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Synthesis and Potential Anti-Inflammatory Activity of Some Tetrahydrophthalazinones

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A solid-phase route for the preparation of 4a,5,8,8a-tetrahydrophthalazinon-1-ones employing the Diels-Alder reaction has been developed. Some of the new compounds have been tested for inhibition of LPS-stimulated TNF-α production in human whole blood from patients with chronic obstructive pulmonary disease (COPD). This evaluation revealed two compounds 17 and 18 of interest, incorporating an arylpiperazine moiety, which were found to inhibit LPS- induced TNF-α release like the well known anti-inflammatory PDE4 inhibitors, rolipram and roflumilast.

Keywords: Solid-phase synthesis; 4a,5,8,8a-tetrahydrophthalazinon-1-ones; Diels-Alder reaction; Cyclative-release; Anti-inflammatory effect; PDE4 inhibitors

INTRODUCTION

Chronic inflammation is a central feature of airway diseases such as bronchial asthma and chronic obstructive pulmonary disease (COPD) whose prevalence is increasing. These lung disorders involve the recruitment and activation of inflammatory cells that increase release and expression of components of the inflammatory cascade including cytokines, chemokines, growth factors…[1] PDE4 isoenzyme is a key metabolizing enzyme for the degradation of intracellular cAMP in pulmonary inflammatory and immune cells. This enzyme, by preventing the accumulation of intracellular cAMP, promotes bronchoconstriction and airway inflammation.[2] Selective inhibition of PDE4 would, therefore, be expected to produce both bronchodilatory and anti-inflammatory effects in patients with bronchial asthma or COPD.[3,4]

In a Search for alternative anti-inflammatory drugs to corticosteroids, selective PDE4 inhibitors have received a considerable amount of attention because they suppress the functions of several cell types involved in allergic and inflammatory disorders. The known PDE4 inhibitors like rolipram and derivatives (eg roflumilast) produce side effects that have hampered their clinical development. The search for potent and selective second generation PDE4 inhibitors represents a critical need.

In this context and bearing in mind the azelastine structure, a synthetic phthalazinone used for the treatment of the asthmatic syndrome, different 4a,5,8,8a-tetrahydrophthalazin-1-ones have been synthesized using solid-phase chemistry and evaluated for inhibition of the LPS-stimulated TNF-α production in human whole blood (Figure 1).

MATERIALS AND METHODS

Chemistry

All reagents were purchased from Aldrich and used directly unless otherwise stated. DMF, THF and CH_2Cl_2 were distilled from CaH_2, Na/benzophenone and P_2O_5, respectively and stored under N_2. Wang resin was commercially available. Infrared spectra were recorded on a 16PC FTIR Perkin Elmer

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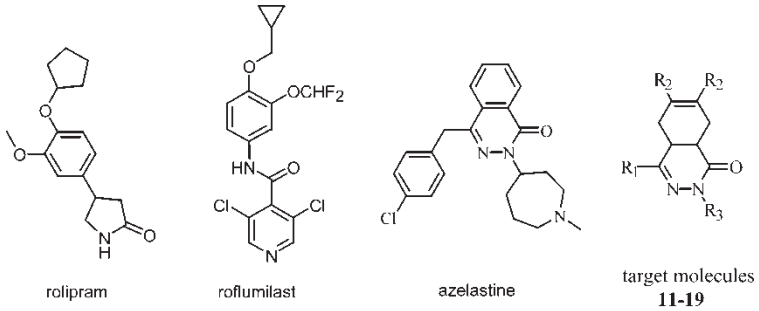


FIGURE 1 Structures of PDE4 inhibitors and target molecules.

spectrometer. Solids were examined with a diffuse reflectance accessory. ^{1}H NMR and ^{13}C NMR were recorded using a Bruker DMX (at 500 MHz and 125 MHz, respectively). Tetramethylsilane was used as the internal standard; chemical shifts (δ) were in ppm. Compounds were purified by column chromatography with silica-gel 60 (70–230 Mesh) purchased from Merck.

Synthesis of Starting Compounds 2–3

Synthesis of Polymer **2**

A solution of 4-oxo-4-phenylbutanoic acid (1.25 mmol) in anhydrous CH_2Cl_2 (3 ml) was added to a suspension of Wang resin (1 g) (Sigma-Aldrich, loading of 0.62 mmol/g) in anhydrous CH_2Cl_2 (8 ml) in the presence of a catalytic amount of dimethylaminopyridine (DMAP, 1%) and diisopropylcarbodiimide (DIC, 1.25 mmol). After stirring for 48 h at room temperature, the resin was washed successively with CH_2Cl_2 (2 × 5 ml), THF (2 × 5 ml), THF/H_2O (1/1, 10 ml), H_2O (10 ml), THF (2 × 5 ml), CH_2Cl_2 (2 × 5 ml) and then dried under reduced pressure at 40°C for 2 h. FT-IR (ν cm^{-1}): 1734 (CO ester), 1700 (CO ketone). To 1 g of resin (0.65 mmol) in 10 ml of THF was added pyridinium tribromide (1.3 mmol) and the resulting mixture was stirred at 50°C for 2 h. The resin was then washed successively with THF (2 × 10 ml), THF/H_2O (10 ml), THF (10 ml) and CH_2Cl_2 (2 × 5 ml). Action of NEt_3 (6.2 mmol) in 10 ml of CH_2Cl_2 at room temperature for 1 h on the intermediate bromide quantitatively afforded the desired polymer supported 4-phenyl-4-oxo-but-2-enoic acid **2**. This step was easily monitored by ^{1}H NMR of the crude product, after cleavage of resin (TFA/CH_2Cl_2: 80/20).

Synthesis of **3**

(*E*)-5-Bromo-4-oxo-pent-2-enoic acid was prepared according to the litterature.[5] It was then anchored on Wang resin using the same conditions as for the starting material **2**. FT-IR (ν cm^{-1}): 1732 (CO ester), 1700 (CO ketone).

General Procedure for the Cycloaddition

To the resins **1**–**3** (1.3 mmol) in dry toluene (20 ml) were added 2,3-dimethylbutadiene or butadiene sulfone (13 mmol). The resulting mixtures were stirred at reflux for 5 h (dimethylbutadiene) or 12 h (butadiene sulfone). The resins **4**–**8** obtained were filtered and washed with toluene, THF and CH_2Cl_2. FT-IR (ν cm^{-1}): 1730 (CO ester), 1700 (CO ketone).

General Procedure for Nucleophilic Substitution with 1-(4-fluorophenyl)-piperazine

Polymers **7**, **8** (0.65 mmol) and amine (6.5 mmol) were stirred in DMSO at room temperature for 2 h. Then, the resins obtained **9**, **10** were filtered off, washed with DMSO, THF/H_2O, H_2O, THF and CH_2Cl_2 and dried under reduced pressure.

Heterocyclisation/cleavage of Compounds 11–19

To the polymers **4**–**6** and **9**, **10** (0.65 mmol) in THF (8 ml) were added solutions of hydrazines (10 eq.) in ethanol (8 ml). The resulting mixtures were stirred at reflux for 15 h. The polymers were then removed by filtration and washed with THF (2 × 5 ml). After removing the solvents under reduced pressure, the residues **11**–**19** were dissolved in CH_2Cl_2, washed twice with water, dried over Na_2SO_4, evaporated to dryness and purified by elution from a silica gel column (CH_2Cl_2/$CH_3COOC_2H_5$: 90/10 for **11**–**16** and CH_2Cl_2/CH_3OH: 90/10 for **17**–**19**).

4-Methyl-4a,5,8,8a-Tetrahydrophthalazin-1(2*H*)-one **11**

Yield: 41%. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.02 (s, 3H), 2.15–2.26 (m, 2H), 2.28–2.35 (m, 1H), 2.42–2.52 (m, 2H), 2.61–2.72 (m, 1H), 5.68–5.82 (m, 2H), 8.55 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 19.8, 25.9, 27.9, 35.9, 36.0, 124.6, 126.2, 155.4, 169.9.

2,4-Dimethyl-4a,5,8,8a-Tetrahydrophthalazin-1(2*H*)-one **12**

Yield: 40%. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.03 (s, 3H), 2.14–2.25 (m, 2H), 2.28–2.35 (m, 1H), 2.42–2.52 (m, 2H), 2.60–2.70 (m, 1H), 3.33 (s, 3H), 5.65–5.75

(m, 2H); ^{13}C NMR ($CDCl_3$) δ (ppm): 19.8, 26.5, 27.9, 36.1, 36.2, 36.2, 124.4, 126.4, 155.2, 168.1.

4,6,7-Trimethyl-4a,5,8,8a-Tetrahydrophthalazin-1(2*H*)-one **13**

Yield: 38%. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.67 (s, 6H), 2.03 (s, 3H), 2.15-2.37 (m, 4H), 2.40–2.50 (m, 1H), 2.52–2.58 (m, 1H), 8.58 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.8, 19.8, 32.0, 34.2, 36.5, 36.6, 123.6, 125.2, 155.7, 170.3.

2,4,6,7-Tetramethyl-4a,5,8,8a-Tetrahydrophthalazin-1(2*H*)-one **14**

Yield: 60%. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.67 (s, 6H), 2.03 (s, 3H), 2.10–2.30 (m, 4H), 2.40–2.45 (m, 1H), 2.50–2.55 (m, 1H), 3.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.8, 19.8, 32.6, 34.3, 36.2, 36.7, 36.9, 123.4, 125.4, 155.5, 168.4.

2-(2-Hydroxyethyl)-4,6,7-Trimethyl-4a,5,8,8a-Tetrahydrophthalazin-1(2*H*)-one **15**

Yield: 41%. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.67 (s, 6H), 2.04 (s, 3H), 2.10–2.30 (m, 4H), 2.40–2.45 (m, 1H), 2.50–2.55 (m, 1H), 3.80 (t, 2H), 3.85 (brs, 1H), 3.90 (t, 2H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.8, 20.0, 32.5, 34.2, 36.6, 36.7, 50.5, 62.0, 123.4, 125.3, 156.5, 169.3.

6,7-Dimethyl-4-Phenyl-4a,5,8,8a-Tetrahydrophthalazin-1(2*H*)-one **16**

Yield: 35%. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.61 (s, 3H), 1.71 (s, 3H), 2.02–2.09 (m, 1H), 2.15–2.25 (m, 2H), 2.80–2.90 (m, 2H), 3.35–3.42 (m, 1H), 7.38–7.45 (m, 3H), 7.75–7.82 (m, 2H), 8.72 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.9, 19.1, 27.8, 29.3, 32.4, 34.8, 123.1, 124.5, 125.8, 128.7, 129.8, 134.8, 154.8, 169.7.

4-[4-(4-Fluorophenyl)-Piperazin-1-Ylmethyl]-4a,5,8,8a-Tetrahydrophthalazin-1(2*H*)-one **17**

Yield: 30%. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.15–2.35 (m, 3H), 2.43–2.60 (m, 2H), 2.62–2.68 (m, 4H), 2.72–2.85 (m, 1H), 3.02 and 3.37 (AB, $J = 13.1$ Hz, 2H), 3.08–3.15 (m, 4H), 5.68–5.73 (m, 2H), 6.84–6.90 (m, 2H), 6.92–7.02 (m, 2H), 8.50 (brs, 1H).

6,7-Dimethyl-4-[4-(4-Fluorophenyl)-Piperazin-1-Ylmethyl]-4a,5,8,8a-Tetrahydro-Phthalazin-1(2*H*)-one **18**

Yield: 41%. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.67 (s, 6H), 2.16–2.35 (m, 3H), 2.48–2.58 (m, 3H), 2.62–2.68 (m, 4H), 3.05 and 3.39 (AB, $J = 12.25$ Hz, 2H), 3.08–3.15 (m, 4H), 6.85–6.89 (m, 2H), 6.92–6.97 (m, 2H), 8.61 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.8, 19.0, 32.0, 33.5, 35.7, 36.8, 50.2, 53.2, 60.3, 115.5 (d, $^2J_{CF} = 22.0$ Hz), 117.8 (d, $^3J_{CF} = 7.5$ Hz), 124.0, 124.8, 147.8, 154.3, 157.2 (d, $^1J_{CF} = 239.1$ Hz), 170.1.

4-[4-(4-Fluorophenyl)-Piperazin-1-Yl-Methyl]-2,6,7-Trimethyl-4a,5,8,8a-Tetrahydro-Phthalazin-1(2*H*)-one **19**

Yield: 45%. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.67 (s, 6H), 2.15–2.27 (m, 3H), 2.43–2.57 (m, 3H), 2.62–2.68 (m, 4H), 3.03 and 3.39 (AB, $J = 13.1$ Hz, 2H), 3.08–3.13 (m, 4H), 3.36 (s, 3H), 6.84–6.89 (m, 2H), 6.93–6.98 (m, 2H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.8, 19.0, 32.6, 33.5, 33.6, 36.0, 36.5, 37.0, 50.3, 53.2, 60.4, 115.5 (d, $^2J_{CF} = 22.0$ Hz), 117.8 (d, $^3J_{CF} = 7.5$ Hz), 123.8, 125.0, 147.9, 154.1, 157.2 (d, $^1J_{CF} = 239.1$ Hz), 168.4.

Anti-inflammatory Effect Evaluation

Some compounds were tested *in vitro* in a model of inflammatory mediator release.

Study Design

A COPD patient according to the Gold Criteria[6] was recruited by the Department of Pneumology (CHU de Rennes, Hôpital de Pontchaillou, France). A volume of 15 ml of whole blood was collected in heparin-tubes. Blood was distributed in 96-well microplate under sterile conditions and incubated 30 min at 37°C in an atmosphere of 5% CO_2 and 95% air. Studied compounds or the PDE4 inhibitors, rolipram and roflumilast, were dissolved in 100% DMSO to a final concentration of 10 mM and then diluted in 70% ethanol to 1 mM. For use in experiments, compounds were then diluted as required with NaCl 0.9%. Compounds (10 μM), PDE4 inhibitors (10 μM) or vehicule were incubated for 1 h. At the end of the incubation period, LPS from *Escherichia Coli* was added to the stimulated well whereas the unstimulated well received sterile saline vehicle. After overnight stimulation, the plasma supernatants were gently transferred to the polypropylene 96-well microplate and stored at −80°C until TNF-α assay.

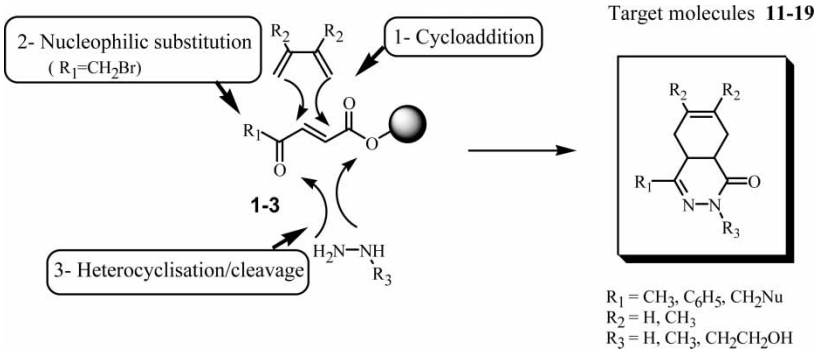
Measurement of TNF-α Release

Plasma TNF-α levels were estimated from diluted samples according to the supplier's instructions (R and D systems Europe, Abingdon, UK). A human TNF-α standard dose-response curve was run on each microplate to allow determination of TNF-α levels in samples. TNF-α concentrations in each sample were determined using an automatic reader plate associated with Genesis software (LabSystems Spectrophotometer, Cambridge, UK) and data were expressed in pg/ml and in percentage following calibration with a reference standard.

RESULTS AND DISCUSSION

Chemistry

The target 4a,5,8,8a-tetrahydrophthalazinon-1-ones **11**–**19** were synthesized on a solid support – Wang



SCHEME 1 General strategy for synthesis of the target compounds **11**–**19**.

resin- in two or three steps. As illustrated in Scheme 1, our strategy was to use cycloaddition of dienes on three polymer-bound dienophiles **1**–**3**. Subsequent treatment of **1** and **2** with substituted hydrazines provided, *via* a cyclisation-cleavage reaction, target phthalazinones **11**–**16**. In the case of phthalazinones **17**–**19** introduction of an amino group at the 4-position of the heterocycle was carried out by nucleophilic substitution, after cycloaddition step, using starting substrate **3** ($R_1 = CH_2Br$).

Synthesis of Polymer-bound Starting Dienophiles 1, 2 and 3

The synthetic strategy used for the preparation of **1** is summarized in Scheme 2. 4-Oxo-pent-2-enoic acid was prepared according to Porter *et al.*,[5] starting from levulinic acid (bromination and elimination of HBr) followed by anchoring to Wang resin under the conditions previously described.[7]

Resin **2** was easily prepared by coupling benzoyl-propionic acid to Wang resin followed by bromination using two equivalents of pyridine hydrobromide perbromide, in THF at 50°C, for 2 h. Treatment of the bromide intermediate with triethylamine afforded the expected compound **2** (Scheme 3).

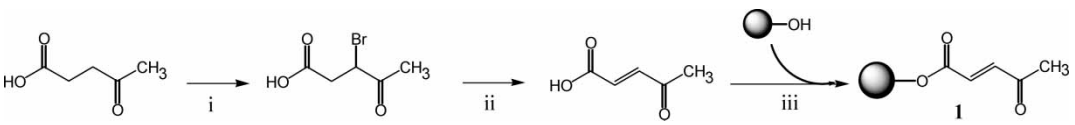
The best route for **3** starts from (*E*)-2,4-pentadienoic acid which can be obtained from acrolein by Knœvenagel condensation with malonic acid.[8] The reaction of diene with aqueous bromine followed by Jone's oxidation gave (*E*)-5-bromo-4-oxo-2-pentenoic acid, according to the method of Jahng and Kim.[9] Anchoring to Wang resin under the usual conditions afforded **3** (Scheme 4).

Synthesis of Phthalazinones 11–19 (Scheme 5)

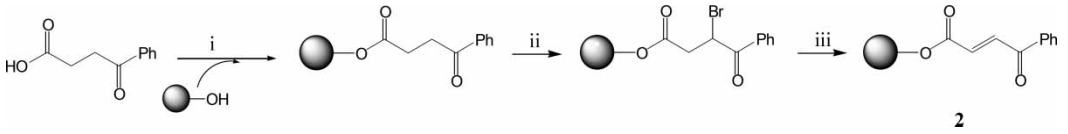
Once the starting materials **1**,**2** and **3** prepared, it was planned to build the cyclohexene ring and introduce diversity (R_2) *via* the Diels-Alder reaction. Thus thermal addition of dienophiles **1**–**3** with two symmetrical dienes (butadiene and 2,3-dimethyl-butadiene) provided the corresponding polymer bound cycloadducts **4**–**8**. Butadiene was generated *in situ* from thermal retroaddition of butadiene sulfone (3-sulfolene). For the synthesis of compounds **9** and **10**, bromo derivatives **7** and **8** were reacted with an excess of 1-(4-fluorophenyl)piperazine in DMSO. In the final step, treatment with diverse hydrazines (R_3 = H, CH_3 and CH_2CH_2OH) caused cyclisation with concomitant release of the desired phthalazinones **11**–**19** into the filtrate in moderate yield (30 to 60%) after purification.

Biology

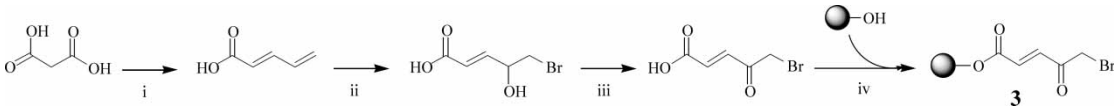
Overnight incubation of human whole blood with LPS induced a marked production of TNF-α, which is a multifunctional cytokine that mediates key roles in acute and chronic inflammation. Cyclic nucleotide PDEs are a family of enzymes that catalyse the degradation of cyclic purine nucleotides (cyclic AMP, cyclic GMP). At least eleven PDE families are currently defined.[2] Among them, cAMP specific isoenzymes, are expressed in macrophages,



SCHEME 2 Synthetic routes to polymer-supported starting material **1**. Reagents and conditions: (i) Bromine, conc. HCl, −15°C → r.t., 5 h; (ii) Sodium acetate, glacial acetic acid, r.t. then 100°C, 45 min; (iii) Wang resin, γ-ketoacid (2 eq.), DIC (2 eq.), DMAP (1%), DMF, r.t., 48 h.

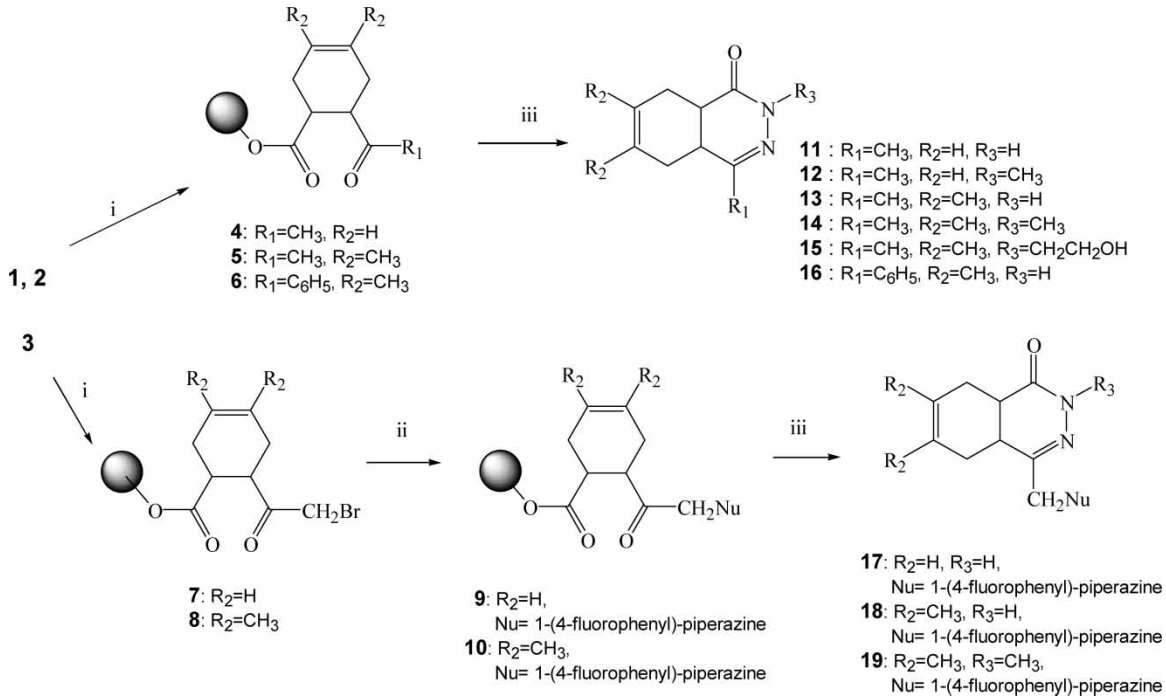


SCHEME 3 Synthetic routes to polymer-supported starting material **2**. Reagents and conditions: (i) Wang resin (0.65 mmol/g) (0.5 eq.), DIC (1 eq.), DMAP (1%), CH_2Cl_2, r.t., 48 h; (ii) Pyridinium tribromide (2 eq.), THF, 50°C, 2 h; (iii) NEt_3 (10 eq.), CH_2Cl_2, r.t., 1 h.



SCHEME 4 Synthetic routes to polymer-supported starting material **3**. Reagents and conditions: (i) Acrolein (1.25 eq.), pyridine, 80°C, 3 h; (ii) $NaHCO_3$ (3 eq.), Bromine (1 eq.), water, r.t., 2 h; (iii) CrO_3 (1.1 eq.), H_2SO_4, 0°C → r.t., 15 h; (iv) Wang resin (0.65 mmol/g), γ-ketoacid (2 eq.), DIC (2 eq.), DMAP (1%), CH_2Cl_2, r.t., 48 h.

neutrophils, CD8+ T cells and airway smooth muscle cells. During the last decade, numerous studies have demonstrated the modulation of inflammatory cell activation by selective PDE4 inhibitors. The fact that these compounds inhibit the hydrolysis of intracellular cyclic AMP may result in bronchodilation and suppression of inflammation. One of the major anti-inflammatory effect of PDE4 inhibitors is their ability to down-regulate LPS-induced TNF-α production. *In vivo*, some studies report the ability of PDE4 inhibitors to reduce TNF-α release in the blood or in BAL fluids of various species.[10–12] *In vitro*, as we have demonstrated in the present study, the selective PDE4 inhibitors, rolipram and roflumilast, have been previously shown to inhibit the release of TNF-α by LPS-stimulated murine mononuclear cells,[13–15] human monocytes or whole blood from healthy individuals.[16–23] Furthermore we have previously shown that PDE4 inhibitors, CI-1044, rolipram and cilomilast, inhibit LPS-stimulated TNF-α production in the whole blood from patients with COPD. [24] In the present study, we have not observed an inhibition of the LPS-induced TNF-α production with compounds **11**, **13** and **16**, at a concentration of 10μM. However the compounds **17** and **18**, in the same way as rolipram and roflumilast, inhibited the release of TNF-α in LPS-stimulated human whole blood from COPD patients. The rank order of inhibition of TNF-α release, induced by efficacious compounds, is



SCHEME 5 Synthetic routes to 4a,5,8,8a-tetrahydrophthalazinon-1-ones **11**–**19**. Reagents and conditions: (i) diene (10 eq.), toluene, 115°C; (ii) amine (10 eq.), DMSO, r.t., 2 h.; (iii) hydrazine (10 eq.), THF/EtOH (50/50), reflux 15 h.

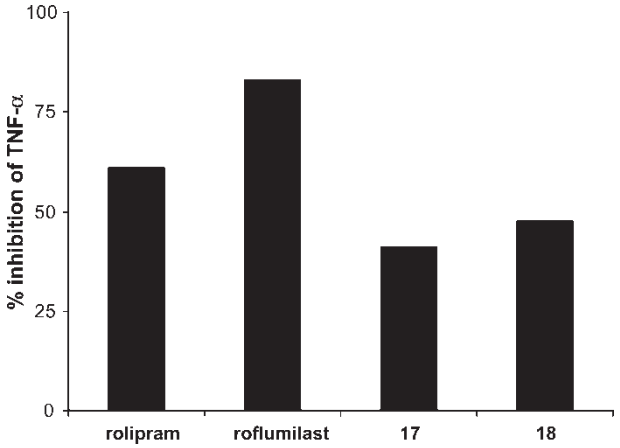


FIGURE 2 Effects of compounds **17**, **18** and PDE4 inhibitors, rolipram and roflumilast, (10 μM) on LPS-induced TNF-α release in whole blood from patient with COPD. Data were expressed in percentage of inhibition of TNF-α release and represent independent experiment in duplicate carried out in blood collected in 1 subject suffering from COPD.

roflumilast (82.9%) > rolipram (60.9%) >**18** (47.4%) > **17** (41.1%) (Figure 2).

In conclusion, although less active than the reference PDE4 inhibitors, the phthalazinones **17** and **18** could represent new lead compounds for further pharmacomodulation and evaluation of their *in vivo* activity in pathophysiological models; corresponding works will be reported in due course.

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