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Functionalized pyrazoles and pyrazolo[3,4-*d*]pyridazinones: Synthesis and evaluation of their phosphodiesterase 4 inhibitory activity

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ABSTRACT

A series of pyrazoles and pyrazolo[3,4-*d*]pyridazinones were synthesized and evaluated for their PDE4 inhibitory activity. All the pyrazoles were found devoid of activity, whereas some of the novel pyrazolo[3,4-*d*]pyridazinones showed good activity as PDE4 inhibitors. The most potent compounds in this series showed an IC_{50} in the nanomolar range. The ability to inhibit TNF-α release in human PBMCs was determined for two representative compounds, finding values in the sub-micromolar range. SARs studies demonstrated that the best arranged groups around the heterocyclic core are 2-chloro-, 2-methyl- and 3-nitrophenyl at position 2, an ethyl ester at position 4 and a small alkyl group at position 6. Molecular modeling studies performed on a representative compound allowed to define its binding mode to the PDE4B isoform.

1. Introduction

In recent years the interest for novel chemotypes of phosphodiesterase 4 (PDE4) inhibitors increased considerably due to their possible therapeutic applications in a wide range of inflammatory and immune disorders[1] like asthma[2], chronic obstructive pulmonary disease (COPD)[3–5], reumathoid arthritis[6], inflammatory bowel disease[7], multiple sclerosis[8] and so on. Moreover, very recently further therapeutic targets emerged since several studies have shown that selective PDE4 inhibitors are able to improve cognitive performances in a variety of assays performed in animal models.[9,10] The main part of PDE4 inhibitors is targeted to asthma and COPD, being these pulmonary diseases among the most common chronic disorders in advanced western countries. Both the pathologies are increasing in incidence and, namely for COPD, which is at the present the fifth cause of death in USA, there is an urgent need for therapeutic treatments.[11] None of the existing medications for COPD demonstrated to modify the long-term decline in lung function which is the hallmark of this disease. Therefore, the goal of COPD pharmacotherapy is to provide relief of symptoms, prevent complications and/or progression of the disease with a minimum of side effects.

Under this point of view, c-AMP elevation mediated by PDE4 inhibitors represents a promising single treatment both for COPD and asthma due to the antiinflammatory and bronchodilatory effect exerted by these agents. Interestingly in COPD patients Cilomilast **A**[12], which together Roflumilast **B**[13] (Fig. 1) is the most advanced PDE4 inhibitor in clinical trials (Phase III), showed evidence for an antiinflammatory and potentially disease modifying effect, which is completely absent in subjects treated with steroids, β_2 agonists and antimuscarinics.[14]

The potential of the approach based on PDE inhibition is clearly demonstrated by the recent success of several chemical entities targeting different PDE subtypes. Thus Cilostazol, which is a

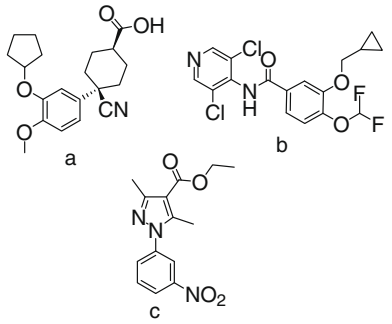

Figure 1. PDE4 inhibitors.

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selective PDE3 inhibitor, is used as peripheral vasodilator[15] and the selective PDE5 inhibitors Sildenafil, Tadalafil and Vardenafil are extensively prescribed for male erectile disfunction.[16] Among PDE4 inhibitors Ibudilast was approved in Japan for the treatment of asthma and stroke.[17]

On these grounds is not surprising the great interest of the pharmaceutical community for identifying novel chemical entities endowed with potent and selective PDE4 inhibitory activity.

As continuation of our previous synthetic efforts in PDE inhibitors area[18–22], we report here the synthesis and evaluation of a series of pyrazoles and pyrazolo[3,4-*d*]pyridazinones some of which are structurally related to compound **C**, belonging to a series of potent PDE4 inhibitors described by researchers from Plexxikon.[23]

2. Chemistry

All compounds were synthesized as reported in Schemes 1–6.

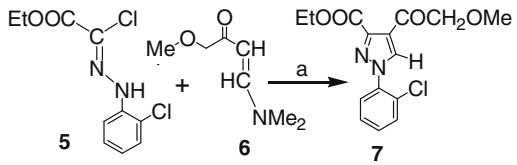
Scheme 1 shows the synthetic procedure affording pyrazoles **3a–e** and **4a–e**. The isoxazolo[3,4-*d*]pyridazinones **2a–e** (**2c**[24]) were prepared by condensing the precursors **1a–d** (**1a**[18], **1b,c**[25] and **1d**[26]) with the opportune arylaldehydes under standard conditions. Treatment of **2a–e** with 2 N NaOH in ethanol at 80 °C afforded pyrazoles **3a–e**[27] which were transformed into the corresponding pyrazoles **4a–e** by reduction with ammonium formate and Pd/C.

Compound **7** was obtained by reacting derivative **5**[28] and **6**[29] in anhydrous toluene under reflux (Scheme 2).

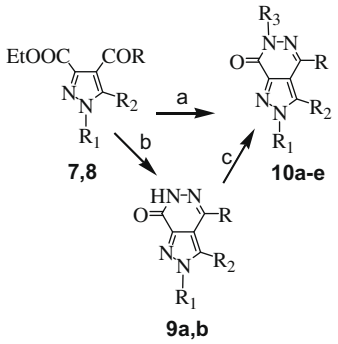
The pyrazolo[3,4-*d*]pyridazinones **10a–e** were obtained as depicted in Scheme 3. Pyrazoles **7** and **8**[30] were treated with hydrazine hydrate in ethanol affording the intermediates **9a,b**, which, were alkylated under standard conditions to give the final products **10a–d**. Compound **10e** was obtained by treatment of the precursor **8** with PPA and ethylhydrazine oxalate.

The synthesis of derivatives **14a–n** and **15a–c** (Scheme 4) was performed starting from the isoxazolo[3,4-*d*]pyridazinones **11a,b**[25,26], which were alkylated under standard conditions (compounds **1a** and **12a**[30], **12b**, **12c**[26] and **12d,e**) and in turn were transformed into the 4-nitro-5-acetyl derivatives **13a–f** (**13a**[18] and **13b**[31]) by oxidative cleavage of the isoxazole ring.[32] Treatment with opportune hydrazines in ethanol at room temperature afforded the final products **14a–n**. Further elaboration of **14d** by alkylation with appropriate arylalkyl halides in anhydrous acetone at 60 °C afforded the final pyrazolo[3,4-*d*]pyridazinones **15a–c**.

Modifications of COOEt group at position 4 of the pyridazine ring (Scheme 5) were performed starting from compounds **14e–g,j**. The intermediates carboxylic acids **16a–d**, obtained by hydrolysis of the corresponding esters, were converted into the secondary and tertiary amides **17a–f** through the intermediate acyl chloride. The synthesis of **18a,b** was carried out through the intermediates **17a,b**, which were transformed into the final 5-cyano derivatives **18a,b**, using $POCl_3$ as reagent. Finally, esterification in



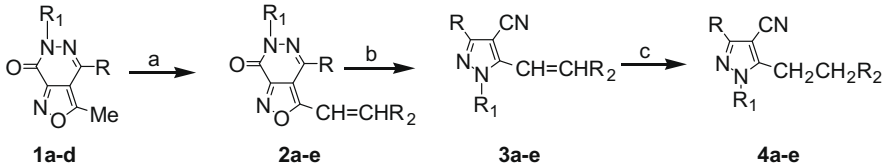
Scheme 2. Synthesis of pyrazole **7**. Reagents and conditions: (a) anhydrous toluene, Et_3N, reflux.



Compd	R	R_1	R_2
7, 9a	CH_2OMe	2-Cl-Ph	H
8, 9b	Me	3-NO_2-Ph	Me

10	R	R_1	R_2	R_3
a	CH_2OMe	2-Cl-Ph	H	Et
b	CH_2OMe	2-Cl-Ph	H	cyclopropylmethyl
c	Me	3-NO_2-Ph	Me	CH_2COOEt
d	Me	3-NO_2-Ph	Me	CH_2CH_2COOEt
e	Me	3-NO_2-Ph	Me	Et

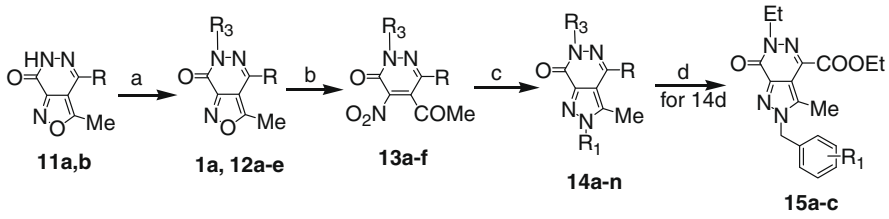
Scheme 3. Synthesis of 2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-ones **10a–e**. Reagents and conditions: (a) ethylhydrazine oxalate, PPA, 80–90 °C; (b) hydrazine hydrate, EtOH, rt; (c) R_3X, anhydrous DMF or MeCOMe, K_2CO_3, reflux.



1	R	R_1
a	Ph	Et
b	Me	Me
c	Ph	Me
d	4-pyridyl	Et

2-4	R	R_1	R_2
a	Ph	Et	α-naphthyl
b	Ph	Et	2-COOH-Ph
c	Me	Me	Ph
d	Ph	Me	3,4-OMe-Ph
e	4-pyridyl	Et	α-naphthyl

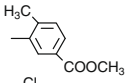
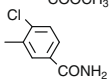
Scheme 1. Synthesis of pyrazoles **3a–e** and **4a–e**. Reagents and conditions: (a) R_2CHO, MeONa, abs MeOH, reflux; (b) NaOH 2 N, EtOH, 80 °C; (c) $HCOONH_4$, Pd/C, EtOH, reflux.



11	R
a	Ph
b	COOEt

Compd.	R	R_3
1a, 13a	Ph	Et
12a, 13b	Ph	CH_2COOEt
12b, 13c	Ph	CH_2CH_2COOEt
12c, 13d	COOEt	Et
12d, 13e	COOEt	n-propyl
12e, 13f	COOEt	cyclopropylmethyl

15	R_1
a	4-CN
b	4-NO_2
c	4-COOMe

14	R	R_1	R_3
a	Ph	3-NO_2-Ph	Et
b	Ph	3-NO_2-Ph	CH_2COOEt
c	Ph	3-NO_2-Ph	$(CH_2)_2COOEt$
d	COOEt	H	Et
e	COOEt	3-NO_2-Ph	Et
f	COOEt	Ph	Et
g	COOEt	2-Cl-Ph	Et
h	COOEt	3-CH_2OH-Ph	Et
i	COOEt	2-CH_3-Ph	Et
j	COOEt	2,6-Cl-Ph	Et
k	COOEt	3-NO_2-Ph	n-propyl
l	COOEt	2-Cl-Ph	cyclopropylmethyl
m	COOEt		Et
n	COOEt		Et

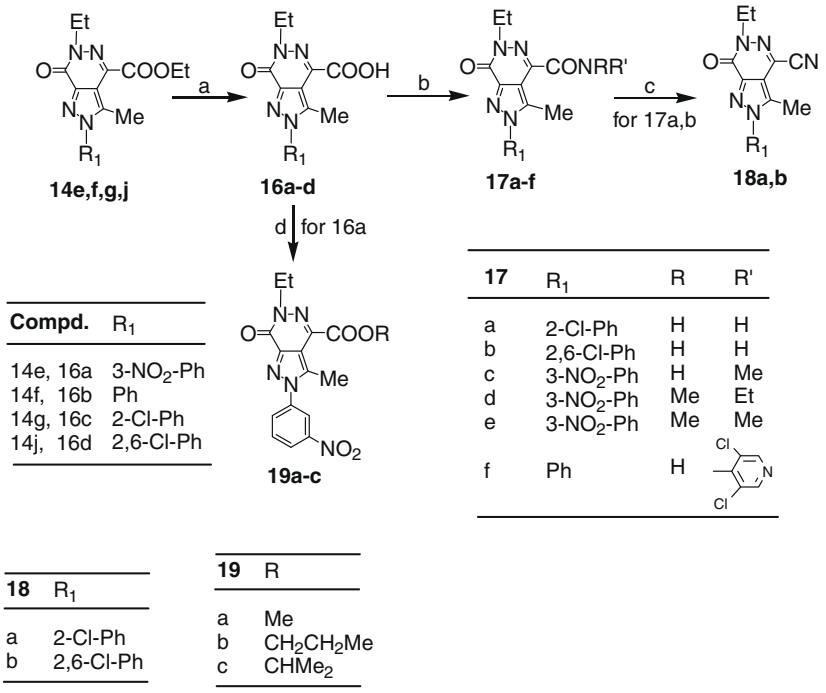
Scheme 4. Synthesis of 3-methyl-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-ones **14a–n** and **15a–c**. Reagents and conditions: (a) R_3X, anhydrous DMF or MeCOMe, K_2CO_3, reflux; (b) CAN, HNO_3, AcOH, 50/55 °C; (c) R_1NHNH_2, EtOH, rt; (d) arylalkyl halides, MeCOMe, K_2CO_3, 60 °C.

standard condition of the carboxylic acid **16a** afforded the final **19a–c**.

Finally, in Scheme 6 is reported a further elaboration of the function at position 4. The precursor **16c** was converted into the primary alcohol **20**, which in turn, was transformed into the ether **21** using NaH and MeI in DMF at room temperature. The final product **23**, bearing a oxadiazole ring at position 4, was synthesized through a three step procedure: treatment of the precursor **16a** with $SOCl_2$ followed by treatment of the crude chloride with acethydrazide (**22**) and cyclization with $POCl_3$. Elemental analyses of all new compounds are reported as Supplementary data.

3. Biological results and discussion

All compounds were evaluated for their ability to inhibit PDE4 from U-937 cells at 1 μM concentration. For the most part of compounds showing more then 50% inhibition at this concentration, dose–response curves were constructed to calculate IC_{50} and the results are reported in Tables 1 and 2. As showed in Table 1, all the pyrazole derivatives resulted inactive at the tested concentration, being only 35% at 1 μM the higher value of inhibition (compound **3a**). With the synthesis of pyrazolopyridazinones (Table 2) we realized bicyclic structures more closely related to the reference pyrazole discovered by Plexxikon (compound **C**). Among them the most potent compound (IC_{50} = 32 nM) was the ethyl ester **14g** (R_1 = 2-Cl, R_2 = Me, R_3 = Et). The analogue **14e** with 3-NO_2–C_6H_4 at position 2 was about twofold less potent. The corresponding methyl ester **19a** was slightly more potent (IC_{50} = 46 nM) suggesting that a minimum size for the ester function is an important requirement for the activity. Support for this hypothesis was done by the results obtained with the corresponding n-propyl and isopropyl esters (**19b** and **19c**, respectively) which proved completely devoid of activity. Replacement of COOEt in compound **14e** with the metabolically stable oxadiazole bioisoster (compound **23**) was associated with a strongly reduced activity (48% inhibition at 1 μM). Any other modification of the ester function provoked loss of activity: thus the carboxylic acid **16a** and the amides **17c–f**, as well as the nitriles **18a,b**, the primary alcohol **20** and the methyl ether **21** showed absence of activity or reduced activity (**21**). In any case the presence of polar and hydrogen-bond donor groups (COOH, CH_2OH, CONH-Me) in this part of the molecule was very detrimental for the activity. Moreover, the importance of a hydrogen-bond acceptor substituent (COOEt) was suggested by the present molecular modeling studies. In fact, the obtained data demonstrated that the replacement of ester function (**14g**) with the methyl ether (**21**) is associated with loss activity (IC_{50} = 32 nM and 41% inhibition at 1 μM, respectively). Replace-



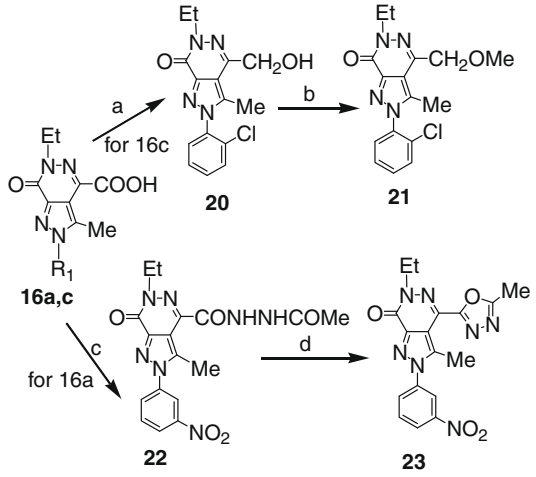
Compd.	R_1
14e, 16a	3-NO_2-Ph
14f, 16b	Ph
14g, 16c	2-Cl-Ph
14j, 16d	2,6-Cl-Ph

17	R_1	R	R'
a	2-Cl-Ph	H	H
b	2,6-Cl-Ph	H	H
c	3-NO_2-Ph	H	Me
d	3-NO_2-Ph	Me	Et
e	3-NO_2-Ph	Me	Me
f	Ph	H	

18	R_1
a	2-Cl-Ph
b	2,6-Cl-Ph

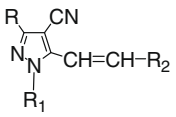
19	R
a	Me
b	CH_2CH_2Me
c	$CHMe_2$

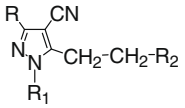
Scheme 5. Synthesis of 3-methyl-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-ones **17a–f**, **18a**,**b** and **19a–c**. Reagents and conditions: (a) NaOH, EtOH, rt; (b) $SOCl_2$, Et_3N, RR′NH, THF; (c) $POCl_3$; (d) RX, anhydrous DMF or MeCOMe, K_2CO_3, reflux.



Scheme 6. Synthesis of 3-methyl-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-ones **20**, **21** and **23**. Reagents and conditions: (a) $NaBH_4$, anhydrous THF, MeOH, 70 °C; (b) NaH, MeI, DMF, rt; (c) $SOCl_2$, Et_3N, acethydrazide; (d) $POCl_3$, 60 °C.

Table 1
PDE4 inhibitory activity of pyrazoles


3a-e


4a-e

	R	R_1	R_2	PDE4[a]
3a	Ph	Et	α-Naphtyl	35
3b	Ph	Et	2-(COOH)Ph	6
3c	Me	Me	Ph	1.3
3d	Ph	Me	3,4-OCH_3-Ph	9
3e	4-pyridyl	Et	α-Naphtyl	19
4a	Ph	Et	α-Naphtyl	-3
4b	Ph	Et	2-(COOH)Ph	11
4c	Me	Me	Ph	2.1
4d	Ph	Me	3,4-OCH_3-Ph	14
4e	4-Pyridyl	Et	α-Naphtyl	2.8

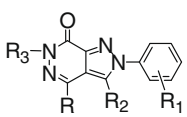
[a] Data are indicated as inhibition percentage at 1 μM concentration.

ment of COOEt with a lipophilic methyl group resulted in compound **10e** (IC_{50} = 385 nM). Despite an order of magnitude reduction of activity, this finding suggests that the introduction of a small alkyl group at position 4 may be an useful approach to overcome the possible metabolic instability of the corresponding esters. Moreover, this result could be interpreted to indicate a different binding mode of these compounds with catalytic site. Moderate activity (47% inhibition at 1 μM) was also found for the analogue **14a** with a phenyl group at position 4.

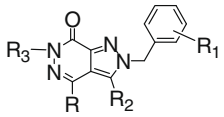
The best substituents at R_3 were ethyl and *n*-propyl. In fact compounds **14e** and **14k** proved equipotent (IC_{50} = 75 and 72 nM, respectively). By comparing the 2-(2-chlorophenyl) derivatives **14g** and **14l** it emerged that replacement of ethyl with cyclopropylmethyl was associated with 1 order of magnitude reduction of activity, suggesting that a small alkyl group is the best arranged in position 6. For compounds substituted at position 4 with Me, the presence of ester function at R_3 (**10c,d**) gave products showing moderate activity (compound **10c**, R_3 = CH_2COOEt, 64% inhibition at 1 μM) or inactivity (**10d**, R_3 = $(CH_2)_2COOEt$). A similar trend was also observed with compounds bearing Ph at position 4, where **14a**, bearing Et at R_3 was more active that **14b** (R_3 = CH_2COOEt) and **14c** (R_3 = $(CH_2)_2COOEt$).

The best substituents in the phenyl group appended at position 2 were 2-chloro and 3-nitro groups. Isosteric replacement of Cl in compound **14g** (IC_{50} = 32 nM) with Me gave a compound (**14i**) showing a slightly reduced activity (IC_{50} = 59 nM). Introduction of a second Cl in position 6 of the phenyl (**14j**) was associated with a strongly reduced activity (IC_{50} = 237 nM). A similar effect was observed for compounds **14m** and **14n** where a second substituent

Table 2
PDE4 inhibitory activity of pyrazolopyridazinones



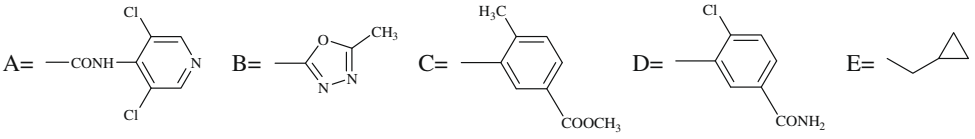
10a-e, 14a-n, 16-19, 20, 21, 23



15a-c

Compd	R	R_1	R_2	R_3	PDE4[a]
10a	CH_2OCH_3	2-Cl	H	Et	65 ± 7%
10b	CH_2OCH_3	2-Cl	H	E[b]	19 ± 7%
10c	CH_3	3-NO_2	CH_3	CH_2COOEt	64.1 ± 7.5%
10d	CH_3	3-NO_2	CH_3	$(CH_2)_2COOEt$	4 ± 3%
10e	CH_3	3-NO_2	CH_3	Et	385
14a	Ph	3-NO_2	CH_3	Et	47 ± 12%
14b	Ph	3-NO_2	CH_3	CH_2COOEt	1 ± 4%
14c	Ph	3-NO_2	CH_3	$(CH_2)_2COOEt$	1 ± 0%
14e	COOEt	3-NO_2	CH_3	Et	75
14f	COOEt	H	CH_3	Et	300
14g	COOEt	2-Cl	CH_3	Et	32
14h	COOEt	3-CH_2OH	CH_3	Et	39 ± 9%
14i	COOEt	2-CH_3	CH_3	Et	59
14j	COOEt	2,6-Cl	CH_3	Et	237
14k	COOEt	3-NO_2	CH_3	*n*-Propyl	72
14l	COOEt	2-Cl	CH_3	E[b]	374
14m	COOEt	C[b]	CH_3	Et	876
14n	COOEt	D[b]	CH_3	Et	757
15a	COOEt	4-CN	CH_3	Et	5% (10 μM)
15b	COOEt	4-NO_2	CH_3	Et	1140
15c	COOEt	4-$COOCH_3$	CH_3	Et	1200
16a	COOH	3-NO_2	CH_3	Et	2 ± 5%
17c	$CONHCH_3$	3-NO_2	CH_3	Et	14 ± 4%
17d	$CON(CH_3)CH_2CH_3$	3-NO_2	CH_3	Et	−2 ± 3%
17e	$CON(CH_3)_2$	3-NO_2	CH_3	Et	4 ± 4%
17f	A[b]	H	CH_3	Et	3 ± 3%
18a	CN	2-Cl	CH_3	Et	−2 ± 9%
18b	CN	2,6-Cl	CH_3	Et	2 ± 13%
19a	$COOCH_3$	3-NO_2	CH_3	Et	46 M
19b	$COO(CH_2)_2CH_3$	3-NO_2	CH_3	Et	14 ± 4%
19c	$COOCH(CH_3)_2$	3-NO_2	CH_3	Et	6 ± 6%
20	CH_2OH	2-Cl	CH_3	Et	−4 ± 1%
21	CH_2OCH_3	2-Cl	CH_3	Et	41 ± 1%
23	B[b]	3-NO_2	CH_3	Et	48 ± 5%

[a] Data are indicated as IC_{50} (nM) or inhibition percentage at 1 μM concentration or at indicated concentration (μM).
[b]



was inserted in the aromatic ring. Finally, elimination of the substituent (compound **14f**) provoked a 10-fold reduction of potency. Thus keeping Me at R_2, COOEt at R and Et at R_3 for the substituent R_1 the following order of efficacy can be established: 2-Cl > 2-CH_3 > 3-NO_2 > 2,6-Cl > H.

Introduction of a methylenic spacer between the aromatic portion and the pyrazole was detrimental for the activity. In fact all the synthesized benzyl derivatives (**15a–c**) were significantly less potent with respect the phenyl analogues, being their IC_{50}, at the best, in the low micromolar range.

As regards R_2 there is only a couple of compounds that may furnish an indication on the role played by the methyl group: thus by comparing compound **21** (41% inhibition at 1 μM) and its nor derivative **10a** (62.9% inhibition at 1 μM) it seems that in this part of the molecule the presence of an hydrogen is better that a methyl group.

Since the inhibition of PDE4 leads to an increase in intracellular cAMP which, in turn, inhibits the release of inflammatory mediators such as TNF-α,[33] we decided to test two selected compounds (**14e** and **14i**) for their ability to inhibit TNF-α release in human peripheral blood mononuclear cells (PBMCs). Both compounds exhibited high activity being their IC_{50} = 383 (170–861) and 127 nM (38–424), respectively (confidence limits in brackets).

Finally, in view of a possible development of these compounds, we examined their drug-like properties following the Lipinski's rule of five.[34] We found that as **14e** and **14i** meet the requirements of this rule being HBD <5, HBA <10, MW <500 and log *P* <5. Evaluation of lipophilicity was performed by measuring log *P* with Molinspiration Free log *P* calculator.[35] The following values were found: 2.263 for **14e** and 2.729 for **14i**.

4. Molecular modeling studies

In order to explain the high potency of the ethyl ester **14g** (IC_{50} = 32 nM) compared to the other less active inhibitors bearing different groups in position 4, the binding mode of **14g** was stud-

ied, using as catalytic site the 1XM4 complex structure of Piclamilast bound to the PDE4B isoform.

Besides the six water molecules directly linked to the metals, an additional water molecule was retained in the hydrophobic clamp, where the selective Gln443 side chain forms important hydrogen bonds with the inhibitors.[36]

Several poses of **14g** were generated in Glide,[37] using the extra precision mode and imposing as docking constraint the hydrogen bond with the Gln443 residue.

In the proposed binding mode of **14g** (Fig. 2) the ethyl ester group sits in a small binding pocket and forms two hydrogen bonds with the Gln443 side chain and with a water molecule. Another hydrogen bond is formed between the nitrogen atom in position 5 of the core structure and the Gln443 side chain. This particular pose of **14g** explains the loss of activity when the ethyl ester is replaced by bulkier groups.

Additional binding energy is found by the 2-chlorophenyl group in position 2, which interacts with the His234, Ile410 and Phe414 residues through significant Van der Waals contacts.

5. Conclusions

In conclusion only the series of pyrazolopyridazinones showed interesting activity; the best arranged groups around the heterocyclic core are 2-chloro-, 2-methyl- and 3-nitrophenyl at position 2, a ethyl ester at position 4 and a small alkyl group (ethyl, *n*-propyl) at position 6. As regards position 3 a more in depth exploration of this part of the molecule is necessary and studies in this sense are in progress.

6. Experimental section

6.1. Chemistry

All melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Avance 400 instruments (Bruker Biospin Version 002 with SGU). Chemical shifts are reported in ppm, using the solvent as internal standard. Infrared spectra (IR) were recorded as Nujol mulls with a Perkin–Elmer spectrometer. Mass spectra (*m/z*) were recorded on a LTQ mass spectrometer (ThermoFisher, San Jose, CA). Extracts were dried over Na_2SO_4 and the solvents were removed under reduced pressure. Merck F-254 commercial plates were used for analytical TLC to follow the course of reaction. Silica Gel 60 (Merck 70–230 mesh) was used for column chromatography.

6.1.1. General Procedure for 2a,b,d,e

A mixture of isoxazolopyridazinones **1a–c**[18,25,26] (**1a**[18], **1b**[25], **1c**[26]) (0.4–0.6 mmol), the appropriate aryl aldehyde (0.4–0.7 mmol), and sodium methoxide (0.5–1 mmol) in anhydrous methanol was heated for 5–10 min at 60–70 °C. After the mixture was cooled, the precipitate was recovered by suction. For compound **2b**, after cooling, 6 N HCl was added, the precipitate recovered by suction and purified by column chromatography using dichlomethane/methanol/acetic acid 9:1:0.1. For compound **2e**, to a mixture of **1d**[26] in absolute EtOH (1 mL), 1-naphthaldehyde (1 mmol), and piperidine (0.5 mL) was added. The mixture was stirred in a sealed tube at 110 °C for 6 h. After evaporation of solvent, ice-water was added and compound **2e** was recovered by suction.

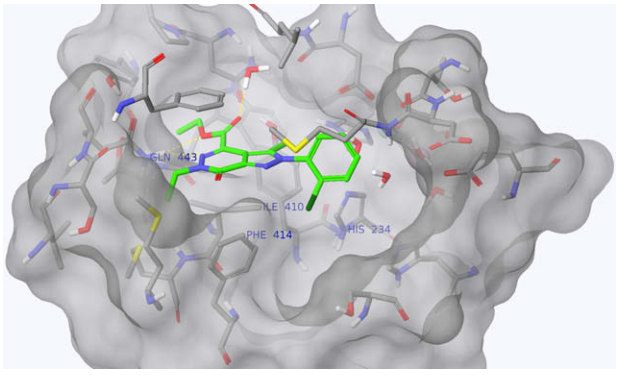


Figure 2. Docking of the potent inhibitor **14g** (IC_{50} = 32 nM) into the PDE4B catalytic binding pocket.

6.1.1.1. 6-Ethyl-3-((*E*)-2-(naphthalen-1-yl)vinyl)-4-phenylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one 2a. Yield = 52%; mp = 195–196 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H, NCH_2CH_3), 4.35 (q, 2H, NCH_2CH_3), 6.90 (d, 1H, CH=*CH*Ar), 7.40–7.70 (m, 9H, Ar), 7.90 (m, 2H, Ar), 8.20 (d, 1H, Ar), 8.50 (d, 1H, *CH*=CHAr).

6.1.1.2. 2-((1*E*)-2-(6-ethyl-6,7-dihydro-7-oxo-4-phenylisoxazolo[3,4-*d*]pyridazin-3-yl)vinyl)benzoic acid 2b. Yield = 40%; mp = 189–192 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, NCH_2CH_3), 4.31 (q, 2H, NCH_2CH_3), 6.70 (d, 1H, CH=*CH*Ar), 7.32 (d, 1H, Ar), 7.45–7.68 (m, 7H, Ar), 8.10 (d, 1H, Ar), 8.58 (d, 1H, *CH*=CHAr).

6.1.1.3. 3-(3,4-dimethoxystyryl)-6-methyl-4-phenylisoxazolo-[3,4-*d*]pyridazin-7(6*H*)-one 2d. Yield = 63%; mp = 234–235 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.95 (s, 3H, NCH_3), 6.60 (d, 1H, CH=*CH*Ar), 6.78 (s, 1H, Ar), 6.87 (d, 1H, Ar), 6.99 (d, 1H, Ar), 7.53–7.67 (m, 6H, (5H, Ar; 1H, *CH*=CHAr)).

6.1.1.4. 6-Ethyl-3-((*E*)-2-(naphthalen-1-yl)vinyl)-4-(pyridin-4-yl)isoxazolo[3,4-*d*]pyridazin-7(6*H*)-one 2e. Yield = 61%; mp = 222–224 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.48 (t, 3H, NCH_2CH_3), 4.35 (q, 2H, NCH_2CH_3), 6.94 (d, 1H, CH=*CH*Ar), 7.45–7.69 (m, 6H, (4H, Ar; 2H, Py)), 7.93 (t, 2H, Ar), 8.21 (d, 1H, Ar), 8.59 (d, 1H, *CH*=CHAr), 8.89 (d, 2H, Py).

6.1.2. General procedure for 3a–e

A mixture of appropriate isoxazolo[3,4-*d*]pyridazinones **2a–e** (**2c**[24]) (0.25–0.45 mmol), 2 N NaOH (2–4 mL) in ethanol (1.5–5 mL) was refluxed under stirring for 2–3 h. Then the mixture was concentrated in vacuo and diluted with cold water, and the precipitate was recovered by suction. For compound **3b**, after dilution with cold water, 6 N HCl was added to the mixture and the precipitate was purified by column chromatography using dichlomethane/methanol/acetic acid 9:1:0.1.

6.1.2.1. 1-Ethyl-5-((*E*)-2-(naphthalen-1-yl)vinyl)-3-phenyl-1*H*-pyrazole-4-carbonitrile 3a. Yield = 34%; mp = 139–140 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.58 (t, 3H, NCH_2CH_3), 4.38 (q, 2H, NCH_2CH_3), 7.05 (d, 1H, CH=*CH*Ar), 7.45–7.60 (m, 5H, Ar), 7.65 (t, 1H, Ar), 7.82 (d, 1H, Ar), 7.92 (d, 2H, Ar), 8.05 (d, 2H, Ar), 8.28 (d, 1H, Ar), 8.60 (d, 1H, *CH*=CHAr).

6.1.2.2. 2-((*E*)-2-(4-Cyano-1-ethyl-3-phenyl-1*H*-pyrazol-5-yl)vinyl)benzoic acid 3b. Yield = 20%; mp = 178–181 °C; IR (Nujol): 1740 (CO), 2200 (CN), 960 (trans CH=CH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.55 (t, 3H, NCH_2CH_3), 4.37 (q, 2H, NCH_2CH_3), 6.85 (d, 1H, CH=*CH*Ar), 7.40–7.51 (m, 4H, Ar), 7.66 (t, 1H, Ar), 7.72 (d, 1H, Ar), 8.01 (d, 2H, Ar), 8.18 (d, 1H, Ar), 8.52 (d, 1H, *CH*=CHAr); MS (ESI): *m/z* 343.38 $[M+H]^+$.

6.1.2.3. 1,3-Dimethyl-5-styryl-1*H*-pyrazole-4-carbonitrile 3c. Yield = 24%; mp = 115 °C (EtOH); IR (Nujol): 2225 (CN), 960 (trans CH=CH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, CCH_3), 3.90

(s, 2H, NCH_3), 6.88 (d, 1H, CH=*CH*Ar), 7.35–7.45 (m, 3H, Ar), 7.54–7.63 (m, 3H, (2H, Ar; 1H, *CH*=CHAr)); MS (ESI): *m/z* 223.27 $[M+H]^+$.

6.1.2.4. 5-(3,4-Dimethoxystyryl)-1-methyl-3-phenyl-1*H*-pyrazole-4-carbonitrile 3d. Yield = 50%; mp = 157–158 °C (EtOH); ^1H NMR ($CDCl_3$) δ 3.87 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.03 (s, 3H, NCH_3), 6.80 (d, 1H, CH=*CH*Ar), 6.92 (d, 1H, Ar), 7.09 (s, 1H, Ar), 7.20 (d, 1H, Ar), 7.40–7.55 (m, 3H, Ar), 7.63 (d, 1H, *CH*=CHAr), 8.00 (d, 2H, Ar).

6.1.2.5. 1-Ethyl-5-((*E*)-2-(naphthalen-1-yl)vinyl)-3-(pyridin-4-yl)-1*H*-pyrazole-4-carbonitrile 3e. Yield = 38%; mp = 238–240 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.60 (t, 3H, NCH_2CH_3), 4.42 (q, 2H, NCH_2CH_3), 7.03 (d, 1H, CH=*CH*Ar), 7.58 (q, 2H, Ar), 7.65 (t, 1H, Ar), 7.83 (d, 1H, Ar), 7.92 (t, 2H, Ar), 8.00 (d, 2H, Py), 8.28 (d, 1H, Ar), 8.60 (d, 1H, *CH*=CHAr), 8.78 (d, 2H, Py).

6.1.3. General procedure for 4a–e

A mixture of appropriate pyrazoles **3a–e** (0.26–0.50 mmol), 10% Pd/C (30–50 mg), and ammonium formate (0.6–1.2 mmol) in EtOH (2–3 mL) was refluxed for 1–2 h. After the mixture was cooled, CH_2Cl_2 (5 mL) was added, the catalyst was filtered off, and the solvent was evaporated in vacuo to afford **4a–e**.

6.1.3.1. 1-Ethyl-5-(2-(naphthalen-1-yl)ethyl)-3-phenyl-1*H*-pyrazole-4-carbonitrile 4a. Yield = 44%; mp = 95–97 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.23 (t, 3H, NCH_2CH_3), 3.28 (t, 2H, CH_2CH_2Ar), 3.55 (t, 2H, CH_2CH_2Ar), 3.76 (q, 2H, NCH_2CH_3), 7.38–7.60 (m, 7H, Ar), 7.80 (d, 1H, Ar), 7.90 (d, 1H, Ar), 8.00 (d, 2H, Ar), 8.10 (d, 1H, Ar).

6.1.3.2. 2-(-2-(4-Cyano-1-ethyl-3-phenyl-1*H*-pyrazol-5-yl)ethyl) benzoic acid 4b. Yield = 33%; mp = 94–98 °C (EtOH); IR (Nujol): 1740 (CO), 2210 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (t, 3H, NCH_2CH_3), 3.20 (t, 2H, CH_2CH_2Ar), 3.40 (t, 2H, CH_2CH_2Ar), 4.13 (q, 2H, NCH_2CH_3), 7.30–7.60 (m, 6H, Ar), 7.96 (d, 2H, Ar), 8.12 (d, 1H, Ar); MS (ESI): *m/z* 345.39 $[M+H]^+$.

6.1.3.3. 1,3-Dimethyl-5-phenethyl-1*H*-pyrazole-4-carbonitrile 4c. Yield = 41%; oil; IR (Nujol): 2220 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.33 (s, 3H, CCH_3), 3.00 (m, 4H, (2H, CH_2CH_2Ar; 2H, CH_2CH_2Ar)), 3.40 (s, 3H, NCH_3), 7.09 (m, 2H, Ar), 7.28 (m, 3H, Ar); MS (ESI): *m/z* 225.29 $[M+H]^+$.

6.1.3.4. 5-(3,4-Dimethoxyphenethyl)-1-methyl-3-phenyl-1*H*-pyrazole-4-carbonitrile 4d. Yield = 30%; mp = 62–64 °C (EtOH); 2220 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.00 (t, 2H, CH_2CH_2Ar), 3.12 (t, 2H, CH_2CH_2Ar), 3.52 (s, 3H, NCH_3), 3.82 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.58 (s, 1H, Ar), 6.68 (d, 1H, Ar), 6.83 (d, 1H, Ar), 7.40–7.50 (m, 3H, Ar), 7.97 (d, 2H, Ar).

6.1.3.5. 1-Ethyl-5-(2-(naphthalen-1-yl)ethyl)-3-(pyridin-4-yl)-1*H*-pyrazole-4-carbonitrile 4e. Yield = 78%; mp = 135–138 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.22 (t, 3H, NCH_2CH_3), 3.30 (t, 2H, CH_2CH_2Ar), 3.57 (t, 2H, CH_2CH_2Ar), 3.73 (q, 2H, NCH_2CH_3), 7.21 (d, 1H, Ar), 7.39 (1H, Ar), 7.50–7.62 (m, 2H, Ar), 7.82 (d, 1H, Ar), 7.93 (d, 1H, Ar), 8.06 (d, 3H, (1H, Ar; 2H, Py)), 8.77 (d, 2H, Py).

6.1.4. Ethyl-1-(2-chlorophenyl)-4-(2-methoxyacetyl)-1*H*-pyrazole-3-carboxylate 7

A mixture of the compound **6**[28] (2.94 mmol) in anhydrous toluene (6 mL), Et_3N (3.6 mmol) and compound **5**[27] (2.95 mmol) was refluxed under stirring for 4 h. The reaction mixture was concentrated in vacuo, diluted with cold water (10 mL) and neutralized with 6 N HCl (10 mL). The suspension was extracted with ethyl acetate (3 × 15 mL) and the solvent was evaporated to afford **7**, which was purified by column chromatography using cyclohexane/ethyl acetate, 1:1, as eluent. Yield = 21%; mp = 66–68 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.44 (t, 3H, OCH_2CH_3), 3.46 (s, 3H, OCH_3), 4.47 (q, 2H, OCH_2CH_3), 4.54 (s, 2H, $COCH_2OCH_3$), 7.43 (m, 2H, Ar), 7.56 (m, 1H, Ar), 7.62 (m, 1H, Ar), 8.40 (s, 1H, Ar).

6.1.5. General procedure for 9a,b

A suspension of **7** or **8**[29] (0.35–0.37 mmol) in absolute ethanol (3–4 mL) and hydrazine hydrate (3–6 mmol) was stirred at room temperature for 1–2 h. Then the mixture was cooled for 1 h and the precipitate was recovered by suction.

6.1.5.1. 2-(2-Chlorophenyl)-4-(methoxymethyl)-2*H*-pyrazolo-[3,4-*d*]pyridazin-7(6*H*)-one 9a. Yield = 74%; mp = 193–194 °C (EtOH); ^1H NMR (DMSO-d_6) 3.35 (s, 3H, CH_2OCH_3), 4.53 (s, 2H, CH_2OCH_3), 7.58–7.69 (m, 2H, Ar), 7.79 (t, 2H, Ar), 8.92 (s, 1H, Ar), 12.39 (exch br s, 1H, NH).

6.1.5.2. 3,4-Dimethyl-2-(3-nitrophenyl)-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-one 9b. Yield = 81%; mp = >300 °C (EtOH); ^1H NMR (DMSO-d_6) 2.50 (s, 3H, 4-CH_3), 2.70 (s, 3H, 3-CH_3), 7.90 (t, 1H, Ar), 8.15 (d, 1H, Ar), 8.46 (m, 2H, Ar), 12.10 (exch br s, 1H, NH).

6.1.6. General procedure for 10a–d

A mixture of **9a** or **9b** (0.14–0.28 mmol), K_2CO_3 (0.29–0.56 mmol), and the appropriate (cyclo)alkyl halide (0.2–0.48 mmol) in anhydrous acetone (4 mL) for **10a,b** or anhydrous DMF (1.5 mL) for **10c,d** was refluxed under stirring for 2–8 h. For compounds **10a,b**, the reaction mixture was concentrated in vacuo and diluted with cold water, and the precipitate **10a** was recovered by suction. For compound **10b**, the suspension was extracted with ethyl acetate (3 × 15 mL) and evaporation of the solvent afforded an oil as final product. For compounds **10c,d**, after cooling, the suspension was diluted with cold water and the precipitates were recovered by suction. Compound **10d** was purified by column chromatography using $CHCl_3$/MeOH, 9.5:0.5, as eluent.

6.1.6.1. 2-(2-Chlorophenyl)-6-ethyl-4-(methoxymethyl)-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-one 10a. Yield = 73%; mp = 58–60 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.41 (t, 3H, NCH_2CH_3), 3.44 (s, 3H, CH_2OCH_3), 4.29 (q, 2H, NCH_2CH_3), 4.62 (s, 2H, CH_2OCH_3), 7.46 (m, 2H, Ar), 7.59 (m, 1H, Ar), 7.71 (m, 1H, Ar), 8.46 (s, 1H, Ar).

6.1.6.2. 2-(2-Chlorophenyl)-6-(cyclopropylmethyl)-4-(methoxymethyl)-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-one 10b. Yield = 42%; oil; ^1H NMR ($CDCl_3$) δ 0.45–0.55 (m, 4H, cC_3H_5), 1.38–1.46 (m, 1H, cC_3H_5), 3.46 (s, 3H, CH_2OCH_3), 4.12 (d, 2H, NCH_2), 4.63 (s, 2H, CH_2OCH_3), 7.48 (m, 2H, Ar), 7.60 (m, 1H, Ar), 7.74 (m, 1H, Ar), 8.47 (s, 1H, Ar).

6.1.6.3. Ethyl 2-(3,4-dimethyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazin-6(7*H*)-yl)acetate 10c. Yield = 58%; mp = 189–190 °C (EtOH); IR (Nujol): 1755 (CO), 1690 (CO), 1520 and 1350 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (t, 3H, $COOCH_2CH_3$), 2.60 (s, 3H, 4-CH_3), 2.78 (s, 3H, 3-CH_3), 4.25 (q, 2H, $COOCH_2CH_3$), 4.95 (s, 2H, NCH_2), 7.80 (t, 1H, Ar), 7.96 (m, 1H, Ar), 8.43 (m, 2H, Ar); MS (ESI): *m/z* 371,35 $[M+H]^+$.

6.1.6.4. Ethyl 3-(3,4-dimethyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazin-6(7*H*)-yl)propanoate 10d. Yield = 33%; mp = 175–176 °C (EtOH); IR (Nujol): 1750 (CO), 1690 (CO), 1530 and 1360 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (t, 3H, $COOCH_2CH_3$), 2.60 (s, 3H, 4-CH_3), 2.75 (s, 3H, 3-CH_3), 2.85 (t, 2H, NCH_2CH_2), 4.20 (q, 2H, $COOCH_2CH_3$), 4.53 (t, 2H, NCH_2CH_2), 7.80 (t, 1H, Ar), 7.95 (m, 1H, Ar), 8.43 (m, 2H, Ar); MS (ESI): *m/z* 385.37 $[M+H]^+$.

6.1.6.5. 6-Ethyl-3,4-dimethyl-2-(3-nitrophenyl)-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-one 10e. Compound **10e** was obtained starting from **8** treating with PPA and ethylhydrazine oxalate at 80 °C for 1 h. After cooling, cold water was added and the precipitate was recovered by suction. Yield = 61%; mp = 215–216 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, NCH_2CH_3), 2.60 (s, 3H, 4-CH_3), 2.77 (s, 3H, 3-CH_3), 4.28 (q, 2H, NCH_2CH_3), 7.80 (t, 1H, Ar), 7.98 (d, 1H, Ar), 8.43 (m, 2H, Ar).

6.1.7. General procedure for 12b,d,e

Compounds **12b,d,e** were obtained starting from **11a**[25],**b**[26] following the same procedure described for **10a–d**. For compound **12b**, the reaction was carried out in anhydrous DMF and the suspension was diluted with cold water and extracted with CH_2Cl_2 (3 × 15 mL) to afford the final product. For compounds **12d,e** anhydrous acetone was used as solvent and the precipitate **12d** was recovered by suction. Compound **12e** was obtained after extraction with ethyl acetate (3 × 15 mL) and evaporation in vacuo.

6.1.7.1. Ethyl 3-(3-methyl-7-oxo-4-phenylisoxazolo[3,4-*d*]pyridazin-6(7*H*)-yl)propanoate 12b. Yield = 75%; mp = 94–96 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, $COOCH_2CH_3$), 2.60 (s, 3H, 3-CH_3), 2.90 (t, 2H, NCH_2CH_2), 4.10 (q, 2H, $COOCH_2CH_3$), 4.55 (t, 2H, NCH_2CH_2), 7.55 (s, 5H, Ar).

6.1.7.2. Ethyl 6,7-dihydro-3-methyl-7-oxo-6-propylisoxazolo[3,4-*d*]pyridazine-4-carboxylate 12d. Yield = 45%; mp = 66–67 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.00 (t, 3H, $NCH_2CH_2CH_3$), 1.46 (t, 3H, $COOCH_2CH_3$), 1.86 (m, 2H, $NCH_2CH_2CH_3$), 3.00 (s, 3H, 3-CH_3), 4.20 (t, 2H, $NCH_2CH_2CH_3$), 4.48 (q, 2H, $COOCH_2CH_3$).

6.1.7.3. Ethyl 6-(cyclopropylmethyl)-6,7-dihydro-3-methyl-7-oxoisoxazolo[3,4-*d*]pyridazine-4-carboxylate 12e. Yield = 48%; mp = 86–87 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 0.61 (m, 4H, cC_3H_5), 1.36 (m, 1H, cC_3H_5), 1.47 (t, 3H, $COOCH_2CH_3$), 3.04 (s, 3H, 3-CH_3), 4.12 (t, 2H, NCH_2), 4.49 (q, 2H, $COOCH_2CH_3$).

6.1.8. General Procedure for 13c–f

To a suspension of **12b–e** (**12c**[26]) (0.8–1.08 mmol), AcOH 50% (7 mL) and HNO_3 65% (1.5–2 mL) heated at 50–60 °C, CAN (6.38 mmol) was added portionwise. The mixture was heated for 1–2 h at 60 °C. After dilution with cold water (10–15 mL) the suspension was extracted with CH_2Cl_2 (2 × 15 mL); evaporation of the solvent afforded compounds **13c,e,f**. Compound **13d** was recovered by suction.

6.1.8.1. Ethyl 3-(4-acetyl–5-nitro-6-oxo-3-phenylpyridazin-1(6*H*)-yl)propanoate 13c. Yield = 67%; oil; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, $COOCH_2CH_3$), 2.15 (s, 3H, $COCH_3$), 2.95 (t, 2H, NCH_2CH_2), 4.15 (q, 2H, $COOCH_2CH_3$), 4.65 (t, 2H, NCH_2CH_2), 7.45 (m, 5H, Ar).

6.1.8.2. Ethyl 4-acetyl-1-ethyl-1,6-dihydro-5-nitro-6-oxopyridazine-3-carboxylate 13d. Yield = 53%; mp = 90–91 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.40 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.61 (s, 3H, $COCH_3$), 4.41 (m, 4H, (2H $COOCH_2CH_3$; 2H, NCH_2CH_3)).

6.1.8.3. Ethyl 4-acetyl-1,6-dihydro-5-nitro-6-oxo-1-propylpyridazine-3-carboxylate 13e. Yield = 47%; mp = 58–60 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.00 (t, 3H, $NCH_2CH_2CH_3$), 1.40 (t, 3H, $COOCH_2CH_3$), 1.80–1.95 (m, 2H, $NCH_2CH_2CH_3$), 2.60 (s, 3H, $COCH_3$), 4.30 (t, 2H, $NCH_2CH_2CH_3$), 4.42 (q, 2H, $COOCH_2CH_3$).

6.1.8.4. Ethyl 4-acetyl-1-(cyclopropylmethyl)-1,6-dihydro-5-nitro-6-oxopyridazine-3-carboxylate 13f. Yield = 45%; mp = 59–61 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 0.59–0.69 (m, 4H, cC_3H_5), 1.30–1.40 (m, 1H, cC_3H_5), 1.42 (t, 3H, $COOCH_2CH_3$), 3.16 (s, 3H, $COCH_3$), 4.23 (t, 2H, NCH_2), 4.44 (q, 2H, $COOCH_2CH_3$).

6.1.9. General procedure for 14a–n

A suspension of **13a–f** (**13a**[18] and **13b**[30]) (0.15–0.57 mmol) and the appropriate hydrazines, which are all commercially available with exception of 1-*m*-methylhydroxyphenylhydrazine[38], (0.39–1.96 mmol) in absolute ethanol (3–10 mL), was stirred for 5 min–12 h at a temperature ranging from 20 to 80 °C. Then the reaction mixture was concentrated in vacuo and diluted with cold water. Compounds **14a–e**,**k** were recovered by suction and recrystallized by ethanol. For compounds **14f–j**,**l–n**, the suspension was extracted with CH_2Cl_2 (2 × 15 mL), the organic layer was washed with 6 N HCl (10 mL), and then evaporated in vacuo. Compounds **14f–j,l–n** were purified by column chromatography, using cyclohexane/ethyl acetate, 1:1 for compounds **14f,i,j,l**; cyclohexane/ethyl acetate, 1:3, for compound **14h** and cyclohexane/ethyl acetate, 2:1, for compounds **14g,m**.

6.1.9.1. 6-Ethyl-3-methyl-2-(3-nitrophenyl)-4-phenyl-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-one 14a. Yield = 57%; mp = 163–164 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.48 (t, 3H, NCH_2CH_3), 2.30 (s, 3H, 3-CH_3), 4.38 (q, 2H, NCH_2CH_3), 7.52–7.63 (m, 5H, Ar), 7.80 (t, 1H, Ar), 8.00 (d, 1H, Ar), 8.43 (m, 2H, Ar).

6.1.9.2. Ethyl 2-(3-methyl-2-(3-nitrophenyl)-7-oxo-4-phenyl-2*H*-pyrazolo[3,4-*d*]pyridazin-6(7*H*)-yl)acetate 14b. Yield = 40%; mp = 207–209 °C (EtOH); IR (Nujol): 1755 (CO), 1690 (CO), 1535 and 1350 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, 3H, $COOCH_2CH_3$), 2.30 (s, 3H, 3-CH_3), 4.27 (q, 2H, $COOCH_2CH_3$), 5.07 (s, 2H, NCH_2), 7.55 (m, 3H, Ar), 7.61 (m, 2H, Ar), 7.80 (t, 1H, Ar), 8.00 (d, 1H, Ar), 8.44 (m, 2H, Ar); MS (ESI): *m/z* 433.42 $[M+H]^+$.

6.1.9.3. Ethyl 3-(3-methyl-2-(3-nitrophenyl)-7-oxo-4-phenyl-2*H*-pyrazolo[3,4-*d*]pyridazin-6(7*H*)-yl)propanoate 14c. Yield = 38%; mp = 152–154 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, $COOCH_2CH_3$), 2.30 (s, 3H, 3-CH_3), 2.92 (t, 2H, NCH_2CH_2), 4.15 (q, 2H, $COOCH_2CH_3$), 4.66 (t, 2H, NCH_2CH_2), 7.57 (m, 5H, Ar), 7.80 (t, 1H, Ar), 8.00 (d, 1H, Ar), 8.43 (m, 2H, Ar).

6.1.9.4. Ethyl 6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14d. Yield = 80%; mp = 40 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, $COOCH_2CH_3$), 1.51 (t, 3H, NCH_2CH_3), 2.63 (s, 3H, 3-CH_3), 4.39–4.49 (m, 4H, (2H, $COOCH_2CH_3$; 2H, NCH_2CH_3)), 6.13 (exch br s, 1H, NH).

6.1.9.5. Ethyl 6-ethyl-6,7-dihydro-3-methyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14e. Yield = 49%; mp = 219–221 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.48 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.82 (s, 3H, 3-CH_3), 4.40 (q, 2H, $COOCH_2CH_3$), 4.52 (q, 2H, NCH_2CH_3), 7.81 (t, 1H, Ar), 7.95 (d, 1H, Ar), 8.45 (m, 2H, Ar).

6.1.9.6. Ethyl 6-ethyl-6,7-dihydro-3-methyl-7-oxo-2-phenyl-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14f. Yield = 53%; mp = 119–121 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.46 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.70 (s, 3H, 3-CH_3), 4.38 (q, 2H, $COOCH_2CH_3$), 4.50 (q, 2H, NCH_2CH_3),7.54 (m, 5H, Ar).

6.1.9.7. Ethyl 2-(2-chlorophenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14g. Yield = 39%; mp = 113–115 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.48 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.62 (s, 3H, 3-CH_3), 4.40 (q, 2H, $COOCH_2CH_3$), 4.51 (q, 2H, NCH_2CH_3), 7.49 (m, 2H, Ar), 7.50–7.60 (m, 1H, Ar), 7.63 (m, 1H, Ar).

6.1.9.8. Ethyl 6-ethyl-6,7-dihydro-2-(3-(hydroxymethyl) phenyl)-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14h. Yield = 30%; mp = 152–154 °C (EtOH); IR (Nujol): 1750

(CO), 1680 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 (t, 3H, $COOCH_2CH_3$), 1.35 (t, 3H, NCH_2CH_3), 2.60 (s, 3H, 3-CH_3), 4.20 (q, 2H, $COOCH_2CH_3$), 4.40 (q, 2H, NCH_2CH_3), 4.62 (d, 2H, CH_2OH), 5.41 (exch br t, 1H, CH_2OH), 7.48–7.61 (m, 4H, Ar); MS (ESI): *m/z* 356.38 $[M+H]^+$.

6.1.9.9. Ethyl 6-ethyl-6,7-dihydro-3-methyl-7-oxo-2-*o*-tolyl-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14i. Yield = 33%; mp = 94–96 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.48 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.05 (s, 3H, 2-CH_3Ar), 2.56 (s, 3H, 3-CH_3), 4.40(q, 2H, $COOCH_2CH_3$), 4.50 (q, 2H, NCH_2CH_3), 7.27 (m, 1H, Ar), 7.36–7.48 (m, 3H, Ar).

6.1.9.10. Ethyl 2-(2,6-dichlorophenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14j. Yield = 43%; mp = 216–218 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.48 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.60 (s, 3H, 3-CH_3), 4.40 (q, 2H, $COOCH_2CH_3$), 4.51 (q, 2H, NCH_2CH_3), 7.47–7.57 (m, 3H, Ar).

6.1.9.11. Ethyl 6,7-dihydro-3-methyl-2-(3-nitrophenyl)-7-oxo-6-propyl-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14k. Yield = 35%; mp = 178–179 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.00 (t, 3H, $NCH_2CH_2CH_3$),1.48 (t, 3H, $COOCH_2CH_3$), 1.90 (m, 2H, $NCH_2CH_2CH_3$), 2.80 (s, 3H, 3-CH_3), 4.30 (t, 2H, $NCH_2CH_2CH_3$), 4.50 (q, 2H, $COOCH_2CH_3$), 7.80 (t, 1H, Ar), 7.93 (d, 1H, Ar), 8.43 (m, 2H, Ar).

6.1.9.12. Ethyl 2-(2-chlorophenyl)-6-(cyclopropylmethyl)-6,7-dhydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14l. Yield = 21%; mp = 132–134 °C (EtOH); ^1H NMR ($CDCl_3$) δ 0.54 (m, 4H, cC_3H_5), 1.22–1.35 (m, 1H, cC_3H_5),1.48 (t, 3H, $COOCH_2CH_3$), 2.62 (s, 3H, 3-CH_3), 4.21 (d, 2H, NCH_2), 4.50 (q, 2H, $COOCH_2CH_3$), 7.49 (d, 2H, Ar), 7.56 (m, 1H, Ar), 7.63 (d, 1H, Ar).

6.1.9.13. Ethyl 2-(5-(methoxycarbonyl)-2-methylphenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14m. Yield = 60%; mp = 192–193 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.42–1.51 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.12 (s, 3H, 2-CH_3Ar), 2.58 (s, 3H, 3-CH_3), 3.94 (s, 3H, $COOCH_3$), 4.41 (q, 2H, $COOCH_2CH_3$), 4.51 (m, 2H, NCH_2CH_3), 7.50 (d, 1H, Ar), 7.97 (dd, 1H, Ar), 8,16 (dd, 1H, Ar).

6.1.9.14. Ethyl 2-(5-carbomoyl-2-chlorophenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 14n. Yield = 60%; mp = 171–172 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.42–1.51 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.60 (s, 3H, 3-CH_3), 4.30–4.48 (q, 2H, $COOCH_2CH_3$), 4.51 (m, 2H, NCH_2CH_3), 6.06 (exch br s, 1H, NH_2), 7.00 (exch br s, 1H, NH_2), 7.69 (d, 1H, Ar), 8.07 (m, 2H, Ar).

6.1.10. General procedure for 15a–c

Compounds **15a–c** were obtained starting from **14d** and appropriate arylalkyl halides, following the same procedure described for **10a–d**. For these compounds, the reaction was carried out in anhydrous acetone at 60 °C for 1–2 h. Compound **15a** was recovered by suction and purified by column chromatography using cyclohexane/ethyl acetate, 1:1, as eluent. Compounds **15b**,**c** were obtained after extraction with CH_2Cl_2 (3 × 20 mL) and purification by column chromatography using cyclohexane/ethyl acetate, 2:1, as eluent for compound **15b** and cyclohexane/ethyl acetate, 3:1, as eluent for compound **15c**.

6.1.10.1. Ethyl 2-(4-cyanobenzyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 15a.

Yield = 14%; mp = 130–131 °C (cyclohexane); IR (Nujol): 2220 (CN), 1745 (CO), 1680 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40–1.50 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.64 (s, 3H, 3-CH_3), 4.35 (q, 2H, $COOCH_2CH_3$), 4.51 (q, 2H, NCH_2CH_3), 5.94 (s, 2H, CH_2Ar), 7.48 (d, 2H, Ar), 7.63 (d, 2H, Ar); MS (ESI): *m/z* 351.36 $[M+H]^+$.

6.1.10.2. Ethyl 2-(4-nitrobenzyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 15b. Yield = 33%; mp = 131–132 °C (cyclohexane); IR (Nujol): 1745 (CO), 1690 (CO), 1530 and 1350 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40–1.50 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.65 (s, 3H, 3-CH_3), 4.36 (q, 2H, $COOCH_2CH_3$), 4.50 (q, 2H, NCH_2CH_3), 6.00 (s, 2H, CH_2Ar), 7.55 (d, 2H, Ar), 8.20 (d, 2H, Ar); MS (ESI): *m/z* 371.35 $[M+H]^+$.

6.1.10.3. Ethyl 2-(3-(methoxycarbonyl)benzyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 15c. Yield = 33%; mp = 131–132 °C (EtOH); ^1H NMR ($CDCl_3$) δ 1.40–1.50 (m, 6H, (3H, $COOCH_2CH_3$; 3H, NCH_2CH_3)), 2.63 (s, 3H, 3-CH_3), 3.92 (s, 3H, $COOCH_3$), 4.38 (q, 2H, $COOCH_2CH_3$), 4.50 (q, 2H, NCH_2CH_3), 5.95 (s, 2H, CH_2Ar), 7.42 (t, 1H, Ar), 7.58 (d, 1H, Ar), 7.98 (d, 1H, Ar), 8.07 (s, 1H, Ar).

6.1.11. General procedure for 16a–d

A suspension of **14e–g** and **14j** (0.15–0.7 mmol) in 2 N NaOH (2–5 mL) was stirred at 25–80 °C for 40 min–2 h. After cooling, the mixture was acidified with 6 N HCl and the precipitate was recovered by suction.

6.1.11.1. 6-Ethyl-6,7-dihydro-3-methyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylic acid 16a. Yield = 68%; mp = 227–229 °C dec (EtOH); IR (Nujol): 1730 (CO), 1680 (CO), 1530 and 1360 (NO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.32 (t, 3H, NCH_2CH_3), 2.60 (s, 3H, 3-CH_3), 4.21 (q, 2H, NCH_2CH_3), 7.95 (t, 1H, Ar), 8.18 (d, 1H, Ar), 8.48 (d, 1H, Ar), 8.53 (s, 1H, Ar), 13.70 (exch br s, 1H, COOH); MS (ESI): *m/z* 343.29 $[M+H]^+$.

6.1.11.2. 6-Ethyl-6,7-dihydro-3-methyl-7-oxo-2-phenyl-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylic acid 16b. Yield = 90%; mp = 230–232 °C dec (EtOH); ^1H NMR (DMSO-d_6) δ 1.29 (t, 3H, NCH_2CH_3), 2.61 (s, 3H, 3-CH_3), 4.18 (q, 2H, NCH_2CH_3), 7.62 (m, 5H, Ar).

6.1.11.3. 2-(2-Chlorophenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylic acid 16c. Yield = 83%; mp = 202–204 °C (EtOH); ^1H NMR (DMSO-d_6) δ 1.32 (t, 3H, NCH_2CH_3), 2.48 (s, 3H, 3-CH_3), 4.20 (q, 2H, NCH_2CH_3), 7.65 (t, 1H, Ar), 7.72 (t, 2H, Ar), 7.84 (d, 1H, Ar).

6.1.11.4. 2-(2,6-Dichlorophenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylic acid 16d.

Yield = 72%; mp = 222–224 °C (EtOH); ^1H NMR (DMSO-d_6) δ 1.33 (t, 3H, NCH_2CH_3), 2.45 (s, 3H, 3-CH_3), 4.20 (q, 2H, NCH_2CH_3), 7.76 (m, 1H, Ar), 7.87 (m, 2H, Ar), 13.70 (exch br s, 1H, COOH).

6.1.12. General Procedure for 17a–f

A mixture of **16a–d** (0.1–0.6 mmol), anhydrous Et_3N (0.15 mL) and $SOCl_2$ (3–27.5 mmol) was stirred at 60 °C for 1–2 h. The excess of $SOCl_2$ was removed in vacuo, and then appropriate amine (0.5–2 mmol) was added to the residue oil dissolved in cold anhydrous THF. For compound **17f** NaH (60% dispersion in mineral oil) (0.6 mmol) was added. The mixture was stirred at room temperature for 20 min–2 h, diluted with cold water (2–5 mL), and the precipitate was recovered by suction for compounds **17a,d**. For compounds **17b,c,e,f**, the suspension was neutralized with 2 N NaOH, extracted with ethyl acetate (3 × 15 mL) and the solvent was evaporated in vacuo. The compounds **17b,c,e** were recrystal-

lized by ethanol, compound **17f** was purified by column chromatography, using cyclohexane/ethyl acetate, 1:1, as eluent.

6.1.12.1. 2-(2-Chlorophenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxamide 17a. Yield = 78%; mp = 203–205 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 1.34 (t, 3H, NCH_2CH_3), 2.52 (s, 3H, 3-CH_3), 4.19 (q, 2H, NCH_2CH_3), 7.65 (t, 1H, Ar), 7.70 (exch br s, 1H, NH_2), 7.72 (s, 1H, Ar), 7.83 (d, 1H, Ar), 8.00 (exch br s, 1H, NH_2).

6.1.12.2. 2-(2,6-Dichlorophenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxamide 17b. Yield = 75%; mp = 243–245 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 1.36 (t, 3H, NCH_2CH_3), 2.52 (s, 3H, 3-CH_3), 4.19 (q, 2H, NCH_2CH_3), 7.74 (exch br s, 1H, NH_2), 7.77 (m, 1H, Ar), 7.85 (s, 1H, Ar), 7.87 (s, 1H, Ar), 8.02 (exch br s, 1H, NH_2).

6.1.12.3. 6-Ethyl-6,7-dihydro-*N*,3-dimethyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxamide 17c. Yield = 64%; mp = 250–252 °C (EtOH); IR (Nujol): 3430 (NH), 1680 (2 CO), 1525 and 1350 (NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (t, 3H, NCH_2CH_3), 2.70 (s, 3H, 3-CH_3), 2.80 (s, 3H, $NHCH_3$), 4.18 (q, 2H, NCH_2CH_3), 7.92 (t, 1H, Ar), 8.15 (d, 1H, Ar), 8.45 (d, 1H, Ar), 8.50 (s, 1H, Ar), 8.55 (exch br s, 1H, $NHCH_3$); MS (ESI): *m/z* 356.34 [M+H]$^+$.

6.1.12.4. *N*,6-Diethyl-6,7-dihydro-*N*,3-dimethyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxamide 17d. Yield = 64%; mp = 250–252 °C (EtOH); IR (Nujol): 1690 (CO), 1670 (CO), 1515 and 1350 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, NCH_2CH_3),1.40 (t, 3H, $N(CH_3)CH_2CH_3$), 2.60 (d, 3H, 3-CH_3), 3.1 5 (d, 3H, $N(CH_3)CH_2CH_3$), 3.48 (q, 1H, $N(CH_3)CH_2CH_3$), 3.65 (q, 1H, $N(CH_3)CH_2CH_3$), 4.30 (q, 2H, NCH_2CH_3), 7.78 (t, 1H, Ar), 7.94 (d, 1H, Ar), 8.41 (m, 2H, Ar); MS (ESI): *m/z* 384.39 [M+H]$^+$.

6.1.12.5. 6-Ethyl-6,7-dihydro-*N*,*N*,3-trimethyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxamide 17e. Yield = 41%; mp = 196–198 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H, NCH_2CH_3), 2.61 (s, 3H, 3-CH_3), 3.1 9 (s, 3H, $N(CH_3)_2$), 3.2 2 (s, 3H, $N(CH_3)_2$), 4.31 (q, 2H, NCH_2CH_3), 7.80 (t, 1H, Ar), 7.94 (d, 1H, Ar), 8.42 (m, 2H, Ar).

6.1.12.6. *N*,2,6-Dichloro(4-pyridin)-6-ethyl-6,7-dihydro-2-phenyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxamide 17f. Yield = 8%; mp = 246–248 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ1.19 (t, 3H, NCH_2CH_3), 2.71 (s, 3H, 3-CH_3), 4.10 (m, 2H, NCH_2CH_3), 7.49–7.59 (m, 7H, Ar), 8.67 (exch br s, 1H, *NH*Ar).

6.1.13. General procedure for 18a,b

A suspension of **17a** or **17b** (0.21 mmol) in $POCl_3$ (5.81 mmol) was stirred at 60 °C for 1–2 h. Then the reaction mixture in the cold water (15 mL) was slowly added. The suspension was extracted with ethyl acetate (3 × 15 mL) and the organic layer evaporated affording a crude precipitate which was recrystallized by ethanol.

6.1.13.1. 2-(2-Chlorophenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carbonitrile 18a. Yield = 91%; mp = 155–158 °C (EtOH); IR (Nujol): 2225 (CN), 1690 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3H, NCH_2CH_3), 2.62 (s, 3H, 3-CH_3), 4.37 (q, 2H, NCH_2CH_3), 7.51 (m, 4H, Ar); MS (ESI): *m/z* 313.74 [M+H]$^+$.

6.1.13.2. 2-(2,6-Dichlorophenyl)-6-ethyl-6,7-dihydro-3-methyl-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carbonitrile 18b. Yield = 70%; mp = 206–209 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.47 (t, 3H, NCH_2CH_3), 2.58 (s, 3H, 3-CH_3), 4.37 (q, 2H, NCH_2CH_3), 7.56 (m, 3H, Ar).

6.1.14. General procedure for 19a–c

Compounds **19a–c** were obtained starting from **16a** following the same procedure described for **10a–d**. For compound **19a**, the reaction was carried out in anhydrous acetone and the suspension was diluted with cold water and extracted with ethyl acetate (3 × 15 mL) to afford the final product. For compounds **19b,c**, the reaction was carried out in anhydrous DMF and after dilution with cold water the final products were recovered by suction.

6.1.14.1. Methyl 6-ethyl-6,7-dihydro-3-methyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 19a. Yield = 96%; mp = 185–188 °C dec (EtOH); IR (Nujol): 1745 (CO), 1690 (CO), 1525 and 1350 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.47 (t, 3H, NCH_2CH_3), 2.84 (s, 3H, 3-CH_3), 4.05 (s, 3H, $COOCH_3$), 4.41 (q, 2H, NCH_2CH_3), 7.82 (t, 1H, Ar), 7.96 (d, 1H, Ar), 8.46 (m, 2H, Ar); MS (ESI): *m/z* 357.32 [M+H]$^+$.

6.1.14.2. Propyl 6-ethyl-6,7-dihydro-3-methyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 19b. Yield = 96%; mp = 184–186 °C dec (EtOH); IR (Nujol): 1745 (CO), 1690 (CO), 1530 and 1350 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.08 (t, 3H, COOCH2CH2*CH3*), *1.*47 (t, 3H, NCH_2CH_3), 1.89 (m, 2H, COOCH2-*CH2*CH3), 2.82 (s, 3H, 3-CH_3), 4.40 (m, 4H, (2H, COO*CH2*CH2CH3; 2H, NCH_2CH_3)), 7.81 (t, 1H, Ar), 7.95 (d, 1H, Ar), 8.45 (m, 2H, Ar); MS (ESI): *m/z* 385.37 [M+H]$^+$.

6.1.14.3. Isopropyl 6-ethyl-6,7-dihydro-3-methyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carboxylate 19c. Yield = 96%; mp = 164–166 °C dec (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.48 (m, 9H, (6H, $COOCH(CH_3)_2$; 3H, NCH_2CH_3), 2.80 (s, 3H, 3-CH_3), 4.40 (m, 2H, NCH_2CH_3), 5.35 (m, 1H $COOCH(CH_3)_2$), 7.80 (t, 1H, Ar), 7.95 (d, 1H, Ar), 8.41 (m, 2H, Ar).

6.1.15. 2-(2-Chlorophenyl)-6-ethyl-4-(hydroxymethyl)-3-methyl-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-one 20

A mixture of **16c** (0.33 mmol), $NaBH_4$ (1.98 mmol) in anhydrous THF was heated at 70 °C for 20 min, then methanol (1 mL) was slowly added. The mixture was stirred for 1 h, and then concentrated in vacuo. After dilution with cold water (10 mL), the suspension was extracted with ethyl acetate (3 × 15 mL) and the solvent was evaporated to afford **20**, which was recrystallized by ethanol. Yield = 85%; mp = 169–171 °C dec (EtOH); IR (Nujol): 1690 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (exch br s, 1H, CH_2OH), 1.43 (t, 3H, NCH_2CH_3), 2.52 (s, 3H, 3-CH_3), 4.30 (m, 2H, NCH_2CH_3), 4.90 (s, 2H, CH_2OH), 7.49 (m, 2H, Ar), 7.55 (m, 1H, Ar), 7.61 (m, 1H, Ar); MS (ESI): *m/z* 318.76 [M+H]$^+$.

6.1.16. 2-(2-Chlorophenyl)-6-ethyl-4-(methoxymethyl)-3-methyl-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-one 21

A mixture of **20** (0.22 mmol) in anhydrous DMF (3 mL) was stirred at 0 °C, NaH (60% dispersion in mineral oil) (0.44 mmol) was added during 10 min and then CH_3I (0.22 mmol) in anhydrous DMF (1 mL) was added dropwise. The suspension was stirred at room temperature for 2 h. After dilution with cold water, the mixture was extracted with CH_2Cl_2 (3 × 15 mL) and the solvent was evaporated in vacuo to afford **21**. Yield = 85%; oil; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, NCH_2CH_3), 2.52 (s, 3H, 3-CH_3), 3.45 (s, 3H, CH_2OCH_3), 4.30 (m, 2H, NCH_2CH_3), 4.52–4.69 (m, 2H, CH_2OCH_3), 7.49 (m, 2H, Ar), 7.54 (m, 1H, Ar), 7.61 (m, 1H, Ar).

6.1.17. *N′*-Acetyl-6-ethyl-6,7-dihydro-3-methyl-2-(3-nitrophenyl)-7-oxo-2*H*-pyrazolo[3,4-*d*]pyridazine-4-carbohydrazide 22

Compound **22** was obtained from **16a** and acethydrazide, following the procedure described for **17a–f**. The mixture was stirred at room temperature for 10 min, diluted with cold water (3 mL), and the precipitate was recovered by suction. Yield = 35%; mp = 146–148 °C dec (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, NCH_2CH_3), 2.20 (s, 3H, $NHCOCH_3$), 2.87 (s, 3H, 3-CH_3), 4.31 (m, 2H, NCH_2CH_3), 7.41 (exch br s, 1H, NH), 7.49 (m, 2H, Ar), 7.80 (t, 1H, Ar), 7.97 (d, 1H, Ar), 8.42 (d, 1H, Ar), 8.50 (s, 1H, Ar), 9.87 (exch br s 1H, NH).

6.1.18. 6-Ethyl-3-methyl-4-(5-methyl-1,3,4-oxadiazol-2-yl)-2-(3-nitrophenyl)-2*H*-pyrazolo[3,4-*d*]pyridazin-7(6*H*)-one 23

Compound **23** was obtained as a precipitate starting from **22** following the same general procedure described for **18a,b**. Yield = 40%; mp = 194–196 °C (EtOH); IR (Nujol): 1690 (CO), 1530 and 1350 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.50 (t, 3H, NCH_2CH_3), 2.73 (s, 3H, 5-CH_3 oxadiazole), 3.03 (s, 3H, 3-CH_3), 4.44 (m, 2H, NCH_2CH_3), 7.83 (t, 1H, Ar), 7.96 (d, 1H, Ar), 8.46 (d, 1H, Ar), 8.49 (t, 1H, Ar); MS (ESI): *m/z* 381.35 $[M+H]^+$.

6.2. Biological assays

6.2.1. PDE4 enzyme preparation. cAMP hydrolysis assay. PDE4 inhibitory activity determination and IC_{50} calculation

The U937 human monocytic cell line was used as source of PDE4 enzyme. Cells were cultured, harvested and supernatant fraction prepared essentially as described in Torphy et al.[39] PDE4 activity was determined in cells supernatants by assaying cAMP disappearance from the incubation mixtures. Cell supernatant (50 μL) were incubated at 30 °C for 30 min in a final volume of 200 μL in the presence of 1.6 μM cAMP with or without the test compound (50 μL). Reactions were stopped by heat inactivation (2.5 min at 100 °C) and residual cAMP was measured using an electrochemiluminescence (ECL)-based immunoassay (Bioveris Corporation, Gaithersburg, MD, USA).

PDE4 activity was calculated as nmol of cAMP disappeared/min mg prot. Percentage of inhibition of PDE4 activity was calculated, assuming cAMP disappearance in the absence of inhibitors as 100% and cAMP disappearance in heat inactivated samples as 0%. IC_{50} were determined by assaying PDE4 activity in the presence of different concentrations of test compound (concentration range: 10^{-12}–10^{-6} M) and calculated with non linear regression (sigmoidal dose–response) by Graph Pad Version 4.03.

6.2.2. In vitro inhibition of TNF-α release in human PBMCs

All of the experiments were carried out using cryopreserved PBMCs, obtained from Cambrex Corporation (New Jersey, USA).

PBMCs (100 μL/well) were incubated in 96-well plates (10^5 cells/well.), for 30 min, in the presence or absence (50 μL) of various concentrations of the compounds of interest. Subsequently, LPS (3 ng/mL) was added. After 18 h culture medium was collected and TNF-α measured by ELISA.

Stock solutions of test compounds were prepared in DMSO and diluted in modified RPMI 1640 medium or DMSO/modified RPMI 1640 medium; the final DMSO concentration in buffer was 0.1% or 0.2% (v/v); this vehicle by itself did not affect TNF-α synthesis. After preincubation with the test inhibitor, 50 μL LPS (Lipopolysaccharides from *Salmonella Enteriditis*), dissolved in modified RPMI 1640 medium, were added to wells (final concentration 3 ng/mL) to stimulate TNF-α synthesis, except that in $n = 3$ wells/plate which served to measure basal TNF-α production. LPS concentration employed as the stimulus (3 ng/mL, corresponding to EC_{84}) was selected on the basis of preliminary experiments, which showed this dose producing nearly maximal stimulation of TNF-α synthesis. In each experimental session, the effect produced by roflumilast (3 nM, corresponding to its EC_{50}) was estimated as control.

The plates were incubated for 18 h at 37 °C in a humidified incubator under an atmosphere of 95% air and 5% CO_2. Afterwards, the culture supernatant was collected and stored at −80 °C until determination of TNF-α contents.

6.2.3. TNF-α measurement

Human TNF-α was measured using a commercially available ELISA kit (Biosource International, Camarillo, CA; Arcus Biologicals, Modena, Italy; detection limit: 1.7 pg/mL); the assay was performed according to the manufacturer's instructions. TNF-α content was expressed as pg/mL.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.066.

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