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Isoxazolo[3,4-*d*]pyridazinones and analogues as *Leishmania mexicana* PDE inhibitors

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Abstract

A series of isoxazolopyridazinones and analogues has been prepared and evaluated as *Leishmania mexicana* phosphodiesterase (PDE) inhibitors. Some of the synthesized compounds showed a moderate PDE inhibitory activity at 100 μM and preliminary structure–activity relationships were discussed.

Keywords: *Leishmania mexicana*; Phosphodiesterase inhibitors; Isoxazolopyridazinones

1. Introduction

In the tropics and subtropics, haemoflagellate protozoan parasites of genus *Leishmania* are causative agents of a variety of diseases representing a major health problem known as leishmaniasis [1].

The only available drugs (stibogluconate, meglumine antimonate, pentamidine and allopurinol) are of limited efficacy and display serious side effects [2–4].

Recently some of us have characterized and purified for the first time a highly specific cAMP phosphodiesterase (PDE) from *Leishmania mexicana* which shows a low sensitivity towards selective and non-selective PDE inhibitors [5]. A similar refractoriness to inhibition has been described for two recently cloned PDEs from another member of the Trypanosomatidae family, *Trypanosoma brucei* (TbPDE2A and TbPDE2B), the causing agent of sleeping sickness [6,7]. Nevertheless, non-specific PDE inhibitors (caffeine, theophylline, papaverine, dipyridamole and allopurinol) (Chart 1), although very weak *Leishmania* PDE inhibitors, are able to inhibit the transformation of amastigotes to promastigotes and the proliferation of promastigotes in *L. donovani* and *L. tropica* [8–10]. A completely structurally different compound, displaying the same activity is Cibacron blue which today represents the most potent inhibitor of *L. mexicana* PDE ($IC_{50} = 8$ μM) [11,12]. Therefore it makes interesting and feasible the discovery of potent and selective PDE inhibitors, which could be therapeutically useful to modulate cAMP levels and proliferation in *Leishmania*, and maybe other trypanosomatids.

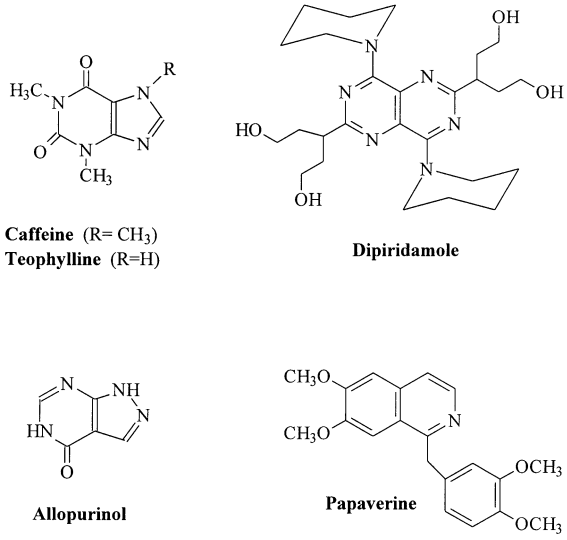

Chart 1

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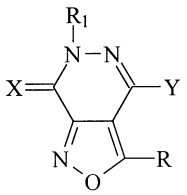
The absence of a useful template for modeling novel agents on one hand and our experience in designing and synthesizing mammalian PDE inhibitors [13,14] bearing the pyridazine fragment on the other, led us to start a research program aimed at the discovery of novel chemical entities belonging to this chemical class as *Leishmania* PDE inhibitors. Thus we evaluated as inhibitors of this enzyme a group of isoxazolo[3,4-*d*]pyridazin-3(2*H*)-ones, some of which had been previously described by us, as well as other related compounds (Tables 1–3).

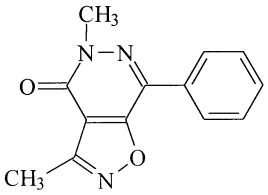
2. Chemistry

The key intermediates for the synthesis of the novel compounds **5a**–**e**, **6** and **9a**–**e** were isoxazolo[3,4-*d*]pyridazinones of type **4**, which have been previously described [13,15,16] with the exception of that with R = *n*-propyl which was prepared as follows: condensation of ethyl chlorooximido acetate **2** with 1-phenylexan-1,3-dione [17] **1** afforded the isoxazole **3** (Scheme 1) which in turn was cyclocondensed with hydrazine to give **4**. Alkylation of the appropriate **4**

Table 1
Chemical and physical data of isoxazolopyridazinones and their inhibitory activity against *Leishmania mexicana* PDE



5a-e, 6, A-O

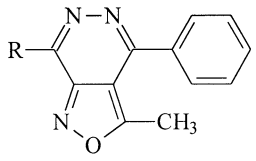


11

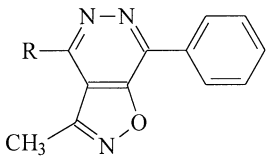
Comp. [a]	R_1	Y	R	X	Yield (%)	m.p. (°C)	Formula [j]	% inhibition [k]
A [b]	H	CH_3	CH_3	O				15.7
B [c]	CH_3	CH_3	CH_3	O				11.0
C [c]	H	C_6H_5	CH_3	O				35.2
D [d]	CH_3	C_6H_5	H	O				11.7
E [c]	CH_3	C_6H_5	CH_3	O				43.0
F [e]	C_2H_5	C_6H_5	CH_3	O				0
G [f]	CH_3	C_6H_5	C_2H_5	O				55.4
5a	CH_3	C_6H_5	nC_3H_7	O	60	122–124	$C_{15}H_{15}N_3O_2$	23.7
5b	CH_3	*m*-ClC_6H_4	CH_3	O	76	163–166	$C_{18}H_{10}ClN_3O_2$	48.5
H [g]	CH_3	*p*-ClC_6H_4	CH_3	O				6.4
I [h]	CH_3	4-pyridyl	CH_3	O				25.0
5c	CH_3	2-thienyl	CH_3	O	57	159–161	$C_{11}H_9ClN_3O_2S$	0
5d	CH_2CN	C_6H_5	CH_3	O	55	140–142	$C_{14}H_{10}N_4O_2$	14.3
5e	$(CH_2)_2OCH_3$	C_6H_5	CH_3	O	72	169–172	$C_{15}H_{15}N_3O_3$	35.9
J [i]	CH_2COOH	C_6H_5	CH_3	O				0
K [i]	$CH_2COOC_2H_5$	C_6H_5	CH_3	O				7.7
L [i]	$(CH_2)_2COOH$	C_6H_5	CH_3	O				32.9
M [i]	$(CH_2)_2COOC_2H_5$	C_6H_5	CH_3	O				16.8
N[i]	$(CH_2)_3COOH$	C_6H_5	CH_3	O				25.2
O [i]		C_6H_5	CH_3	O				17.3
6		C_6H_5	CH_3	S	62	187–189	$C_{13}H_{11}N_3OS$	4.8
11		C_6H_5	CH_3		75	135–137	$C_{13}H_{11}N_3O_2$	58.7
Cibacron		C_6H_5	CH_3					$IC_{50} = 8$ μM

[a] New compounds were rechrystallized from EtOH, with the exception of **11** which was from cyclohexane.
[b] Ref. [21].
[c] Ref. [22].
[d] Ref. [24].
[e] Ref. [13].
[f] Ref. [25].
[g] Ref. [26].
[h] Ref. [27].
[i] Ref. [20].
[j] C, N, H, analysis within ±0.4%.
[k] All compounds were tested at 100 μM.

Table 2
Chemical and physical data of isoxazolo[3,4-*d*]pyridazine and of isoxazolo[4,5-*d*]pyridazine and their inhibitory activity against *Leishmania mexicana* PDE



P, 8, 9a,b



13a-e

Comp.	R	Yield (%)	m.p. (°C)	Formula [f]	% inhibition [g]
P [a]	OMe				5.1
8 [a]	SMe				5.5
9a [b]	NHMe	60	149–152	$C_{13}H_{12}N_4O$	24.1
9b [c]	NHEt	70	114–116	$C_{14}H_{14}N_4O$	22.0
9c [c]	NH*n*-Pr	80	130–133	$C_{15}H_{16}N_4O$	28.0
9d [c]	NH*n*-But	67	115–117	$C_{16}H_{18}N_4O$	51.8
9e [d]	piperidine	76	130–132	$C_{17}H_{18}N_4O$	22.0
12 [d]	Cl	72	184–187	$C_{12}H_8N_3OCl$	NT [h]
13a [e]	NHMe	81	182 dec	$C_{13}H_{12}N_4O$	0
13b [e]	NHEt	77	165–167	$C_{14}H_{14}N_4O$	0
13c [e]	NH*n*-Pr	82	146–149	$C_{15}H_{16}N_4O$	22.0
13d [e]	NH*n*-But	69	160–162	$C_{16}H_{18}N_4O$	22.0
13e [b]	piperidine	67	130–134	$C_{17}H_{18}N_4O$	26.0
Cibacron					$IC_{50} = 8$ μM

[a] Ref. [18].
[b] Crystallized from EtOH–H_2O 1:1.
[c] Purified by column chromatography using cyclohexane–ethyl acetate 1:2 as eluent.
[d] Crystallized from EtOH.
[e] Crystallized from cyclohexane.
[f] C, N, H, analysis within ± 0.4%.
[g] All compounds were tested at 100 μM.
[h] Not tested.

with the required halo derivative afforded the final compounds **5a**–**e**. When compound **5** with $R = R_1 = CH_3$ and $Ar = C_6H_5$ was refluxed with P_2S_5 in toluene, compound **6** was obtained in good yield. In similar conditions using Lawesson's reagent, the intermediate **7** [18] was isolated and converted into the final **9a**–**e** in two steps: alkylation with iodomethane [18] and nucleophilic replacement with the appropriate (cyclo)-alkylamine.

The synthesis of isoxazolo[4,5-*d*]pyridazinones **11** and **13a**–**e** is depicted in Scheme 2: treatment of **10** [19] with $POCl_3$ in the presence of triethylamine afforded the corresponding 5-chloroderivative **12** which was converted in the final **13a**–**e** in the same reaction conditions described for **9a**–**e**. Compound **11** was prepared from **10** by alkylation in standard conditions.

Finally the open models **15a**,**b**, reported in Scheme 3, were easily obtained by treatment of 5-acetyl-4-nitro pyridazinones **14a**,**b** [20] by nucleophilic displacement with chlorine using TEBA in CH_3CN.

^{1}H NMR spectra of new compounds are shown in Table 4.

3. Experimental procedures

3.1. Chemistry

All melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Varian Gemini 200 instruments. Chemical shifts are reported in ppm, using the solvent as internal standard. Extracts were dried over Na_2SO_4 and the solvents were removed under reduced pressure. E. 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.

All common reagents were purchased from Aldrich Chemical and used without purification.

3.1.1. Synthesis of ethyl 4-benzoyl-5-n-propylisoxazolo-3-carboxylate (3) ($R = n\text{-}C_3H_7$, $Ar = C_6H_5$)

To a cooled and stirred solution of sodium ethoxide (13 mmol) in anhydrous EtOH (20 ml) was added a

solution of 1-phenylhexane-1,3-dione [17] (13 mmol) in the same solvent (70 ml). Then a solution of ethyl chlorooximido acetate [21] (13.3 mmol) in anhydrous EtOH (10 ml) was added dropwise over a 1 h period. After solvent evaporation, the residue was washed with cold 0.5 N NaOH and water and extracted with CH_2Cl_2 (3×20 ml): 70% yield; oil (column chromatography, eluent: cyclohexane/ethyl acetate 1:1). Anal. ($C_{16}H_{17}NO_4$) C, H, N.

*3.1.2. Synthesis of 4-phenyl-3-n-propylisoxazolo-[3,4-d]pyridazin-7(6H)-one (**4**) (R = n-C_3H_7, Ar = C_6H_5)*

The isoxazole **3** (R = *n*-C_3H_7, Ar = C_6H_5) (0.5 mmol) was dissolved in EtOH (5 ml) and then 0.15 ml of hydrazine hydrate was added. After 1 h the crude product was collected by suction from the cooled mixture: 40% yield; m.p. 146–148 °C (EtOH). Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

*3.1.3. General procedure for the synthesis of isoxazolo[3,4-d]pyridazinones **5a–e** and of 3,5-dimethyl-7-phenylisossazolo[4,5-d]pyridazinone (**11**)*

A mixture of isoxazolopyridazinone **4** or **10** (1.6 mmol), anhydrous K_2CO_3 (13.0 mmol) and the appropriate haloderivative (15.0 mmol) in anhydrous DMF (5 ml) was heated at 70–90 °C for 1–2 h under stirring. After dilution with cold water, the crude precipitate was recovered by suction.

*3.1.4. Synthesis of 3,6-dimethyl-4-phenylisoxazolo-[3,4-d]pyridazin-3(2H)-thione (**6**)*

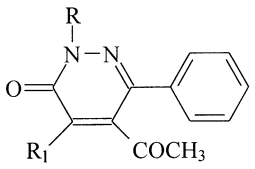
A mixture of 3,6-dimethyl-4-phenylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one [22] (1.6 mmol), P_2S_5 (10.0 mmol) in toluene (10 ml) was heated at 100 °C for 90 min. After cooling, the residue was filtered off and toluene was evaporated in vacuo affording **6** as yellow precipitate.

*3.1.5. General procedure for the synthesis of isoxazolo[3,4-d]pyridazine **9a-e***

7-Methylthiopyridazine **8** [18] (1.5 mmol) and the appropriate (cyclo)alkylamine (30.0 mmol) in EtOH (2 ml) were heated at 110–120 °C for 4–7 h in a sealed tube. After cooling the mixture was diluted with cold water. Compound **9a** was recovered by suction, **9b–d** were obtained after extraction with CH_2Cl_2 (3×20 ml) and evaporation in vacuo.

For compound **9e** the reaction was carried out in toluene at 130 °C for 7 h. After cooling, the organic layer was washed with water (10 ml) and the resultant aqueous layer extracted with ethyl acetate (3×15 ml). Compound **9e** was obtained by the evaporation in vacuum of the collected organic layers.

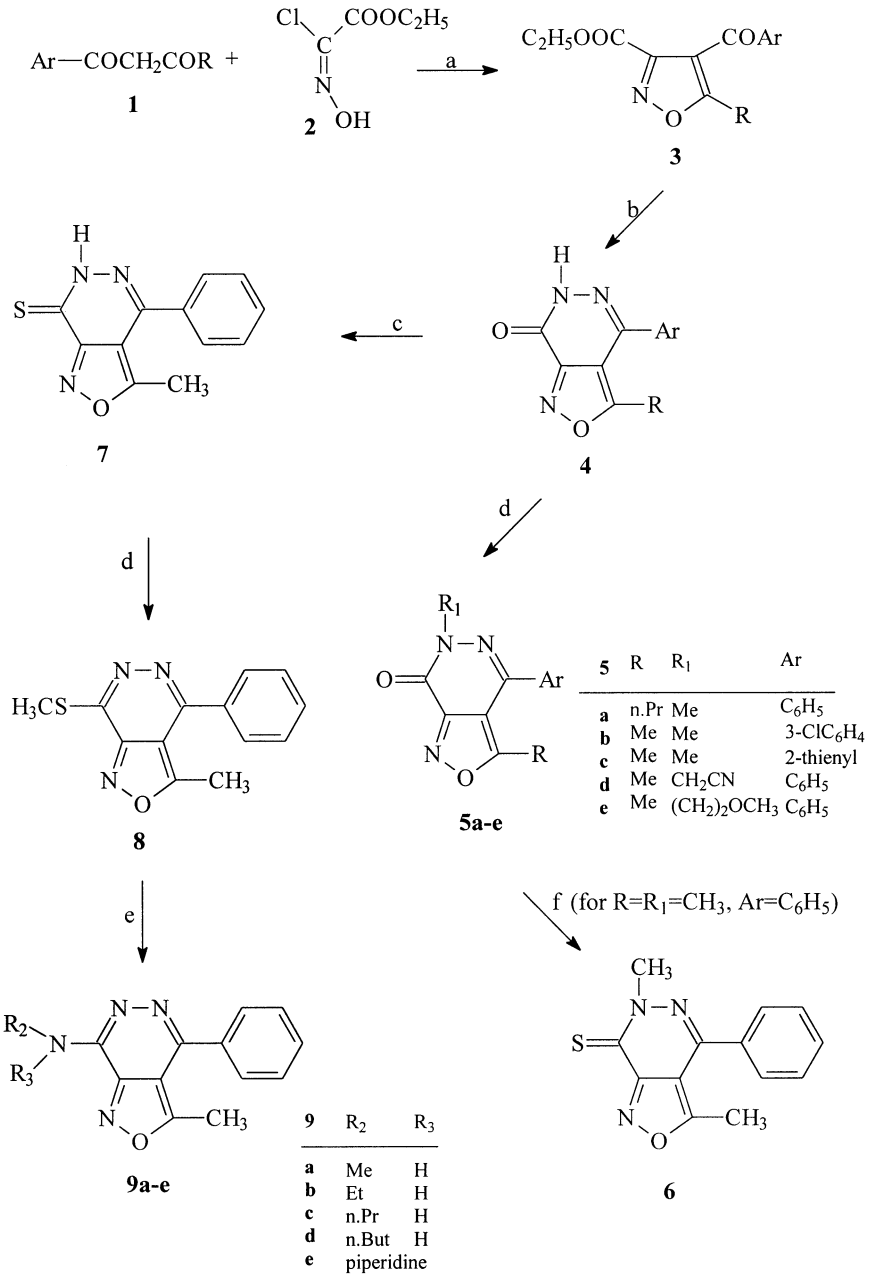
Table 3
Chemical and physical data of pyridazinones and their inhibitory activity against *Leishmania mexicana* PDE



(Q-V)

Comp.	R	R_1	Yield (%)	m.p. (°C)	Formula [f]	% inhibition [g]
Q [a]	CH_3	NO_2				0
R [b]	CH_3	Cl				10.7
S [b]	CH_3	Br				39.0
T [c]	CH_3	I				31.1
U [b]	CH_3	SCH_3				42.9
V [d]	CH_3	NHC_6H_5				0
15a [e]	$CH_2COOC_2H_5$	Cl	93	113-115	$C_{16}H_{15}ClN_2O_4$	42.5
15b [e]	$(CH_2)_2COOC_2H_5$	Cl	95	oil	$C_{17}H_{17}ClN_2O_4$	51.9
Cibacron						$IC_{50} = 8$ μM

[a] Ref. [25].
[b] Ref. [28].
[c] Ref. [29].
[d] Ref. [27].
[e] Compounds were purified by column chromatography using tolune–ethyl acetate 9:1 as eluent.
[f] C, N, H, analysis within ±0.4%.
[g] All compounds were tested at 100 μM.



Scheme 1. (a) EtONa, abs. EtOH; (b) hydrazine hydrate, EtOH; (c) Lawesson's reagent, toluene; (d) RX, DMF, K_2CO_3; (e) (cyclo)alkylamine; (f) P_2S_5, toluene.

*3.1.6. Synthesis of 4-chloro-3-methyl-7-phenylisoxazolo[4,5-d]pyridazinone (**12**)*

Et_3N (0.1 mmol) was added to a cooled suspension of compound **10** [19] (1.2 mmol) in $POCl_3$ (3 ml). Then the mixture was slowly heated at 100 °C under stirring for 7 h. The precipitate was recovered by suction after cooling and neutralization with 6 N NaOH.

*3.1.7. Synthesis of isoxazolo[4,5-d]pyridazines **13a–e***

Compounds **13a–e** were obtained following the procedure described above for **9a–d**. All products were recovered by filtration after heating at 110 °C for 1 h and the addition of water.

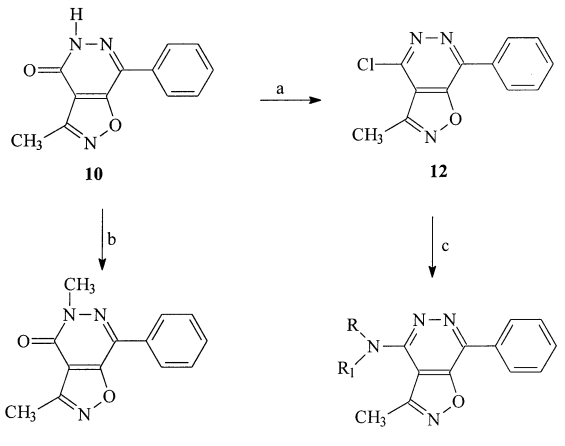
*3.1.8. General procedure for the synthesis of 5-acetyl-4-chloropyridazinones **15a,b***

A mixture of the appropriate 5-acetyl-4-nitroderivative **14** [20] (0.8 mmol), TEBA (24.0 mmol) in CH_3CN (3 ml) was refluxed for 1–2 h. After concentration, ice cold water was added and the mixture was extracted with CH_2Cl_2 (3 × 20 ml). Evaporation of the solvent in vacuo afforded the crude **15a,b** which were purified by column chromatography.

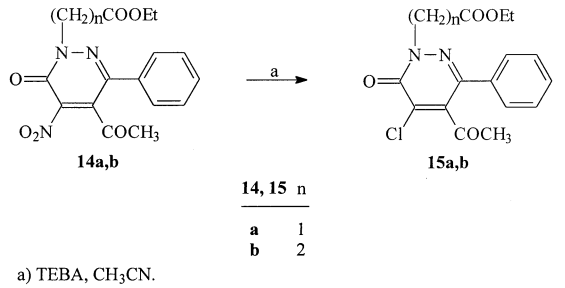
3.2. Biology

3.2.1. Phosphodiesterase assay

PDE activity was assayed at 100 μM [^{3}H]cAMP ([2,8-^{3}H]cAMP, 37 Ci.mmol^{-1}, from Amersham, UK) according to the method of Kinkaid and Manganiello [23]. The reactions were performed in a buffer containing 50 mM Hepes (pH 7.5), 0.1 mM EGTA, 8.3 mM Mg chloride and 0.2 mg/ml BSA in a final reaction volume of 300 μl, at 30 °C for 15 min. Hydrolysis of substrate did not exceed 20% under these conditions and cAMP–PDE activity was proportional to time and enzyme concentration. The soluble fraction of *L. mexicana* was used as a source of enzyme prepared as described in Rascón et al. [5]. Assays were run in triplicates and results are the average of at least two independent assays. For inhibition studies, each synthetic compound was dissolved in dimethyl sulfoxide (DMSO) and serially diluted to a final concentration of 100 μM in the assay. Controls in presence of DMSO were run parallel to samples. Special care was taken to avoid a final concentrations of DMSO over 1% in the assay which otherwise inactivates the enzyme.



Scheme 2. (a) $POCl_3$, Et_3N; (b) CH_3I, DMF, K_2CO_3; (c) (cyclo)alkylamine.



Scheme 3. (a) TEBA, CH_3CN.

Table 4
^{1}H NMR spectra of new compounds

Comp.	^{1}H NMR
3	1.00 (m, 6H, $CH_2CH_2CH_3$); 1.80 (m, 2H, $CH_2CH_2CH_3$); 2.80 (t, $J = 7.8$ Hz, 2H, $CH_2CH_2CH_3$); 7.50 (m, 5H, Ar); 10.0 (exch., br. s., 1H, NH).
4	0.90 (t, 3H, $CH_2CH_2CH_3$ and $COOCH_2CH_3$); 1.80 (m, 2H, $CH_2CH_2CH_3$); 2.90 (t, $J = 7.8$ Hz, 2H, $CH_2CH_2CH_3$); 4.10 (q, J = 7.8 Hz, 2H, $COOCH_2CH_3$); 7.50 (m, 5H, Ar).
5a	0.80 (t, $J = 7.8$ Hz, 3H, $(CH_2)_2CH_3$); 1.60 (m, 2H, CH_2CH_3); 2.80 (t, $J = 7.9$ Hz, $CH_2CH_2CH_3$); 3.80 (s, 3H, NCH_3); 7.55 (s, 5H, Ar).
5b	2.60 (s, 3H, CCH_3); 3.80 (s, 3H, NCH_3); 7.40–7.60 (m, 4H, Ar).
5c	2.85 (s, 3H, CCH_3); 3.80 (s, 3H, NCH_3); 7.30–7.60 (m, 4H, Ar).
5d	2.60 (s, 3H, CCH_3); 5.15 (s, 2H, NCH_2); 7.60 (m, 5H, Ar).
5e	2.55 (s, 3H, CCH_3); 3.40 (s, 3H, OCH_3); 3.80 (t, $J = 8.2$ Hz, NCH_2CH_2); 4.45 (t, $J = 8.1$ Hz, 2H, NCH_2CH_2); 7.55 (s, 5H, Ar).
6	2.60 (s, 3H, CCH_3); 4.20 (s, 3H, NCH_3); 7.60 (s, 5H, Ar).
9a	2.65 (s, 3H, CCH_3); 3.70 (d, $J = 6.5$ Hz, 2H, $NHCH_3$); 7.55 (s, 5H, Ar); 11.30–11.50 (exch. br. s., 1H, NH).
9b	1.50 (t, $J = 8.0$ Hz, 3H, CH_2CH_3); 1.80–2.10 (exch. br. s., 1H, NH); 2.70 (s, 3H, CCH3); 4.10–4.25 (m, 2H, CH_2CH_3); 7.55 (s, 5H, Ar).
9c	1.05 (t, $J = 8.1$ Hz, 3H, CH_2CH_3); 1.70–1.90 (m, 2H, $CH_2CH_2CH_3$); 2.65 (s, 3H, CCH_3); 3.75 (t, $J = 8.1$ Hz, 2H, CH_2NH); 5.40–5.65 (exch. br. s., 1H, NH); 7.45–7.75 (m, 5H, Ar).
9d	1.00 (t, $J = 8.0$ Hz, 3H, $(CH_2)_3CH_3$); 1.40–1.60 (m, 2H, $(CH_2)_2CH_2CH_3$); 1.70–1.85 (m, 2H, $CH_2CH_2CH_2CH_3$); 2.75 (s, 3H, CCH_3); 3.75 (t, $J = 7.9$ Hz, 2H, $NHCH_2$); 5.40–5.80 (exch. br. s., 1H, NH); 7.45–7.55 (m, 3H, Ar); 7.60–7.70 (m, 2H, Ar).
9e	2.70–2.85 (m, 6H, piperidine); 2.60 (s, 3H, CCH_3); 4.10–4.25 (m, 4H, piperidine); 7.45–7.65 (m, 5H, Ar).
11	2.75 (s, 3H, CCH_3); 3.95 (s, 3H, NCH_3); 7.45–7.55 (m, 3H, Ar); 8.10–8.20 (m, 2H, Ar).
12	2.85 (s, 3H, CH_3); 7.55–7.65 (m, 3H, Ar); 8.25–8.35 (m, 2H, Ar).
13a	2.75 (s, 3H, CCH_3); 3.35 (d, $J = 7.7$ Hz, 3H, $NHCH_3$); 4.85–5.00 (exch. br. s., 1H, NH); 7.45–7.60 (m, 3H, Ar); 8.35–8.45 (m, 2H, Ar).
13b	1.45 (t, $J = 8.0$ Hz, 3H, CH_2CH_3); 2.75 (s, 3H, CCH_3); 3.80–3.95 (m, 2H, CH_2CH_3); 4.85–5.00 (exch. br. s., 1H, NH); 7.40–7.60 (m, 3H, Ar); 8.35–8.45 (m, 2H, Ar).
13c	1.10 (t, $J = 8.1$ Hz, 3H, CH_2CH_3); 2.75–2.90 (m, 2H, CH_2CH_3); 2.75 (s, 3H, CCH_3); 3.75 (q, $J = 8.1$ Hz, CH_2NH); 4.90–5.05 (exch. br. s., 1H, NH); 7.40–7.60 (m, 3H, Ar); 8.35–8.45 (m, 2H, Ar).
13d	1.00 (t, $J = 8.0$ Hz, 3H, $(CH_2)_3CH_3$); 1.20–1.40 (m, 2H, $(CH_2)_2CH_2CH_3$); 1.70–1.85 (m, 2H, $CH_2CH_2CH_2CH_3$); 2.75 (s, 3H, CCH_3); 3.80 (t, $J = 7.9$ Hz, 2H, $NHCH_2$); 4.90–5.10 (exch. br. s., 1H, NH); 7.45–7.60 (m, 3H, Ar); 8.35–8.45 (m, 2H, Ar).
13e	1.60–1.90 (m, 6H, piperidine); 2.75 (s, 3H, CCH_3); 3.50–3.65 (m, 4H, piperidine); 7.45–7.60 (m, 3H, Ar); 8.40–8.50 (m, 2H, Ar).
15a	1.35 (t, $J = 8.1$ Hz, 3H, $COOCH_2CH_3$); 2.25 (s, 3H, $COCH_3$); 4.30 (q, $J = 8.1$ Hz, 2H, $COOCH_2CH_3$); 5.00 (s, 2H, NCH_2); 7.45 (s, 5H, Ar).
15b	1.25 (t, $J = 8.0$ Hz, 3H, $COOCH_2CH_3$); 2.20 (s, 3H, $COCH_3$); 2.95 (t, $J = 8.0$ Hz, 2H, $CH_2COOCH_2CH_3$); 4.15 (q, $J = 8.0$ Hz, 2H, $COOCH_2CH_3$); 4.65 (t, 7.9 Hz, 2H, NCH_2); 7.40–7.55 (m, 5H, Ar).

4. Result and discussion

All compounds were tested at 100 μM concentration for their ability to inhibit the activity of PDE extracted from *Leishmania mexicana* [5]. Cibacron blue was tested in the same conditions and used as reference compound. Results are reported in Tables 1–3, where the data obtained by testing all novel compounds and several previously synthesized analogues (**A**–**V**) [24–29] are indicated. Although none of the tested compounds showed a similar level of activity in comparison with Cibacron blue, some interesting results could be emphasized and preliminary structure–activity relationships could be obtained.

In the series of isoxazolo[3,4-*d*]pyridazinones (Table 1) the best results were furnished by compounds **E**, **G** and **5b**. The presence of a phenyl group in 4-position of the bicyclic system plays an important role. In fact replacement of this group with a methyl (**B**), 4-pyridyl (**I**) or the introduction in *para* position of a chlorine (**H**), was detrimental. The presence of a 2-thienyl group completely abolished the activity (**5c**). On the other hand, moving chlorine in the *meta* position (**5b**) completely restored the activity. It is interesting to observe that compound **11** which is the isomer of the previously examined **E**, was the most potent in this group (58.7% inhibition), probably indicating that two methyl and a phenyl group are the best arranged in this bicyclic system.

In position 3, the length of the carbon chain plays a fundamental role, the presence of the ethyl group being associated with the best activity (**G**, 55.4% inhibition). Homologation or elimination of this carbon chain resulted in the loss of activity (compounds **D** and **5a**). As regards the substituent in position 6, the methyl represents the best group (**E**), its homologation being very detrimental (**F**). Introduction in the same position of a functionalized alkyl chain gave interesting results for $R_1 = (CH_2)_2OCH_3$ (**5e**) and $(CH_2)_2COOH$ (**L**). It is interesting to observe that, with the exception of compound **K**, the carboxylic derivatives are more potent than the corresponding esters. Finally, replacement of the carbonyl dipole with C=S group led to a dramatic loss of activity (**6**), indicating that the hydrogen bond acceptor properties could play a significant role in the activity.

In Table 2 the data related to isoxazolo[3,4-*d*]pyridazines and their [4,5-*d*]condensed isomers **13a**–**e** are reported. Obtained data seem to indicate that the presence of the alkoxy and methylthio groups in position 7 of the isoxazolo[3,4-*d*]pyridazine system is detrimental for the activity. Among the different alkyl- and cycloalkylamino derivatives **9a**–**e**, compound **9d** emerged (51.8% inhibition). On the contrary, in the isomeric series **13a**–**e** no compounds showed interesting activity. The data obtained for a group of 5-acetyl-6-phenylpyridazinones are reported in Table 3. In the series of compounds **Q**–**V** can be observed that the presence of both oxidated and reduced nitrogen functions is detrimental (**Q** and **V**); among the halo-derivatives the 4-bromo was the most potent. Finally, the presence of a methylthio group in position 4 (**U**) was associated with a good level of activity. When in compound **R** the methyl group at position 2 was replaced with an ethylacetate (**15a**) or ethylpropionate (**15b**) residue, a significant improvement of activity was observed.

Further studies are in progress in order to obtain more potent and selective inhibitors.

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