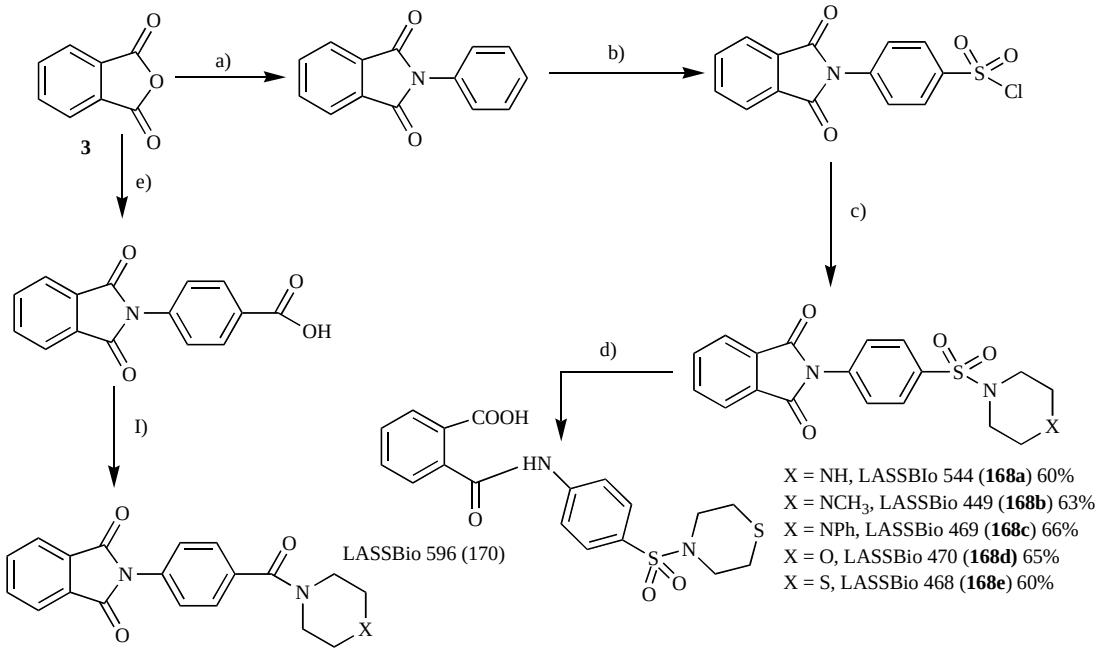


Fig. (5). Mechanism of TNF-α signalling.



Scheme 11. (a) $C_6H_5NH_2$, reflux, 1 h; 86%; (b) $ClSO_3H$, PCl_5, 50 ^{O}C, 30 min, 70%; (c) functionalized piperazines, CH_2Cl_2, rt, 30 min, 60–66%; (d) LiOH, THF, MeOH, H_2O, rt, 10 min, 77%; (e) 4-$CO_2HC_6H_4NH_2$, AcOH, reflux, 1 h, 91%; (f) (1) SO_2Cl, DMF (cat), reflux, 1 h; (2) CH_2Cl_2, functionalized piperazines, rt, 30 min, 81–97%.

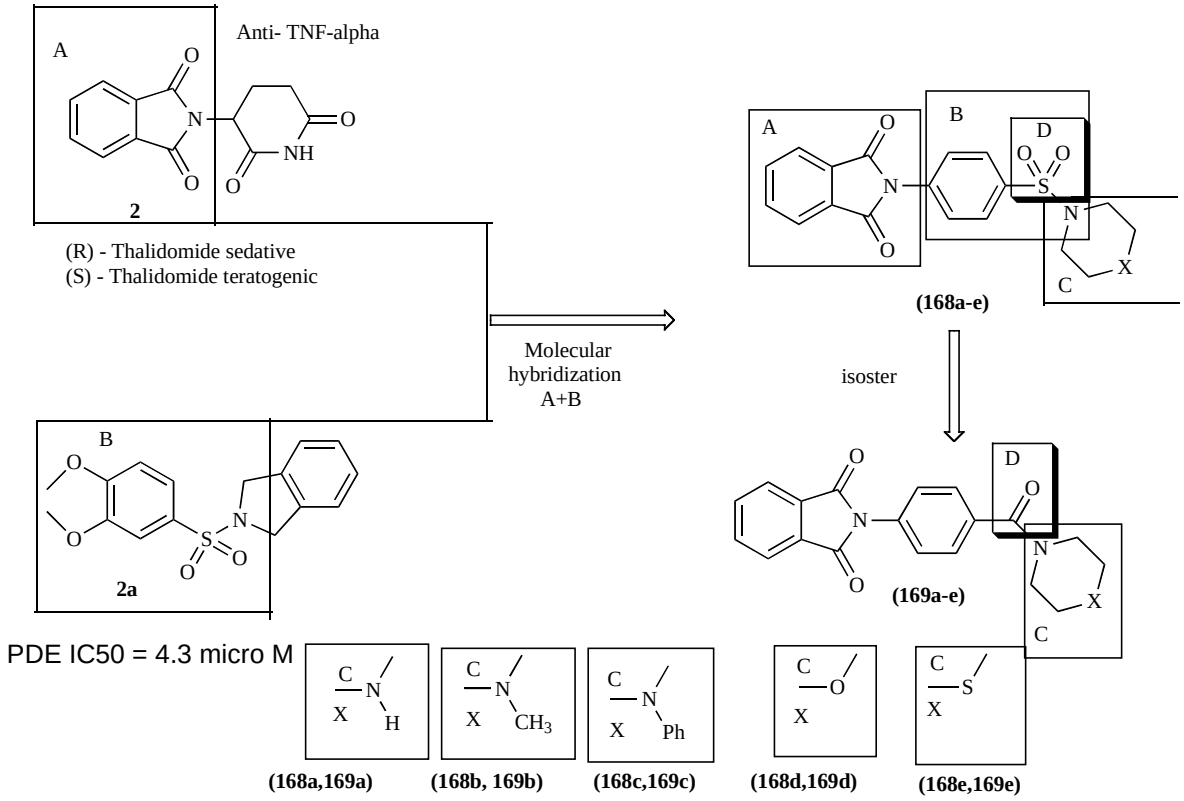


Fig. (6). Design of hybrids of thalidomide (1) and aryl sulfonamide phosphodiesterase (2).

as previously described [51-52]. GFP expression, as a measure of TNF promoter activity, was quantitated by flow cytometry by measuring the fluorescence intensity of individual cells. This method had the added advantage of being able to easily assess cellular toxicity of each compound by comparing forward and side scatter (as a measure of cellular size and granularity) during flow cytometry. The cell population in each assay that exhibited low granularity was considered to be dead. This was confirmed by staining with propidium iodide. All thalidomide and *N*-phenylphthalimide derivatives were assessed in triplicate at concentrations of 100 μM and the percentage inhibition of TNF expression (relative to TNF expression from solvent treated control cells) for each compound was measured.

3.2. Phthalimide Derivative as Anti-Angiogenic Agents

Angiogenesis is the process of the formation of new blood vessels, and involves the proliferation of endothelial cells in response to specific growth stimuli such as vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF). It is controlled by a balance of endogenous inhibitors and stimulators (Fig. (**7**)). It is an important natural process occurring in the body, both in health (wound healing [53], female reproductive cycle [54]) and disease (cancer [55], rheumatoid arthritis [56], diabetic retinopathy [57]). The growth and maintenance of solid tumours is highly dependent on neovascularization and can be regulated by agents that interfere with either the stimulation or proliferation of endothelial cells [58]. As a result, the control of angiogenesis continues to be an attractive area for the development of novel therapeutic agent [55].

One such agent is thalidomide (**2**) ((Fig. (**1**)), which was developed in the 1950's by Chemie Grunenthal of Germany as a non-toxic sedative [59]. In addition to its sedative effects in humans, an association was reported of teratogenic limb defects from maternal thalidomide usage [59]. Thalidomide has significant anti-angiogenic activity, and the effects of thalidomide on corneal angiogenesis induced by VEGF have been reported [60]. Additionally, thalidomide has demonstrated inhibitory effects on angiogenesis in the bFGF induced rabbit corneal micro pocket assay [61] and orally in mice models [62]. Prostate cancer is the most common malignancy in American men and is the second leading cause of cancer mortality [63]. Prostate cancer, as is the case with numerous types of cancer depends on the growth of new blood vessels. In fact, in prostate cancer, an increased micro vessel density correlates to poorer prognosis [64]. As long as the cancer is confined to the prostate, it can be successfully controlled by radiation or surgery. However, in metastatic disease, few treatment options are available beyond androgen ablation [65]. Clinical trials of thalidomide in patients with androgen-independent prostate cancer have been reported, and they showed that thalidomide has modest activity in patients with metastatic prostate cancer [66]. Depending on their mechanism of action the inhibitors of angiogenesis can exert their effect in many ways. Some inhibit endothelial cells directly, while others inhibit the angiogenesis signalling cascade or block the ability of endothelial cells to break down the extra cellular matrix. Therapeutic agents that can inhibit more than one of these

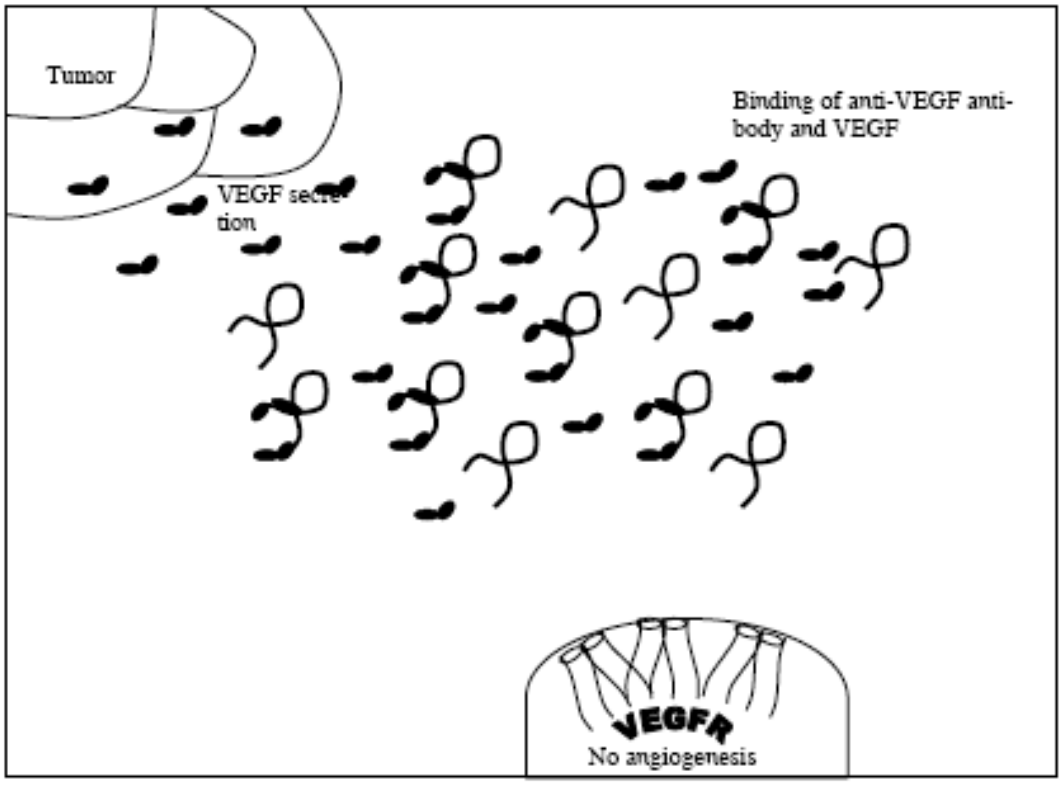


Fig. (7). Mechanism of inhibition of angiogenesis.

pathways could prove to have a distinct advantage over conventional anti-angiogenic therapies in which only one mechanism of action is targeted. On the basis of the latter statement, number of research groups has been engaged in the structural modification of thalidomide with the aim of developing novel anti-angiogenic/anti-cancer analogues that possess superior biological action relative to thalidomide.

3.2. Phthalimide Derivative as Anti-Cancerous Agents

Kok *et al.* in 2008 [67] synthesized benzothiazole substituted phthalimide derivative as shown in Scheme **12** and evaluated its anti-cancerous activity on human carcinoma cell lines.

The synthesised phthalimide derivative showed significant cytotoxicity on three human cancer cell lines including SKHep1, Burkitt's lymphoma cell line (B cell type) CA46 and chronic myelogenous leukemia (CML) K562. It was observed that both caspase-dependent and independent apoptotic pathway are important for the action of this phthalimide derivative. Inspired by these results the same group in 2009 [68] synthesized α-phthalimide ketone derivatives (Scheme **13**) and evaluated *in vitro* antiproliferative activity on MDAMB-231 and SKHep-1 human carcinoma cell lines.

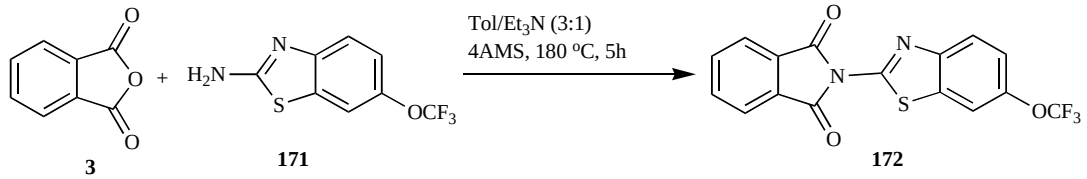
It was observed that both compounds **176** and **177** could induce cell rounding and cell shrinkage on both MDAMB-231 breast cancer cells and SKHep-1 hepatoma cells.

4. STRUCTURE ACTIVITY RELATIONSHIP (SAR):

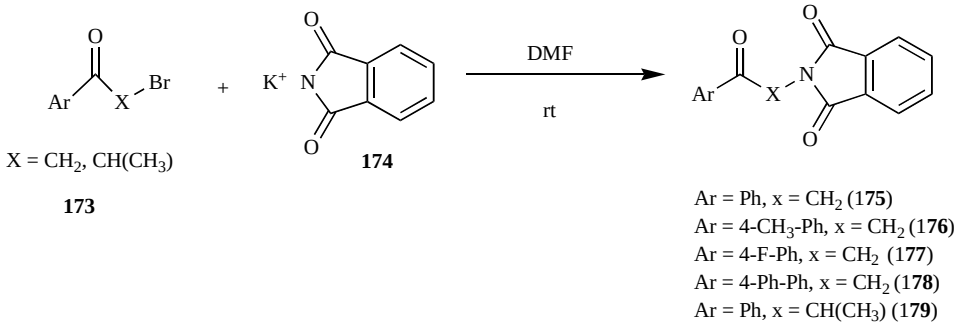
4.1. SAR of Phthalimide Derivatives as TNF-α Inhibitors

Structure activity relationship of phthalimide derivatives was well reviewed by Hashimoto in 2003 [69] and the compilation of their work also appeared. To the best of our knowledge no other review is available till date. Therefore, an attempt has been made for compilation of work so far in this field.

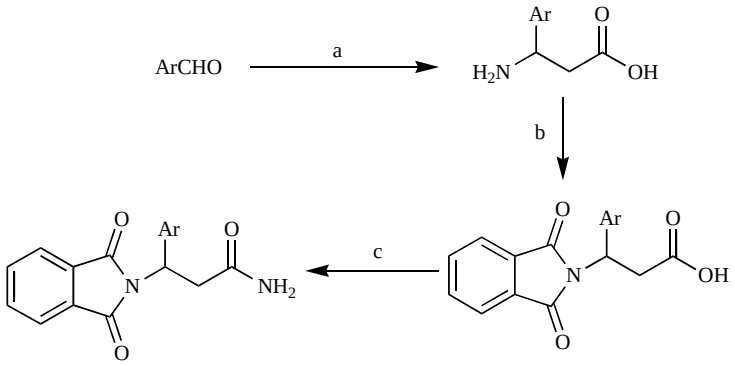
At physiological pH, thalidomide hydrolyzes at both the phthalimide as well as glutarimide rings [70]. It has therefore postulated that thalidomide may act as a prodrug for one of its hydrolysis product. Hydrolysis of the glutarimide ring



Scheme 12.



Scheme 13.



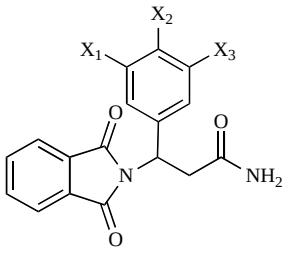
Scheme 14. [a]Reagent: (a) NH_4OAc, $CH_2(CO_2H)$, EtOH, reflux: (b) N-carbethoxyphthalimide, Na_2CO_3, CH_3CN/H_2O: (c) (1) CDI/THF, (2) Con. NH_4OH.

affords either *N*-phthaloylglutamine or *N*-phthaloylisoglutamine. Analogues were therefore prepared based on the hydrolysis of the glutarimide ring of thalidomide (Scheme **14**) [71].

TNF-α inhibition was measured in the supernatant of human PBMCs stimulated with lipopolysaccharides (LPS). Simple phthalimidoalkyl amides which might mimic glutarimide hydrolysis products of thalidomide were evaluated for their ability to inhibit TNF-α. In exploring the effects of substitution of simple phthalimidoalkyl amides, the phthalimido amide of 3-phenylpropionic acid (**180**), was found to be nearly equipotent to thalidomide. Some examples are shown in Table **8**.

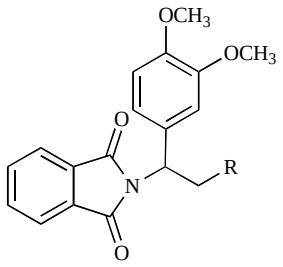
The study on the effect of substitution on TNF-α inhibition showed that the presence of an electron-withdrawing group or an electron-donating group at the *meta* or *para* position was found to increase the activity as shown in Table **8**. Substitution with a 3-cyano group (**181**) or a 4-cyano group (**182**) also increased the activity. Substitution with an electron-donating group such as 3-methoxy (**183**) or 4-methoxy (**184**) was observed to increase the activity. Thus, substitution effects appear to be mediated by steric effects. Disubstituted compound like 3,4-dimethoxy analogue (**185**) was found to be 15-fold more potent than thalidomide due to the synergistic effect. The 3,5-dimethoxyphenyl analogue (**190**) was 3 times less active than **185**. The study of 3,4-dimethoxy substitution of **185** observed that substituents larger than diethoxy lead to decrease in activity. The diethoxy analogue (**186**) was found to be 2 times more active than **185**. Unlike thalidomide, these compounds can inhibit 100% of the TNF-α formed by LPS stimulation. Phthalimido amides such as **185** were initially explored because of their relationship to

Table 8. N-phthaloyl β-amino β-aryl Amide Analogues



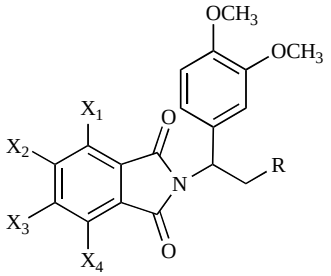
Compound	X_1	X_2	X_3
180	H	H	H
181	H	H	CN
182	H	CN	H
183	H	H	OCH_3
184	H	OCH_3	H
185	H	OCH_3	OCH_3
186	H	OEt	OEt
187	H	OPr	OPr
188	H	Cl	Cl
189	H	H	Ph
190	OCH_3	H	OCH_3

Table 9. Different Isosteric of Amide Moiety



Compound	R
191	$CONHCH_3$
192	$CONHCH_2CH_3$
193	CONHBn
194	CO_2H
195	CH_2OH
196	CO_2CH_3

Table 10. Ring-Substituted Phthaloyl Analogues



Compound	X_1	X_2	X_3	X_4
197	H	H	NO_2	H
198	H	H	NH_2	H
199	H	H	H	NO_2
200	H	H	H	NH_2
201	H	H	H	OH
202	H	H	Ph	H
203	H	Cl	Cl	H
204	Cl	H	H	Cl
205	H	H	tBu	H

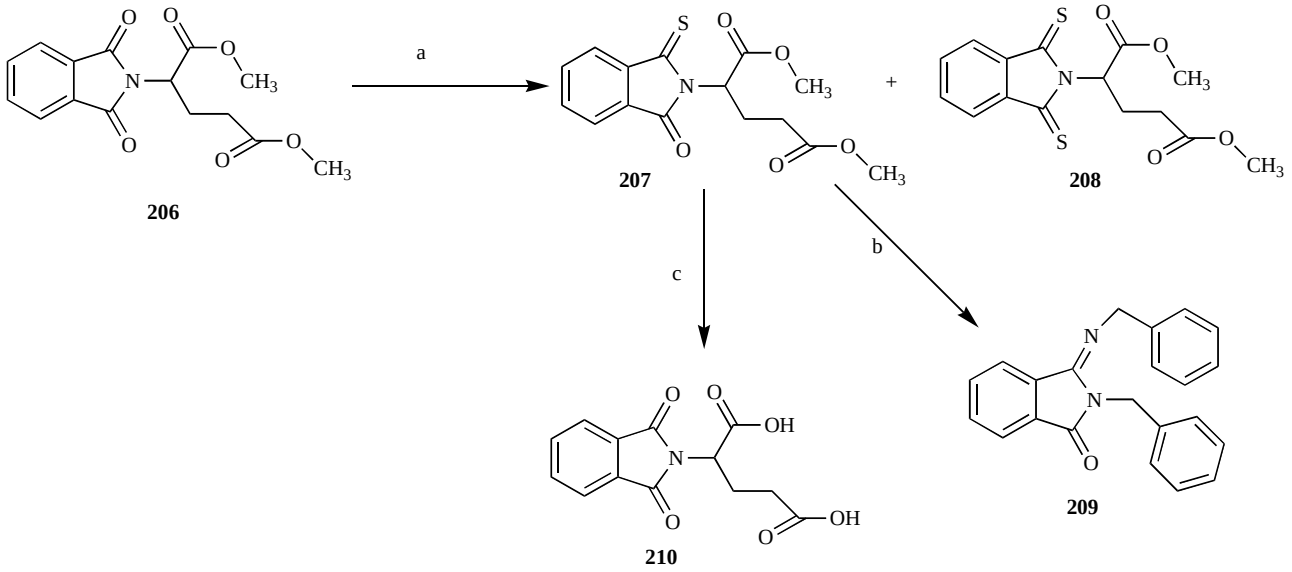
thalidomide hydrolysis products. Isosteric replacement of the amide moiety revealed that the amide moiety was not optimal as shown in Table **9**. Compound **185** was chosen as the parent compound because of its high activity. The *N*-methyl analogue (**191**) of **s**ubstituted amides resulted in decreased activity. The free carboxylic acid analogue (**194**) was found to be 5-fold less active. The primary alcohol analogue (**195**) also has shown slight increase in the activity. Replacement of the amide moiety with a carboxy methyl group (**196**) showed 5-fold increased activity. To improve the activity of **196,** the effect of substitution of the aromatic ring of the phthaloyl ring was explored as shown in Table **10.** Dihalo substitution of the aromatic ring (**203** and **204**) led to the decrease in activity. Alkyl substitution had only a minor effect. Nitro group substitution in the 3- or 4-position decreased the activity by more than 1 order of magnitude. However, 3- or 4-amino substitution (**198** and **200**, respectively) yielded compounds with submicromolar IC_{50}'s. Replacement of the amino glutarimide portion of thalidomide with β-amino β-aryl amino acid derivatives and substitution of the phthaloyl ring have resulted in analogues having 500 times more TNF-α inhibition efficacy than thalidomide. Unlike thalidomide, analogues in this series inhibit 100% of the TNF-α production in LPS-stimulated PBMCs.

In 2003 Zu *et al.* synthesized different phthalimide derivative (Scheme **15** and **16**) [72]. Same method was applied, as discussed above for the evaluation of TNF-α inhibition.

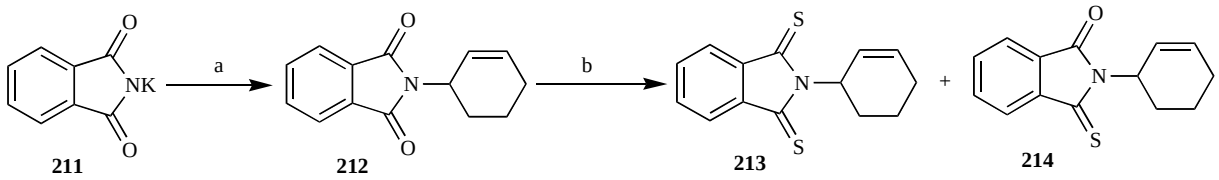
The action of the described phthalimide analogues to inhibit TNF-α secretion was assessed in human PBMC. The compounds **207**, **208**, and **210** are thio analogues of hydrolysis metabolites of thalidomide. The monothio analogue (**207**) has an IC_{50} of 20 μM without any toxicity; demethylation (**210**) lowered the potency. The dithio analogue (**208)** was 2-fold more potent than **207**, but it induced cellular toxicity at lower concentrations. The thio analogues **213** and **214**, with a simplified glutarimide ring, were potent TNF-α inhibitors with some toxicity at 30 μM, with IC_{50} values 15 μM and 16 μM, respectively. The high lipophilicity and loss of hydrogen bond acceptor capability potentially allows the attainment of higher intracellular drug levels which is responsible for the elevated potency of these derivatives. Monothiophthalimide (**214**) showed a marginal TNF-α activity at a concentration of 30 μM without toxicity. Interestingly, however, dithiophthalimide **(213)** was found to possess potent activity with an IC_{50} of 3 μM. Although it was associated with toxicity at 30 μM, its inhibition of TNF-α occurred at lower concentration that was well tolerated.

Lima L. M. *et al.* in 2002 designed (Fig. (**6**)) and synthesized (Scheme **11**) hybrids of thalidomide (**2**) and aryl sulfonamide phosphodiesterase (**2a**) [45]. These phthalimide analogues (**168a–e**) were screened for their ability to inhibit the acute inflammatory response, measured by LPS-induced TNF-α inhibition and neutrophil infiltration into mice lungs, with thalidomide as standard. It was found that the most active compound was **168e** (LASSBio 468). Compound **168e** (LASSBio 468) which possesses the thiomorpholine ring at the aryl moiety of the 4-sulfonylphenylphthalimide framework, was selected to study the dose dependent response. This compound inhibited the neutrophil infiltration induced by LPS with ED_{50} 2.5 mg kg^{-1}.

In order to investigate the pharmacophoric moiety of this compound which is responsible for the anti-inflammatory activity of this compound, they hydrolysed compound **168e** (LASSBio 468) to furnish compound **170** in 77% yield (Scheme **11**). The pharmacological evaluation of this compound (**170**) revealed a remarkable decrease in anti-



Scheme 15. [a]Reagent: (a) Lawesson's reagent/ toluene; (b) benzylamine; (c) HCl/HOAc; (d) F_3CCONH_2, HOBT, EDCl, Et_3N/CH_2Cl_2.



Scheme 16. [a]Reagent: (a) 3-bromocyclohexene/DMF; (b) Lawesson's reagent/toluene.

inflammatory activity which indicates that phthalimide ring plays a vital role in the anti-inflammatory activity.

Authors investigated the role of sulfonyl group by its isosteric replacement [present in the aryl-sulfonamide moiety found in the prototype (2)] with a carbonyl unit, as in compounds **169a–e** (Scheme **11**). The pharmacological evaluation of amide compound **169e** (LASSBio 596), designed upon the bioassays results from **168e**, revealed a significant loss of activity when compared with the original compound **168e**, suggesting the crucial role of the phenyl-sulfonyl-piperazine framework in the investigated bioactivity.

The preliminary SAR study with **168e** (LASSBio 468) compound revealed the importance of the sulfonyl group, the nature of the N-terminal piperazine ring, also indicated the role of the phthalimide ring in the anti-inflammatory activity of compound (**168e**). Further, it was investigated for the inhibitory activity on neutrophil recruitment and elevation of TNF-α level in BALF of mice lungs treated with LPS. Moreover, the TNF-α inhibitory activity of compound **168e** (LASSBio 468) was not correlated with inhibitory activity of PDE-4 or PDE-3, major isoforms found in all pro-inflammatory and immunocompetent cells.

Since thalidomide exists as a mixture of R and S (responsible for teratogenicity) isomer at physiologic pH and that is not issue with LASSBio-468 which is achiral molecule. This molecule does not associate with the side effects and therefore could be a useful tool to modulate inflammatory diseases, such as septic shock and rheumatoid arthritis.

In the same year further attempt was made to synthesize some more thalidomide derivatives [73]. A process was designed for keeping its beneficial action and avoiding its side effects (Fig. (**8**)).

Different series of compounds were synthesised by using the paths as shown in (Scheme **17**). In the series of 2-phenoxy-phthalimide derivatives (**217a–d**), the racemate (**217b**) was the most active, inhibiting 60% of TNF-α production and neutrophil influx, similar to the effect of thalidomide. Results indicated that the substitution in the *ortho* position of the phenyl ring of N-phenyl-phthalimide moiety led to the same inhibitory profile as in the *para* position [72]. For investigating the contribution of the methylenedioxy subunit in the anti-inflammatory activity the target phthalimide-amides **215a** and **215b** were evaluated and data showed that these compounds were equipotent to the prototype **169e** (48% inhibition of the neutrophils recruitment induced by LPS) [72] as inhibitors of the inflammatory response. Taken together, these data indicated that the anti-inflammatory profile is closely dependent of the substitution pattern in the phenyl ring, considering that no activity for N-phenyl-phthalimide was found. The *ortho* or *para* substitution in the phenyl ring, for sulfonamide, amide, (**215a**, **b**, ester **217a, b, d**), or either by carboxylic acid (**217c**) are essential for the modulation of the production of TNF-α and ultimately for the anti-inflammatory activity. A hybrid derivative **216b** (LASSBio 867) was synthesized

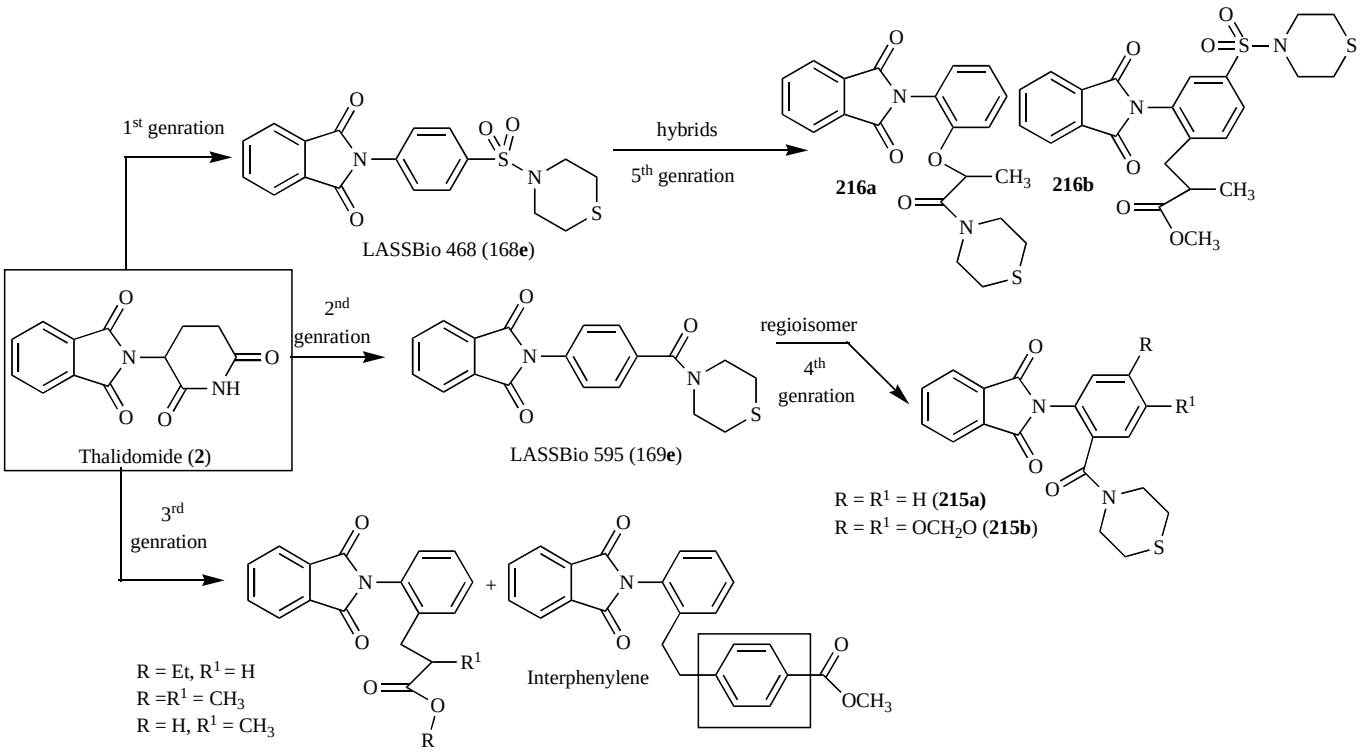
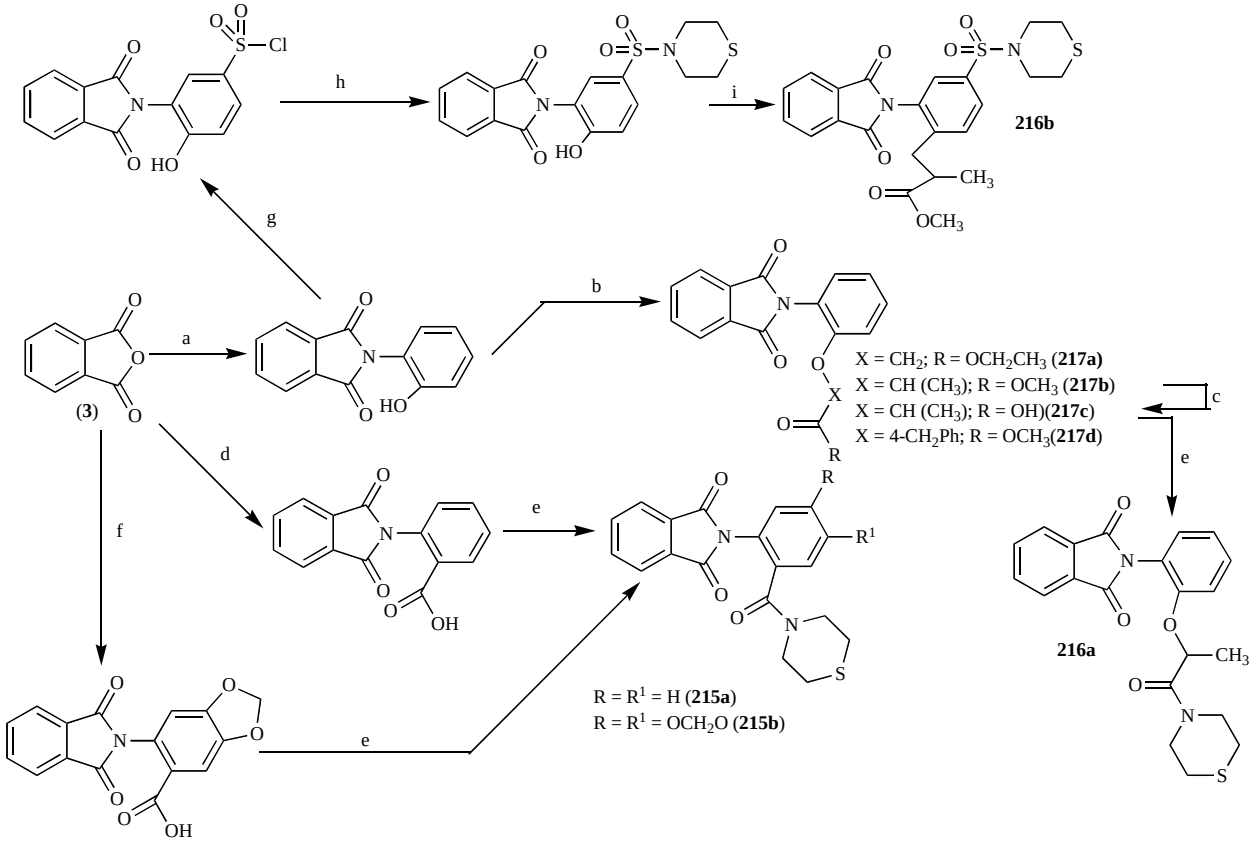
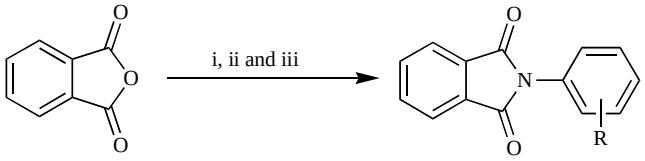


Fig. (8). Designs for the synthesis of thalidomide derivatives in order to keep its beneficial action and to avoid its side effects.



Scheme 17. Conditions: (a) 2-NH_2PhOH, AcOH, reflux, 1h, 91%; (b) EtO_2CCH_2Br or $MeO_2CCH(CH3)Br$ or MeO_2CPhCH_2Br, K_2CO_3, DMF, rt, 72h, 74–83%; (c) HCl:AcOH (1:1), rt, 1h, 85%; (d) 2-NH_2PhCO_2H, AcOH, reflux, 1h, 83%; (e) 1-$SOCl_2$, DMF(cat.), reflux, 1h; 2-thiomorpholine, CH_2Cl_2, rt, 1h, 80% (5a), 85% (6a) and 87% (6b); (f) 6-aminobenzo[d][1,3]dioxole-5-carboxylic acid, AcOH, reflux, 1 h, 80%; (g) $ClSO_3H$, 90 ^{O}C, 2h, 89%; (h) thiomorpholine, CH_2Cl_2, rt, 2h, 78%; (i) $MeO_2CCH(CH_3)Br$, K_2CO_3, DMF, rt, 72h, 60%.



3

2' R = I (**218**)
3' R = I (**219**)
4' R = I (**220**)

Scheme 18. Reagent and condition: (i) 2-iodoaniline, toluene, 115 ^{o}C, 23h, 60 %; (ii) 3-iodoaniline, toluene, 115 ^{o}C, 24h, 88%; (iii) 4-iodotoluene, toluene, 115 ^{o}C, 24 h, 53%.

between **168e** (LASSBio 468) and **217b** (LASSBio 542) by the double substitution in the N-attached phenyl ring for the possible optimization of the anti-inflammatory activity. Treatment with 10 mg/kg of compound **216b** for 1 h before inhalation of LPS did not improve the anti-inflammatory activity, even considering the presence of the sulphonamide group in C-3 and the phenoxy-ester group in C-2 positions. However, the substitution in more than one position in the phenyl ring of phthalimide system (**215b** and **216b**), led to a decrease in the inhibitory effect on TNF-α levels and neutrophil recruitment.

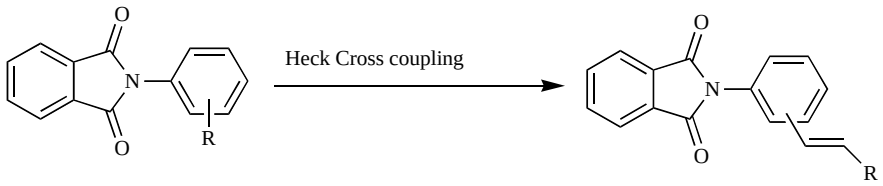
Stewart *et al.* in 2007 synthesized new phthalimide derivative by using Heck cross coupling (Scheme **18**, **19**) [74].

The N-arylphthalimide derivatives prepared by various substitutions as shown in Table **11** provide different valuable analogues required for increasing the inhibition of TNF expression. Specifically, the substitution on *ortho* (or C2') position provided enhanced inhibitory qualities when compared to thalidomide (**1**) at 100 μM. Iodo derivative **218** and methyl or butyl esters (**221** and **222**) displayed a 40%, 48% and 52% inhibition respectively, compared to thalidomide (**2**) at 38%. With these three groups the C2 substitution provided a slightly better inhibitory activity as opposed to C3 and C4. The *meta*-substituted 2-cyclohexen-1-one 40 also showed promising TNF inhibitory activity at 47%. This method for the analysis of thalidomide analogues provided an accurate, simple and more targeted method for the inhibition of TNF expression and ultimately to the determination of the effects on signalling through the NFκB inflammatory. Heck cross coupling was an excellent method for the attachment of olefins to the thalidomide and phthalimide ring. The compounds **222** and **226** showed the high potential and had a TNF expression inhibitory activity 52% and 50%, respectively.

4.2. SAR of Phthalimide Derivatives as Anti-Angiogenic Agents

Capitosti *et al.* in 2004 [75] synthesized some phthalimide derivatives which showed inhibition of both angiogenesis and prostate cancer. All the compounds were synthesized by condensation of phthalic anhydride with appropriate amine as shown in Scheme **20** and **21**.

Thalidomide analogues **234-259** (Tables **12**, **13**) were first screened for their ability to inhibit the proliferation of human micro vascular endothelial cells (HMEC's), both in the presence and absence of VEGF, with thalidomide as the standard. It has been previously demonstrated that inhibition of angiogenesis by thalidomide requires metabolic activation [76]; however, inactivated thalidomide was used as a standard for proliferation assays to simply demonstrate the ability of synthesized analogues to inhibit angiogenesis without the need for prior metabolic activation. Additionally, these analogues were also screened for inhibitory activity against the proliferation of PC-3 and DU-145 prostate cancer cell lines. In the non-fluoro-substituted phthalimide class (**234–242, 252, 254, 256, 258**), the most active compound against HMEC proliferation (6) exhibited low micro molar IC_{50} values both in the presence and absence of VEGF. Analogue **238** demonstrated potent ability to overcome the proliferation of endothelial cells mediated by growth factor. The non-fluoro substituted analogues (with the exception of **235**, **238**, and **239**) possessed either comparable or only slightly more potent anti-angiogenic activity with and without VEGF present as compared to thalidomide. The compound **235** showed an increase in potency over thalidomide against prostate cancer cell lines and this effect was only seen with the DU-145 cell line. These results suggest it is not advantageous to substitute phenyl ring within this class. However, this structural class have efficacy against growth factor mediated micro vessel endothelial cell proliferation. In the tetrafluorophthalimido class (**243-251**, **253**, **255**, **257**, **259**), compounds **248** and **249** were the most active analogues, as they both revealed a remarkable increase in potency over thalidomide in the HMEC screen with IC_{50} values ranging from 100–330 nm. Furthermore, all tetrafluoro-substituted analogues possessed a considerable increase in potency in antiangiogenic activity compared to thalidomide. Similarly, all tetrafluoro-analogues demonstrated significant increases in potency over thalidomide in both PC-3 and DU-145 cell lines in prostate cancer. Compounds **248** and **249** possess *p*- and *m*-methyl groups on the phenyl ring attached to the imide nitrogen respectively (Table **12**). Replacement of the *p*-methyl group

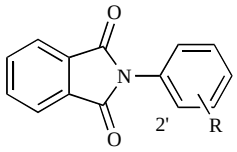


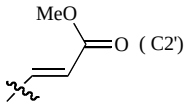
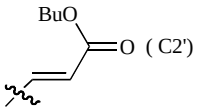
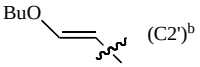
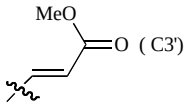
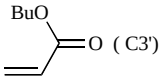
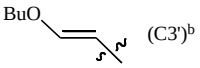
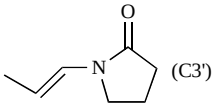
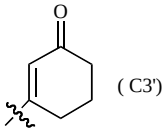
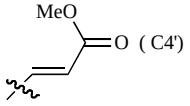
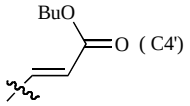
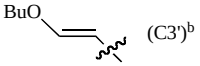
2' R = I (**218**)
3' R = I (**219**)
4' R = I (**220**)

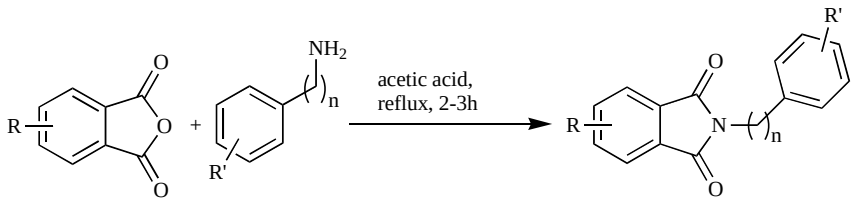
R = ester, ether, etc

Scheme 19.

Table 11. C20, C30 and C40 N-aryl Phthalimide Analogues

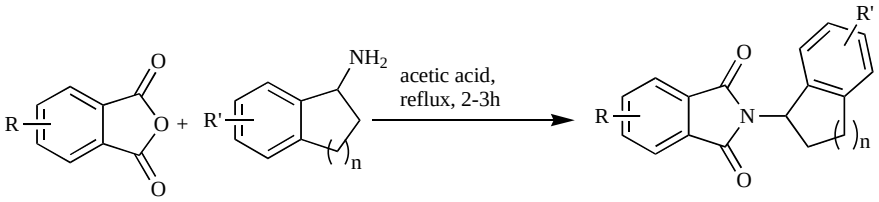


Compound	R (Carbon Substitution)
2	Thalidomide
218	I (C2')
221	
222	
223	
219	I (C3')
224	
225	
226	
227	
228	
229	
220	I (C4')
230	
231	
232	
233	



234-251, 253

Scheme 20.



254-259

Scheme 21.

Table 12. Target Phthalimides in Series 1

Compound	R					
	1	**2**	**3**	**4**	**R'**	**n**
234	H	H	H	H	H	0
235	H	H	H	H	4-Cl	0
236	H	H	H	H	3-Cl	0
237	H	H	H	H	2-Cl	0
238	H	H	H	H	3,4-Cl	0
239	H	H	H	H	4-CH3	0
240	H	H	H	H	3-CH3	0
241	H	H	H	H	2-CH3	0
242	H	H	H	H	2-CH3	0
243	F	F	F	F	4-OCH3	0
244	F	F	F	F	H	0
245	F	F	F	F	4-Cl	0
246	F	F	F	F	3-Cl	0
247	F	F	F	F	2-Cl	0
248	F	F	F	F	3,4-Cl	0
249	F	F	F	F	4-CH3	0
250	F	F	F	F	3-CH3	0
251	F	F	F	F	2-CH3	0
252	H	H	H	H	4-OCH3	0
253	F	F	F	F	H	1

Table 13. Target Phthalimides in Series 2

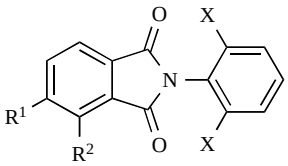
Compound	R					
	1	2	3	4	R'	n
254	H	H	H	H	H	1
255	F	F	F	F	H	1
256	H	H	H	H	H	2
257	F	F	F	F	H	2
258	H	H	H	H	H	3
259	F	F	F	F	H	3

of **249** with a *p*-chloro group (**244**) resulted in a slight loss in activity, raising the IC_{50} to the low micro molar range. Replacement of the *m*-methyl group of **249** with a *m*-chloro group (**245**) results in only a modest decrease in activity to the high nanomolar range. Both methyl and chloro groups added similar contributions to the overall lipophilicity of the compound; therefore, it was anticipated that the electron donating properties of the methyl group may led to the increased activity of compounds **248** and **249**. The unsubstituted tetrafluoro-analogue (**243**) showed activity similar to that of compounds **244** and **245**, lending more support to the electrostatic effect of the methyl group. Compound **226**, incorporating a *p*-methoxy group, also, have similar activity to **244** and **245**, possibly due to the methoxy groups moderately inductive electron withdrawing properties. Tetrafluoro-analogue (**253**) with a methylene insertion between the phenyl group and the imide nitrogen also showed significant anti-angiogenic and anti-prostate cancer activity in the high nanomolar to low micromolar range. Additionally, the tetrafluoro-substituted restricted analogues (**255**, **257**, **259**) showed similar to slightly increased anti-angiogenic and anti-prostate cancer activity relative to both **253** and the tetrahydro counterparts **254**, **256** and **258** (Table **13**).

This preliminary SAR study of these compounds revealed the importance of tetrafluorination of the phthalimide core, in addition to *p*- or *m*-methyl substitution on the opposite phenyl ring. Furthermore, methylene insertion between the phenyl ring and the imide nitrogen in the tetrafluorophthalimido class neither abrogate in vitro activity nor restricting the rotation of the freely rotatable phenyl group. The presence of the fluorine substituents on the phthalimide core drastically increases the potency. Incorporation of fluorine in the place of hydrogen often improves their potency because fluorine sterically mimics the hydrogen [77]. Such improvements have been attributed to either the enhanced lipophilicity of fluorinated compounds or to stereoelectronic changes imparted to compounds by the strongly electronegative fluorine substitution resulting in more effective binding to their respective target. Additionally, the fluorinated compounds presented herein represent analogues that would be resistant to the phenyl metabolism that has been reported for thalidomide [78]. These compounds demonstrated increased effectiveness against prostate cancer in comparison to thalidomide and showed ability to inhibit both phenotypes. In particular, the fluorinated analogues seem to be more active against the androgen independent PC-3/DU-145 cells, and it is precisely this androgen independent prostate cancer that proves to be more fatal to patients because of a current lack of effective treatment strategies.

Noguchi *et al.* in 2005 [79] synthesized 2,6-diisopropylphthalimide analogues as shown in Table **14** and evaluated their anti-angiogenesis activity by using human umbilical vein endothelial cell (HUVEC) assay system.

Table 14. 2, 6-Diisopropylphthalimide Analogues



Compound	R^1	R^2	X
PP-00 (260)	H	H	H
PP-11 (261)	H	H	CH_3
PP-33 (262)	H	H	$CH(CH_3)_2$
5HPP-33 (263)	OH	H	$CH(CH_3)_2$
4HPP-33 (264)	H	OH	$CH(CH_3)_2$
5APP-33 (265)	NH_2	H	$CH(CH_3)_2$
4APP-33 (266)	H	NH_2	$CH(CH_3)_2$
5NPP-33 (267)	NO_2	H	$CH(CH_3)_2$
4NPP-33 (268)	H	NO_2	$CH(CH3)_2$

The effect of substituents introduced at the 4- or 5-position of the phthalimide moiety of PP-33 (**262**) was investigated. The hydroxyl group at the position corresponding to that of 5-hydroxytryptamine (5-HT) (**234**),

showed quite potent anti-angiogenic activity in a dose dependent manner (ca. 59% inhibition at 100 lM, ca. 41% inhibition at 30 lM, and ca. 8% inhibition at 10 lM). Its isoelectronic amino derivative, 5APP-33 (**265**), also showed potent anti-angiogenic activity, though it was less potent than 5HPP-33 (**263**). The regio-isomers of 5HPP-33 (**263**) and 5APP-33 (**265**), that is, 4HPP-33 (**264**) and 4APP-33 (**266**), respectively, were less active than the corresponding 5-substituted analogues. Interestingly, analogues substituted with an electron-withdrawing nitro group 5NPP-33 (**267**) and 4NPP-33 (**268**) showed position dependency, in contrast to the analogues bearing an electron-donating group, i.e. 4NPP-33 (**268**) was more potent than 5NPP-33 (**267**).

Lima *et al*. in 2002 [80] based on Zafirlukast(**269**) (Fig. (**9**)), which is an example of selective and competitive D_4 receptor antagonist, design and synthesise (Scheme **22**) leukotriene D_4 receptor antagonists.

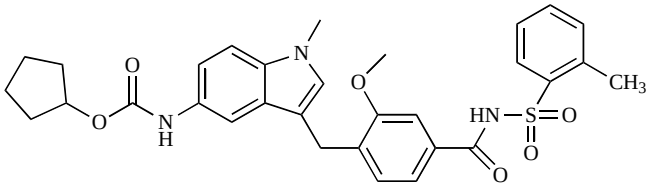


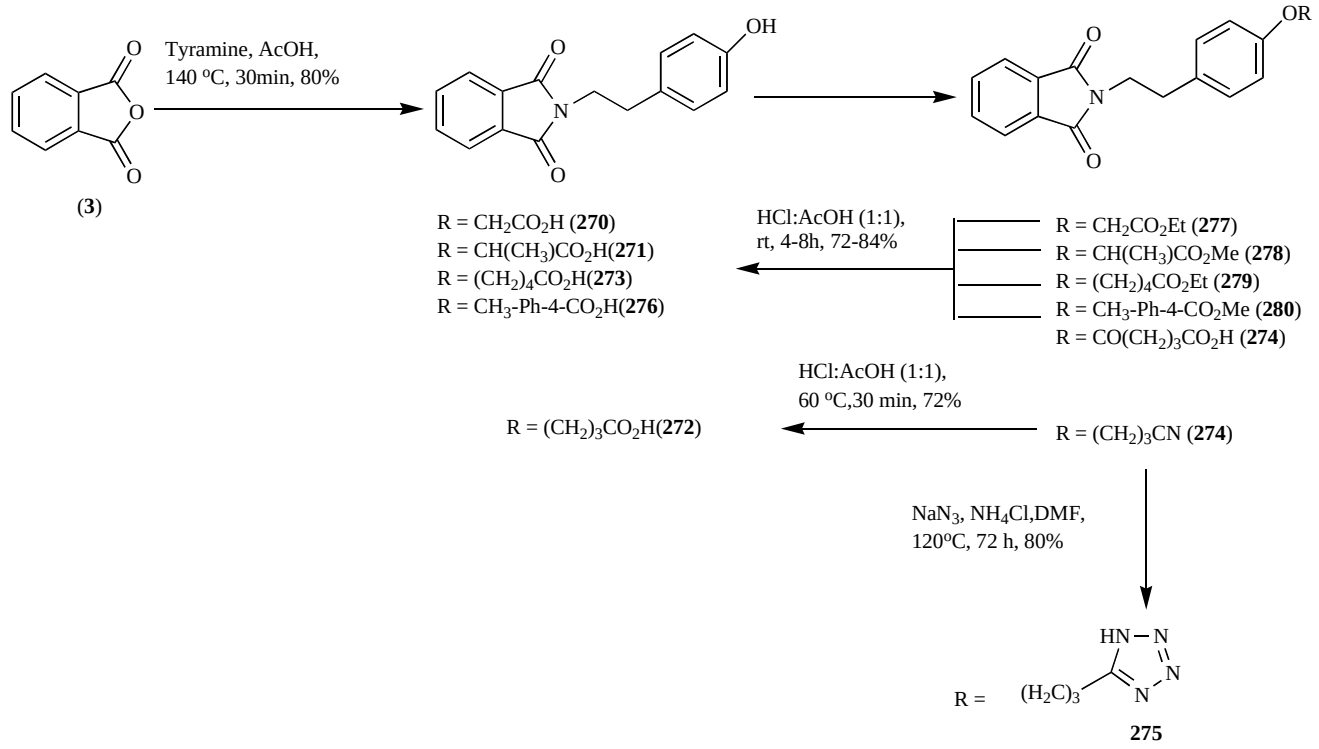
Fig. (9). Chemical structure of Zafirlukast (**269**).

All compounds were evaluated in vitro using the leukotriene D_4 (LTD_4) induced contraction of guinea-pig trachea strips bioassay and zafirlukast (**269**) as standard. The analysis of these screening data showed a significant inhibitory activity for compounds LASSBio 553 (**272**) and LASSBio 483 (**274**), and a highlighting activity observed for compound **275** (LASSBio 552). On the basis of these results, LASSBio 552 (**275**) was selected to study the concentration–response relationship to inhibit the contractile activity induced by 100nM of LTD4, presenting an IC50=31.2 mM (10 nM–400 mM). Although, LASSBio 552 has been showed to be less potent than zafirlukast (IC50=1.03nM [0.1 nM–100 mM]), it was able to evoke a maximum inhibitory response, contrasting with zafirlukast that presented a maximum response of effect in the range of 58%. These results suggest that LASSBio 552 presented a better efficacy profile than the standard zafirlukast.

4.3. SAR of Phthalimide Derivatives as Anti Cancerous Agents

The benzothiazole derivatives (Scheme **12**) were reported to have anti-cancerous activity on human carcinoma cell lines. Inspired by these results Chan, S.H. *et. al*. in 2009 [67] synthesized α-phthalimide ketone derivatives and evaluated in vitro antiproliferative activity on MDAMB-231 and SKHep-1 human carcinoma cell lines. α-Phthalimide ketone were synthesized by condensation of bromoketone and potassium phthalimide as shown in Scheme **13**.

It was observed that both compounds **176** and **177** could induce cell rounding and cell shrinkage on both MDAMB-231 breast cancer cells and SKHep-1 hepatoma cells. The presence of strong electronegative fluorine group at the *para* position of the aryl ring in compound **177** may contribute to its significant inhibitory activity. When the activity of compound **178** or **176** was compared with compound **175** it concluded that the steric effect was not account for the activity. Further investigation revealed a significant decrease



Scheme 22.

in biological activity when the X residue changed from a methylene (CH2) to –CH (CH3)– group (**179**).

CONCLUSIONS

Phthalimide analogues as TNF-α inhibitors have been found very effective for the treatment of various immuno-inflammatory conditions. These analogues selectively constitute a different class of TNF-α inhibitors. Some of these analogues such as thalidomide and LASS Bio 468 have shown remarkable effect on TNF-α production and hence, used for manifestation of number of immunomodulatory disorders. The main mechanism of these drugs is directly linked to their ability to regulate the TNF-α production in body. Synthesis of new kind of phthalimide analogues with novel N-alkyl substitution may lead to the discovery of new drug candidate for treatment of TNF-α generated disease such as rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, bronchial asthma, allergic conditions and cancers. Several other phthalimide analogues were also found to exhibit bidirectional activity on TNF-α. The structure activity relationship of these analogues may help in development of specific analogue with desired activity. The small size and simple method of synthesizing for these analogues make them of pharmaceutical interest. In conclusion these potential findings of phthalimide and its analogues provide a platform to synthetic chemists as well as biologists for the development of new pharmaceutical agent. Hopefully this is the beginning of the end of TNF-α induced disorder.

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ABBREVIATION

TNF-α	=	Tumor necrosis factor-α
BRMs	=	Biological response modifiers
HIV	=	Human immunodeficiency virus
AIDS	=	Acquired immunodeficiency syndrome
GVHD	=	Graft-*versus*-host disease
TPA	=	12-O-tetradecanoylphorbol 13-acetate
HL-60	=	Human promyelocytic leukemia cells
DABCO	=	1,4-diazabicyclo[2,2,2] octane
GABA	=	Gamma-aminobutyric acid
MES	=	Maximal electroshock seizure
scPTZ	=	Subcutaneous pentylenetetrazole
scSTY	=	Subcutaneous strychnine
ipPIC	=	Intraperitoneal picrotoxin
MAOS	=	Microwave-assisted organic synthesis
SAR	=	Structure activity relationship
ILs	=	Interleukins
CD8+	=	Cluster of differentiation
PBMCs	=	Peripheral blood mononuclear cells
LPS	=	Lipopolysaccharide
BALF	=	Bronchoalveolar lavage fluid
cAMP	=	Cyclic 3',5'-adenosine monophosphate
PDE	=	Phosphodiesterase
RNA	=	Ribonucleic acid
mRNA	=	Messenger ribonucleic acid
NFκB	=	Nuclear factor kappa-light-chain-enhancer of activated B cells
GFP	=	Green fluorescent protein
FRT	=	FLP recombinase target
VEGF	=	Vascular endothelial growth factor
bFGF	=	Basic fibroblast growth factor
HMEC's	=	Human micro vascular endothelial cells
HUVEC	=	Human umbilical vein endothelial cell
5-HT	=	*5*-hydroxytryptamine
LTD_4	=	Leukotriene D4
CML	=	Chronic myelogenous leukaemia

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