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Aza Analogues of Thalidomide: Synthesis and Evaluation as Inhibitors of Tumor Necrosis Factor-α Production In Vitro

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Abstract—A synthetic entry to derivatives of the new classes of 5-phthalimidouracils and 5-phthalimidobarbituric acids is reported. These 5-phthalimidopyrimidines as well as phthalimido-2,4-difluorobenzenes were designed as analogues of thalidomide, a well known inhibitor of TNF-α production. A preliminary in vitro investigation of the compounds as inhibitors of the TNF-α production was performed. Among the compounds of the present series, 5-ethyl-1-phenyl-5-(tetrafluorophthalimido)barbituric acid and 2-(2,4-difluorophenyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3(2*H*)-dione were proved to be potent inhibitors. Both compounds showed inhibitory activity in the lower micromolar range on the LPS-induced TNF-α production in human monocytes.

Introduction

Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine produced by numerous cell types among which monocytes/macrophages play a major role. Overproduction of TNF-α is associated with a wide range of pathological conditions.[1] This has led to much effort in finding ways to downregulate its production. Attention has focused on the use of low molecular weight agents as TNF-α inhibitors, such as thalidomide (**1**, Fig. 1) which was reported to be a selective inhibitor of TNF-α production in monocytes stimulated with bacterial lipopolysaccharide (LPS).[2] Despite the teratogenicity of thalidomide, there is a remarkable resurgence of interest in the drug in recent years due to its usefulness for the treatment of erythema nodosum leprosum and other inflammatory and autoimmune disease states such as chronic graft-versus-host disease, Behcet's disease, rheumatoid arthritis, HIV-associated aphthous ulceration and wasting syndrome.[3–5] The effectiveness of the drug in these diseases has mostly been attributed to its specific inhibitory activity on TNF-α production. It was found that the regulation of TNF-α production by thalidomide and some of its derivatives is bidirectional and specific to cell type and to inducer.[6] Both thalidomide enantiomers racemize with relatively high velocity under physiological conditions;[4,7] therefore, enantio-dependence of the TNF-α production-regulating activity was also investigated with nonracemizable analogues, for example, 3′-methylthalidomide.[6,8] Modifications of the thalidomide structure led to several analogues with improved anti-TNF-α activities. Examples include amino substitution at the phthaloyl moiety,[9] phenyl substitution at 5′-position (e.g., **2**),[10] and phthaloyl β-amino amide derivatives;[11] the latter compounds were designed as analogues of hydrolysis products of **1**. Several tetrafluorophthalimides were found to be superior to non-fluorinated analogues in blocking TNF-α production in LPS-stimulated monocytes with inhibitor **3** being the most potent.[12]

In the present paper, we report our initial results on the preparation of novel 5-phthalimidopyrimidines and their effects on TNF-α production. This approach is based on replacing the –CH–CH_2–CH_2– unit in **1** by –C=CH–NH–, resulting in 5-phthalimidouracil (**15**, Scheme 4). In contrast to **1**, the dehydrogenated aza analogue **15** contains no chiral centre. With respect to structure **2**, 5-phthalimidouracils with a phenyl substituent

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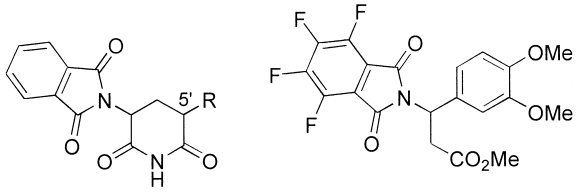
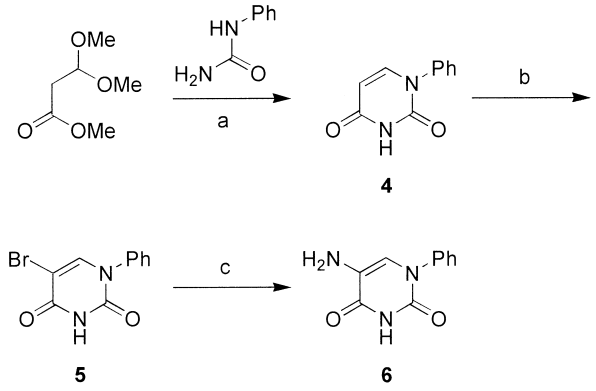


Figure 1. Phthalimide derived inhibitors of TNF-α production.



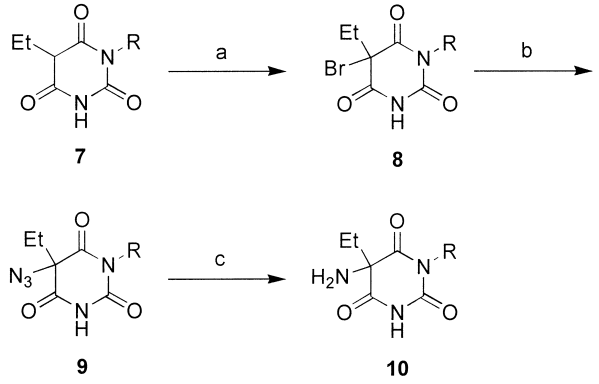
Scheme 1. (a) NaH, dioxane, reflux, 4 N H_2SO_4, reflux; (b) Br_2, acetic acid, room temperature; (c) NH_3, MeOH, 120 °C.

at N−1 are included in the present study. Furthermore, the uracil unit of 5-phthalimidouracil was replaced by the 2,4-difluorophenyl substituent thus leading to a nonpolar isostere.[13] Another structural modification included the introduction of a barbituric acid moiety. For these derivatives, 5-alkyl substitution was applied in order to prevent a labile hydrogen at the chiral carbon.

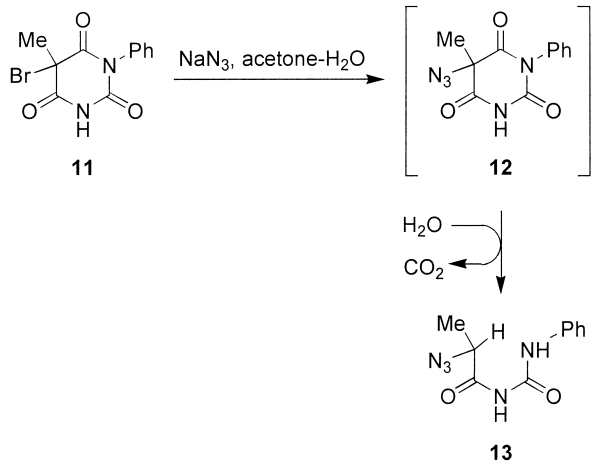
Results and Discussion

Chemistry

The synthesis of the uracil precursor **6** is shown in Scheme 1. Methyl 3,3-dimethoxypropanoate was condensed with phenylurea to 1-phenyluracil (**4**).[14] The 1-phenyl substitution was confirmed by MS, considering the reverse Diels-Alder fragmentation (M^+–HNCO) and the absence of the (M^+–PhNCO) fragment, and ^1H NMR: On irradiation of the 6-H resonance, a NOE enhancement in the multiplet of the phenyl protons was observed. Selective bromination in the 5-position was accomplished on treatment of **4** with bromine in acetic acid by a procedure that turned out to be advantageous compared to the described photolytic[15] or oxidative[16] bromination of uracils. Attempts to convert the bromouracil **5** to the desired primary amine **6** by Gabriel synthesis, or via the corresponding 5-azido derivative were not successful, since **5** failed to react both with potassium phthalimide or sodium azide. However, the aminouracil **6** could be prepared from **5** with a solution of ammonia in methanol on performing the reaction in a closed vessel at 120 °C for 20 h. The synthesis of 5-aminobarbituric acids **10** (Scheme 2) followed a route that has recently been described.[17] Whereas 5-ethyl-5-bromobarbituric acids **8** when subjected to the conditions of the azidolysis[18] produce azido derivatives **9** (R = Ph, cyclohexyl), conversion of the corresponding 5-methyl derivative **11** (Scheme 3) to the desired azido compound **12** was not successful. The urea **13** was obtained, probably as a result of hydrolytic ring opening of the intermediate **12** followed by decarboxylation. The synthesis of the final 5-phthalimidopyrimidines **15**–**22** is depicted in Scheme 4. Phthalimidouracils **15**–**18** were prepared by reacting phthalic anhydrides **14** with 5-aminouracil or **6**, respectively, in glacial acetic acid. Accordingly, aminobarbituric acids **10** were transformed to phthalimidobarbituric acids **19**–**22**, and 2,4-difluoroaniline was converted to the corresponding phthaloyl derivatives **23**–**25**.



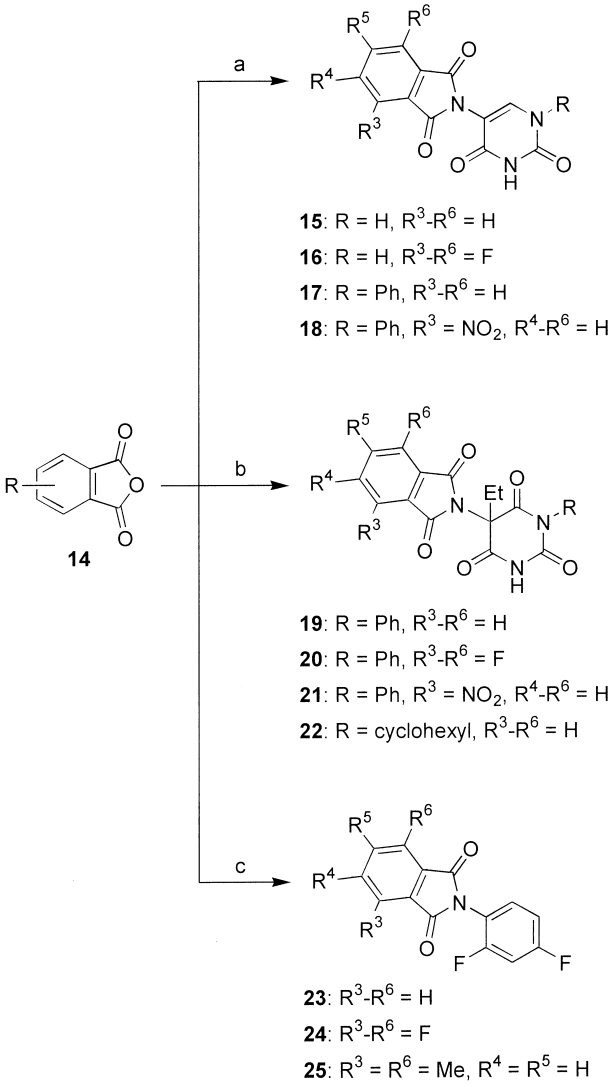
Scheme 2. (a) Br_2, acetic acid, room temperature; (b) NaN_3, acetone-H_2O, reflux; (c) H_2, Pd/C, acetic acid, room temperature.



Scheme 3.

Inhibition of the TNF-α production

The inhibitory potential of 5-phthalimidopyrimidines (**15**–**22**) and 2,4-difluorophenyl phthalimides (**23**–**25**) was assessed by determining the TNF-α production of LPS-stimulated monocytes (Table 1). 5-Phthalimidouracil (**15**), whose structure resembled that of thalidomide most compared to the other compounds of this series, did show only weak activity (19% inhibition at 50 μM). Introduction of a phenyl, or cyclohexyl residue at position **1** of the pyrimidine moiety resulted in a loss



Scheme 4. (a) 5-Aminouracil or **6**, acetic acid, reflux or 130 °C; (b) **10** (R = Ph or cyclohexyl), acetic acid, reflux or 170 °C; (c) 2,4-difluoroaniline, acetic acid, reflux.

of activity, provided that the phthalimido portion was unsubstituted (**17**, **19**, **22**) or 3′-nitro substituted (**18**, **21**). The tetrafluoro phthalmidopyrimidine **20** exhibited strong inhibitory activity. Among the three difluorophenyl phthalimides (**23**–**25**), only **24** was active. Thus, in the present series, tetrafluoro substitution at the phthalimide unit was necessary for strong inhibitory activity (**20** versus **19**; **24** versus **23**), but not sufficient, since **16** was found to be inactive. The replacement of the unsubstituted uracil moiety by a difluorophenyl rest (**16** versus **24**) remarkably increased the inhibitory potential. The difluorobenzene derivative **24** can be regarded as a closed steric mimic[13] for the uracil derivative **16**. Thus, the increased activity of **24** might be attributed to the enhanced lipophilicity and the loss of hydrogen bond donor capability, respectively, that results from the isosteric replacement. Noteworthy, in a series of phthaloyl β-amino amides, the TNF-α lowering ability required a lipophilic group within the side chain, and 3-phenylpropionic acid derivatives proved to be

Table 1. Inhibition of LPS-induced TNF-α production in human monocytes

	% Inhibition[a] (and cell viability[b])			
compd	at 50 μM	at 25 μM	at 5 μM	at 0.5 μM
1 (thalidomide)	15 (98)	< 10		
15	19 (99)			
16	< 10 (91)	< 10	< 10	
17	< 10 (95)			
18	< 10 (95)			
19	< 10 (90)	< 10		
20	100 (68)	100 (73)	88 (80)	12 (87)
21	16 (68)			
22	< 10 (77)			
23	< 10 (87)			
24	100 (78)	100 (88)	84 (95)	< 10 (92)
25	< 10 (74)			

[a]Human monocytes (1×10^6/mL) were incubated in the presence of different concentrations of the indicated compounds for 15 min before addition of LPS (100 ng/mL). After 4 h, TNF-α concentration in the culture supernatants was determined by ELISA. Percentage of inhibition refers to the amount of TNF-α produced in the presence of LPS alone (100% activation). Values represent results of three independent experiments.

[b]Cytotoxic effects on LPS-treated monocytes are indicated in parentheses as percentage of surviving cells. Values represent results of three independent experiments.

highly potent inhibitors.[11] Compared to thalidomide, which was reported to inhibit 50–60% of the LPS-induced TNF-α release at 200 μM,[6,13] IC_{50} values of approximately 3 μM for the analogues **20** and **24** could be estimated. These compounds showed complete inhibition at 25 μM. However, at this concentration cell viability was slightly affected by both compounds (Table 1).

The mechanisms underlying the action of compounds **20** and **24** are not clear. Potential intervention sites in TNF-α production have been reviewed (for example, see ref 1a) and include both the induction of TNF expression, as well as TNF synthesis, processing and release. Similar to thalidomide,[19,20] the compounds may exert their effect by intervening with the transcriptional regulation of the TNF-α gene. Another target of thalidomide has been identified as α_1-acid glycoprotein (AGP).[21] However, tetrafluoro thalidomide derivatives did not compete for the thalidomide binding site on AGP and additional pieces of evidence also support the conclusion that fluorinated and nonfluorinated phthalimides might interact with distinct target proteins to modulate TNF-α production.[21] Moreover, inhibition of cAMP phosphodiesterases (PDEs) is one mechanism by which TNF-α production may be reduced. PDE4 is the major PDE isoenzyme in monocytes and macrophages. Among thalidomide analogues with TNF-α inhibitory activity, certain inhibitors indeed acted as PDE4 inhibitors,[22,23] but others, including thalidomide itself, failed to inhibit PDE4.[9,23]

In summary, we have developed a synthetic entry to certain derivatives of the new classes of 5-phthalimidouracils and 5-phthalimidobarbituric acids. The synthetic routes reported will be applicable to prepare various

analogues in order to obtain structural diversity of potential inhibitors of TNF-α production. Since, as shown within the present series, high activity depended on the presence of a tetrafluorinated phthalimide unit, combination of the fixed tetrafluorophthalimide portion with various pyrimidinetrione and difluorophenyl substituents appears as a promising concept to develop potent phthalimide derived inhibitors of TNF-α production. For such syntheses, 5-substituted 5-aminobarbituric acids can be utilized and these precursors can be separated to non-racemizable enantiomers by standard procedures.[24] We are currently investigating this approach.

Experimental

Chemistry

Melting points were determined on a Boetius apparatus and are not corrected. Thin-layer chromatography was performed on Merck aluminium sheets, silica gel 60 F_{254}. ^{13}C NMR spectra (75 MHz), ^{1}H NMR spectra (300 MHz), and ^{19}F NMR spectra (188 MHz) were recorded on a Varian Gemini 300. IR spectra were measured with a Perkin-Elmer 16 PC FTIR spectrometer. Mass spectra (70 eV) were obtained using a Varian MAT CH6 spectrometer. 5-Aminouracil, tetrafluorophthalic anhydride, 3-nitrophthalic anhydride, and 2,4-difluoroaniline were purchased from Aldrich (Steinheim, Germany). 5-Aminobarbituric acids **10**, and 5-bromo-5-methyl-1-phenylbarbituric acid (**11**) were prepared as described.[17]

Cell separation and stimulation

Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated by centrifugation over a Ficoll-Paque (Pharmacia, Freiburg, Germany) density gradient. After repeated washing in phosphate-buffered saline containing 0.3 mM EDTA (PBS/EDTA) the PBMCs were further separated by counterflow centrifugation using the J6-MC elutriator system (Beckmann Instruments, Palo Alto, USA) as described previously.[25] Monocytes of >90% purity were incubated at a density of 1×10^6/mL overnight in RPMI 1640 (Sigma Aldrich, Deisenhofen, Germany), containing 10% FCS (Sigma Aldrich, Deisenhofen, Germany), 2 mM glutamine (Biochrom KG, Berlin, Germany), 100 U/mL penicillin (Biochrom KG, Berlin, Germany) and 100 μg/mL streptomycin (Biochrom KG, Berlin, Germany) in 96 well-plates (Greiner, Trickenhausen, Germany). Inhibitor stock solutions were prepared in DMSO. Dilutions were done with RPMI 1640 containing 10% FCS to a final concentration of 0.05% DMSO in the cell culture. For solvent controls, DMSO was added to obtain the same final concentration as in the inhibitor-treated cultures. After preincubation of the monocytes with the inhibitors for 15 min, LPS (100 ng/mL) (Sigma Aldrich, Deisenhofen, Germany) was added, and after 4 h supernatants were collected and analyzed for TNF-α concentrations. Cell viability was assayed by Trypan blue exclusion.

Detection of TNF-α in culture supernatants

The content of TNF-α in the cell culture supernatants was determined by Enzyme-Linked Immunosorbent Assay (ELISA). The primary anti-TNF-α antibody as well as the secondary purified rabbit polyclonal anti-TNF-α antibody were kindly provided by W. Buurmann (Maastricht University, Maastricht, The Netherlands). The peroxidase labelled goat-anti-rabbit antibody was from Sigma Aldrich, Deisenhofen, Germany.

1-Phenylpyrimidine-2,4(1*H*,3*H*)-dione (4). Phenylurea (2.72 g, 20 mmol) was added in small portions to a mixture of sodium hydride (675 mg of a 80% suspension in oil, 22.5 mmol) and anhydrous dioxane (33 mL) at argon atmosphere. When the hydrogen evolution has ceased, methyl 3,3-dimethoxypropanoate (3.3 g, 22.5 mmol) was added. The mixture was refluxed for 90 min, poured into 4 N sulfuric acid (200 mL) and refluxed again for 15 min, cooled and extracted with CH_2Cl_2 (3×200 mL). The organic layer was dried (Na_2SO_4), evaporated to dryness under reduced pressure and the residue was recrystallized from EtOH to obtain **4** (1.4 g, 37%): mp 244–246 °C (lit.[26] mp 247 °C); ^{1}H NMR (DMSO-d_6) δ 5.68 (dd, 1H, $J=7.9$, 2.3 Hz), 7.40–7.52 (m, 5H), 7.70 (d, 1H, $J=7.9$ Hz), 11.42 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 101.60, 126.79, 128.20, 129.10, 138.88, 145.49, 150.36, 163.67; MS (EI) *m*/*z* (rel intensity) 188 (M^+, 100), 145 (M^+-HCNO, 78).

5-Bromo-1-phenylpyrimidine-2,4(1*H*,3*H*)-dione (5). A solution of bromine (2.4 g, 15 mmol) in glacial acetic acid (10 mL) was added dropwise over 2 h to a solution of **4** (2.82 g, 15 mmol) in glacial acetic acid (150 mL). The mixture was stirred at room temperature overnight and evaporated to dryness under reduced pressure. The residue was washed with H_2O and dried to give **5** (3.61 g, 90%): mp 273–275 °C (lit.[15] mp 271–273 °C); ^{1}H NMR (DMSO-d_6) δ 7.40–7.55 (m, 5H), 8.26 (s, 1H), 11.96 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 95.81, 126.79, 128.48, 129.05, 138.36, 144.93, 149.85, 159.76; MS (EI) *m*/*z* (rel intensity) 268, 266 (M^+, 45), 225, 223 (M^+-HNCO, 44), 77 (100).

5-Amino-1-phenylpyrimidine-2,4(1*H*,3*H*)-dione (6). Compound **5** (1 g, 3.74 mmol) was dissolved in a 20% methanolic solution of NH_3 (30 mL) and heated for 20 h in a sealed tube at 120 °C. The solution was evaporated to dryness, redissolved in EtOH (100 mL), treated with charcoal and filtrated. The filtrate was concentrated, cooled, and the precipitate was filtered off to obtain **6** (290 mg, 38%): mp 223–225 °C; ^{1}H NMR (DMSO-d_6) δ 4.19 (s, 2H), 6.85 (s, 1H), 7.35–7.50 (m, 5H), 11.34 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 120.04, 123.26, 126.55, 127.61, 129.05, 139.73, 148.55, 161.11; MS (EI) *m*/*z* (rel intensity) 203 (M^+, 100), 133 (35), 105 (69). Anal. calcd for $C_{10}H_9N_3O_2$: C, 59.11; H, 4.46; N, 20.68. Found: C, 58.81; H, 4.40; N, 20.35.

1-(2-Azidopropionyl)-3-phenylurea (13). Compound **11** was reacted with sodium azide in 50% aqueous acetone as described[18] to obtain compound **13**: mp 177–180 °C; ^{1}H NMR (DMSO-d_6) δ 1.45 (d, 3H, $J=6.9$ Hz), 4.13 (q,

1H, $J = 6.9$ Hz), 7.07–7.13 (m, 1H), 7.30–7.40 (m, 2H), 7.50–7.60 (m, 2H), 10.21 (s, 1H), 10.90 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 16.36, 57.09, 119.85, 123.86, 128.90, 137.32, 150.25, 172.98; IR (KBr, cm^{-1}) 2104 (N_3), 1702 (CO); MS (EI) m/z (rel intensity) 233 (M^+, 22), 119 (100). Anal. calcd for $C_{10}H_{11}N_5O_2$: C, 51.50; H, 4.75; N, 30.03. Found: C, 51.30; H, 4.69; N, 30.30.

5-Phthalimidopyrimidine-2,4(1*H*,3*H*)-dione (15). A mixture of phthalic anhydride (592 mg, 4 mmol), 5-aminouracil (508 mg, 4 mmol) and glacial acetic acid (25 mL) was refluxed for 2 h. After cooling, the precipitate was filtered off, washed with H_2O and dried to give **15** (820 mg, 80%): mp > 320 °C, dec. (lit.[27] mp > 300 °C); ^{1}H NMR (DMSO-d_6) δ 7.90 (s, 1H), 7.91–8.03 (m, 4H), 11.41 (s, br, 1H), 11.64 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 105.34, 123.61, 131.33, 135.02, 143.44, 150.69, 160.70, 166.83; MS (EI) m/z (rel intensity) 257 (45, M^+), 104 (60), 44 (100). Anal. calcd for $C_{12}H_7N_3O_4$: C, 56.04; H, 2.74; N, 16.34. Found: C, 55.70; H, 3.06; N, 16.00.

5-(Tetrafluorophthalimido)pyrimidine-2,4(1*H*,3*H*)-dione (16). A mixture of tetrafluorophthalic anhydride (440 mg, 2 mmol), 5-aminouracil (254 mg, 2 mmol) and glacial acetic acid (50 mL) was refluxed for 6 h. After cooling, the precipitate was filtered off, washed with H_2O and dried to give **16** (260 mg, 40%): mp 350–355 °C; ^{1}H NMR (DMSO-d_6) δ 7.89 (d, 1H, $J = 6.2$ Hz), 11.47 (d, 1H, $J = 6.2$ Hz), 11.72 (s, 1H); ^{19}F NMR (DMSO-d_6/$CFCl_3$) δ −142.0 (m, 2F), −136.5 (m, 2F); MS (EI) m/z (rel intensity) 329 (M^+, 50), 176 (100). Anal. calcd for $C_{12}H_3N_3O_4F_4$: C, 43.79; H, 0.92; N, 12.77. Found: C, 43.60; H, 1.16; N, 12.50.

1-Phenyl-5-phthalimidopyrimidine-2,4(1*H*,3*H*)-dione (17). A mixture of compound **6** (203 mg, 1 mmol), phthalic anhydride (163 mg, 1.1 mmol) and glacial acetic acid (10 mL) was refluxed for 90 min. The solvent was evaporated under reduced pressure and the residue was recrystallized twice from EtOH to give **17** (200 mg, 60%): mp 262–270 °C; ^{1}H NMR (DMSO-d_6) δ 7.48–7.54 (m, 5H), 7.92–8.01 (m, 4H), 8.28 (s, 1H), 12.04 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 106.56, 123.68, 126.71, 128.65, 129.28, 131.27, 135.10, 138.29, 146.26, 149.66, 160.24, 166.66; MS (EI) m/z (rel intensity) 333 (58, M^+), 104 (100). Anal. calcd for $C_{18}H_{11}N_3O_4$: C, 64.86; H, 3.33; N, 12.61. Found: C, 64.59; H, 3.22; N, 12.22.

5-(3-Nitrophthalimido)-1-phenylpyrimidine-2,4(1*H*,3*H*)-dione (18). A mixture of compound **6** (508 mg, 2.5 mmol), 3-nitrophthalic anhydride (580 mg, 3 mmol) and glacial acetic acid (40 mL) was heated in a sealed tube at 130 °C for 17 h. The solvent was evaporated under reduced pressure and the oily residue was triturated with EtOH (10 mL) to obtain a precipitate which was filtered off. The crude product was recrystallized from EtOH with charcoal to give **18** (650 mg, 69%): mp 232–235 °C; ^{1}H NMR (DMSO-d_6) δ 7.47–7.55 (m, 5H), 8.13–8.20 (m, 1H), 8.24 (s, 1H), 8.29–8.32 (m, 1H), 8.38–8.42 (m, 1H), 12.09 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 105.98, 122.37, 126.67, 127.54, 128.74, 129.06, 129.31, 132.89, 137.11, 138.26, 144.57, 146.49, 149.58, 160.01, 162.03, 164.62; MS (EI) m/z (rel intensity) 378 (M^+, 4), 44 (100). Anal. calcd for $C_{18}H_{10}N_4O_6$: C, 57.15; H, 2.66; N, 14.81. Found: C, 57.01; H, 2.55; N, 14.46.

5-Ethyl-1-phenyl-5-phthalimidobarbituric acid (19). A mixture of 5-amino-5-ethyl-1-phenylbarbituric acid (1.24 g, 5 mmol), phthalic anhydride (814 mg, 5.5 mmol) and glacial acetic acid (60 mL) was refluxed for 5 h. The solvent was evaporated under reduced pressure and the residue was recrystallized from EtOH to give **19** (1.2 g, 64%): mp 212–214 °C; ^{1}H NMR (DMSO-d_6) δ 1.11 (t, 3H, $J = 7.3$ Hz), 2.89 (q, 2H, $J = 7.3$ Hz), 7.21–7.38 (m, 2H), 7.42–7.59 (m, 3H), 7.95 (s, 4H), 12.43 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 9.41, 27.02, 67.66, 123.84, 128.56, 128.99, 129.26, 130.30, 134.04, 135.64), 149.08, 167.54, 167.84, 167.90; MS (EI) m/z (rel intensity) 377 (M^+, 31), 230 (50), 104 (100). Anal. calcd for $C_{20}H_{15}N_3O_5$: C, 63.66; H, 4.01; N, 11.14. Found: C, 63.33; H, 4.01; N, 11.01.

5-Ethyl-1-phenyl-5-(tetrafluorophthalimido)barbituric acid (20). A mixture of 5-amino-5-ethyl-1-phenylbarbituric acid (250 mg, 1 mmol), tetrafluorophthalic anhydride (260 mg, 1.2 mmol) and acetic acid (7 mL) was refluxed for 3 h. The solvent was evaporated to dryness under reduced pressure. The residue was recrystallized from EtOH to give **20** (270 mg, 60%): mp 218–220 °C; ^{1}H NMR (DMSO-d_6) δ 1.09 (t, 3H, $J = 7.2$ Hz), 2.86 (q, 2H, $J = 7.3$ Hz), 7.20–7.32 (m, 2H), 7.45–7.59 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 9.36, 26.90, 68.10, 112.20, 112.65, 128.05, 128.56, 129.27, 133.91, 141.10, 147.05, 148.88, 162.51, 166.85, 167.18; MS (EI) m/z (rel intensity) 449 (M^+, 62), 421 (M^+, 20), 230 (39), 176 (70), 119 (100). Anal. calcd for $C_{20}H_{11}N_3O_5F_4$: C, 53.46; H, 2.47; N, 9.35. Found: C, 53.26; H, 2.78; N, 9.04.

5-Ethyl-5-(3-nitrophthalimido)-1-phenylbarbituric acid (21). A mixture of 5-amino-5-ethyl-1-phenylbarbituric acid (990 mg, 4 mmol), 3-nitrophthalic anhydride (772 mg, 4 mmol) and glacial acetic acid (40 mL) was heated in a sealed tube at 170 °C for 15 h. The solvent was evaporated to dryness under reduced pressure. The residue was recrystallized from ethyl acetate to give **21** (390 mg, 23%): mp 214–216 °C; ^{1}H NMR (DMSO-d_6) δ 1.11 (t, 3H, $J = 7.3$ Hz), 2.90 (q, 2H, $J = 7.3$ Hz), 7.27–7.33 (m, 2H), 7.46–7.55 (m, 3H), 8.13–8.19 (m, 1H), 8.26–8.30 (m, 1H), 8.39–8.43 (m, 1H), 12.50 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 9.40, 27.08, 68.06, 121.49, 127.93, 128.57, 129.03, 129.26, 129.81, 132.34, 133.94, 137.55, 144.46, 148.96, 163.49, 165.55, 167.04, 167.46; MS (EI) m/z (rel intensity) 422 (M^+, 35), 289 (30), 230 (32), 177 (30), 119 (100). Anal. calcd for $C_{20}H_{14}N_4O_7$: C, 56.88; H, 3.34, N, 13.27. Found: C, 56.90; H, 3.33; N, 12.90.

1-Cyclohexyl-5-ethyl-5-phthalimidobarbituric acid (22). A mixture of 5-amino-1-cyclohexyl-5-ethylbarbituric acid (500 mg, 2 mmol), phthalic anhydride (300 mg, 2 mmol) and glacial acetic acid (20 mL) was refluxed for 5 h. The mixture was cooled, the precipitate was filtered off, washed with H_2O and dried to give **22** (470 mg, 61%): mp 248–253 °C; ^{1}H NMR (DMSO-d_6) δ 0.99 (t, 3H, $J = 7.4$ Hz), 1.01–2.21 (m, 10H), 2.68 (q, 2H, $J = 7.4$ Hz), 4.13–4.29 (m, 1H), 7.90 (s, 4H), 12.09 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 9.10, 24.83, 25.62, 25.71,

27.23, 27.92, 29.05, 54.66, 67.83, 123.74, 130.32, 135.56, 149.24, 167.20, 167.79, 168.28; MS (EI) *m/z* (rel intensity) 383 (M^+, 5), 302 (M^+, 100), 105 (52). Anal. calcd for $C_{20}H_{21}N_3O_5$: C, 62.65; H, 5.52; N, 10.96. Found: C, 62.30; H, 5.85; N, 10.89.

2-(2,4-Difluorophenyl)-1*H*-isoindole-1,3(2*H*)-dione (23). A mixture of 2,4-difluoroaniline (2 g, 15.5 mmol), phthalic anhydride (2.07 g, 14 mmol) and acetic acid (100 mL) was refluxed for 3.5 h. The solvent was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (150 mL), washed with 0.1 M HCl (3×50 mL) and H_2O (50 mL), and dried (Na_2SO_4). After removal of the solvent, the residue was recrystallized twice from EtOH to give **23** (2.8 g, 77%): mp 167–168 °C; ^{1}H NMR (DMSO-d_6) δ 7.25–7.33 (m, 1H), 7.48–7.56 (m, 1H), 7.62–7.71 (m, 1H), 7.90–8.03 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 104.98 (dd, ^2J = 26.8, 24.0 Hz, C-3′), 112.17 (dd, ^2J = 22.7, ^4J = 3.5 Hz, C-5′), 115.83 (dd, ^2J = 13.0, ^4J = 3.9 Hz, C-1′), 123.70 (C-5, C-6), 131.38 (C-3a, C-7a), 131.94 (C-6′), 134.95 (C-4, C-7), 157.73 and 162.23 (dd, ^1J = 251.1, ^4J = 13.28 Hz and dd, ^1J = 247.7, ^3J = 11.7 Hz, C-2′ and C-4′), 166.17 (C-1, C-3); MS (EI) *m/z* (rel intensity) 259 (M^+, 100). Anal. calcd for $C_{14}H_7NO_2F_2$: C, 64.87; H, 2.72; N, 5.40. Found: C, 65.20; H, 2.61; N, 5.22.

2-(2,4-Difluorophenyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3(2*H*)-dione (24). A mixture of 2,4-difluoroaniline (1 g, 7.75 mmol), tetrafluorophthalic anhydride (1.54 g, 7 mmol) and glacial acetic acid (50 mL) was refluxed for 3.5 h. The solvent was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (75 mL), washed with 0.1 M HCl (3×25 mL) and H_2O (2×25 mL), and dried (Na_2SO_4). After removal of the solvent, the residue was recrystallized from EtOH to give **24** (980 mg, 42%): mp 145–146 °C; ^{1}H NMR (DMSO-d_6) δ 7.22–7.38 (m, 1H), 7.50–7.66 (m, 2H); ^{19}F NMR (DMSO-d_6/$CFCl_3$) δ −142.3 (m, 2F), −136.9 (m, 2F), −113.8 (m, 1F), −105.6 (m, 1F); MS (EI) *m/z* (rel intensity) 331 (M^+, 65), 287 (68), 148 (100). Anal. calcd for $C_{14}H_3NO_2F_6$: C, 50.78; H, 0.91; N, 4.23. Found: C, 50.60; H, 0.83; N, 3.95.

2-(2,4-Difluorophenyl)-4,7-dimethyl-1*H*-isoindole-1,3(2*H*)-dione (25). A mixture of 2,4-difluoroaniline (2 g, 15.5 mmol), 3,6-dimethylphthalic anhydride[28] (2.46 g, 14 mmol) and glacial acetic acid (100 mL) was refluxed for 3.5 h. The solvent was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (150 mL), washed with 0.1 M HCl (3×50 mL) and H_2O (2×50 mL), and dried (Na_2SO_4). After removal of the solvent, the residue was recrystallized from EtOH to give **25** (1.27 g, 32%): mp 212–212.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.59 (s, 6H), 7.24–7.32 (m, 1H), 7.46–7.56 (m, 1H), 7.54 (s, 2H) 7.56–7.66 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 16.87 (CH_3), 104.93 (dd, ^2J = 27.1, 24.2 Hz, C-3′), 112.08 (dd, ^2J = 22.6, ^4J = 3.6 Hz, C-5′), 115.93 (dd, ^2J = 13.2, ^4J = 3.9 Hz, C-1′), 128.15 (C-4, C-7), 132.07 (dd, ^3J = 10.1, 2.1 Hz, C-6′) 135.37 (C-3a, C-7a), 136.62 (C-5, C-6), 157.81 and 162.08 (d, J = 264.1 Hz and d, J = 235.6 Hz, C-2′ and C-4′), 166.64 (C-1, C-3); MS (EI) *m/z* (rel intensity) 287 (M^+, 100), 259 (81). Anal. calcd for $C_{16}H_{11}NO_2F_2$: C, 66.90; H, 3.86; N, 4.88. Found: C, 67.20; H, 3.77; N, 4.59.

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