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CONCISE ARTICLE

(Pd/C-mediated)coupling–iodocyclization–coupling strategy in discovery of novel PDE4 inhibitors: a new synthesis of pyrazolopyrimidines†

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Pyranones fused with a pyrazolopyrimidine moiety were prepared *via* regioselective construction of pyranone ring using (Pd/C-mediated)coupling–iodocyclization followed by Sonogashira/Heck/Suzuki reactions. The pyrazolopyrimidine based reactant required was obtained *via* a new H_3PO_3 mediated condensation reaction. This strategy has led to the discovery of a novel and potentially safe PDE4 inhibitor.

Phosphodiesterase type 4 (PDE4), exists in four different isoforms (PDE4A, B, C and D), is one of eleven isozymes, and is specific for the hydrolysis of cAMP to AMP.[1–3] In inflammatory and immune cells, the inhibition of cellular responses, including the production and/or release of proinflammatory mediators, cytokines, and active oxygen species, is associated with elevated levels of cAMP. Thus, inhibition of PDE4 was expected to be beneficial for the treatment of inflammatory and immunological diseases including asthma and chronic obstructive pulmonary disease (COPD).[1,3–5] However, many PDE4 inhibitors have been discontinued from clinical trials due to a narrow window between efficacy and the undesired side effects such as nausea and emesis.[1,2,4–7] More recently, cardiovascular effects of PDE4 inhibitors have been reported.[8] While roflumilast (Daxas®, Nycomed) was launched in Europe recently, the search for a new chemical class for the evaluation of selective PDE4 inhibitory properties with improved therapeutic indexes is desirable.

The PDE4B mRNA has been reported to be up-regulated due to the exposure of monocytes to bacterial endotoxin LPS.[9]

Further, circulating leukocytes from PDE4B but not PDE4A or PDE4D knockout mice displayed ~90% reduction in LPS induced TNF-α production indicating the key role of the PDE4B subtype in promoting inflammation.[10] Thus, targeting PDE4B may be a more balanced approach to treat the inflammatory component of COPD without causing or minimizing the emetic side effects.[1] Notably, design of PDE4D allosteric modulators

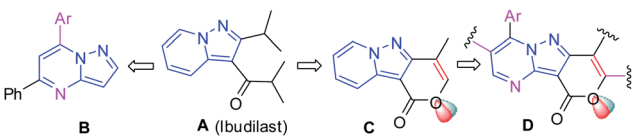
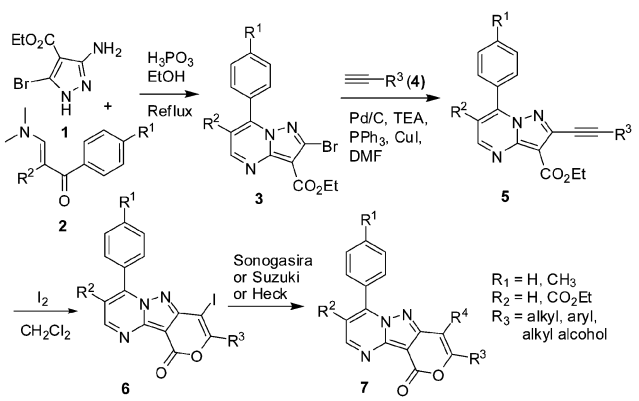


Fig. 1 Design of a novel chemical class **D** from **A** and **B**.



Scheme 1 Preparation of the other starting compound **2**.

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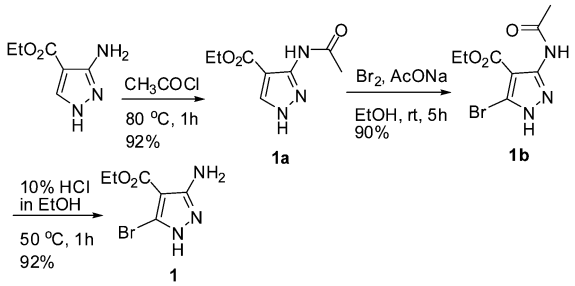
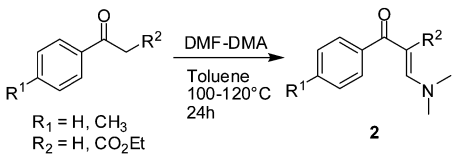
† Electronic supplementary information (ESI) available: Experimental procedures, spectral data and (^{1}H and ^{13}C NMR, MS and HRMS) spectra for all new compounds. CCDC 832934 and 832935. For ESI and crystallographic data in CIF or other format see DOI: 10.1039/c2md00273f



Scheme 2 Preparation of the starting pyrazole derivative **1**.

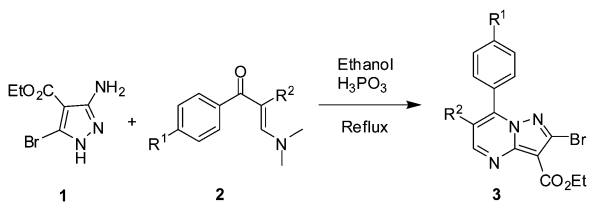


Scheme 3 Synthesis of novel pyranopyrazolopyrimidine derivatives (**7**).

for enhancing cognition with improved safety has been reported recently.[11]

Ibudilast **A** (Fig. 1), approved in Japan for the treatment of asthma and stroke,[12] is a pyrazole based PDE4 inhibitor. Recently, we have reported 7-(hetero)aryl substituted pyrazolopyrimidine **B** (Fig. 1) derived from **A** as a potential scaffold for the development of novel PDE4 inhibitors.[13a] In continuation of this effort we became interested in design, synthesis and evaluation of novel compounds represented by **D** (Fig. 1) for their PDE4 inhibitory properties.[13] The fused heterocyclic structure **D** was arrived at *via* **C** by (i) connecting the two side chains of **A** in an intramolecular fashion followed by introducing an oxygen and a double bond in the newly formed six-membered ring and (ii) introducing some of the structural features of **B**. Since the new scaffold **D** possesses four potential positions including the aryl group to introduce diversity a library generation based on **D** was therefore undertaken. Herein, we report a (Pd/C-mediated)coupling–iodocyclization–coupling strategy and a new synthesis of pyrazolopyrimidine to generate a compound library based on **D** leading to the identification of a novel inhibitor of PDE4B.

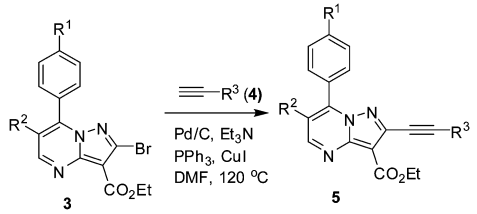
Table 1 Preparation of 2-bromopyrazolo[1,5-*a*]pyrimidines (**3**)[a]

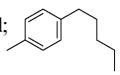


Entry	Ketone (**2**) R^1, R^2	Time (h)	Product (**3**)	Yield[b] (%)
1	H, H (**2a**)	4.0	**3a**	75
2	CH_3, H (**2b**)	4.0	**3b**	74
3	H, CO_2Et (**2c**)	5.0	**3c**	70

[a] All the reactions were carried out by using compounds **1** (1.0 mmol), **2** (1.0 mmol) and H_3PO_3 (1.0 mmol) in ethanol at refluxing temperature. [b] Isolated yield.

Table 2 Pd/C-mediated synthesis of 2-alynyl pyrazolo[1,5-*a*]pyrimidines (**5**)[a]



Entry	Bromopyrazolo-pyrimidine (**3**)	Alkyne (**4**; R^3 =)	Time (h)	Product (**5**)	Yield[b] (%)
1	**3a**	**4a**; *n*-Hexyl	15	**5a**	70
2	**3a**	**4b**; *n*-Pentyl	15	**5b**	65
3	**3a**	**4c**; 	12	**5c**	65
4	**3a**	**4d**; 	18	**5d**	68
5	**3a**	**4e**; 	12	**5e**	60
6	**3a**	**4f**; 	10	**5f**	70
7	**3a**	**4g**; $-CH_2CH_2OH$	10	**5g**	67
8	**3b**	**4a**; *n*-Hexyl	15	**5h**	70
9	**3b**	**4e**	12	**5i**	65
10	**3c**	**4a**	15	**5j**	60

[a] All the reactions were carried out by using 3 (1.0 mmol), terminal alkyne (1.5 mmol), 1 : 4 : 2 ratio of Pd/C–PPh_3–CuI and Et_3N (3 equiv) in DMF at 120 °C. [b] Isolated yield.

The synthesis of target compounds (**7**) is shown in Scheme 1. The key compound (**1**) required for our synthesis was prepared from ethyl 3-amino-1*H*-pyrazole-4-carboxylate *via* *N*-acylation followed by bromination and subsequent deacylation

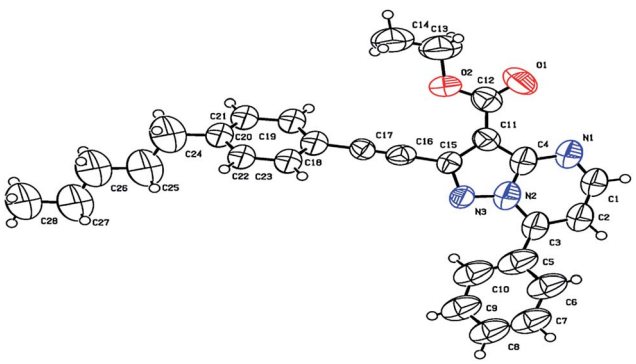
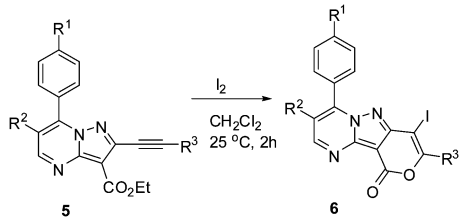


Fig. 2 X-ray crystal structure of **5d** (ORTEP diagram). Thermal ellipsoids are drawn at 50% probability level.

Table 3 Synthesis of 10*H*-pyrano[4′,3′:3,4]pyrazolo [1,5-*a*]pyrimidin-10-one derivatives (**6**) *via* iodocyclization of alkynes **5**[a]



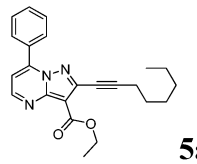
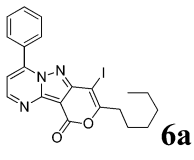
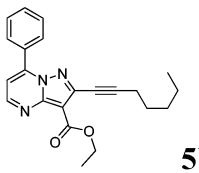
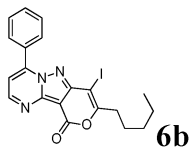
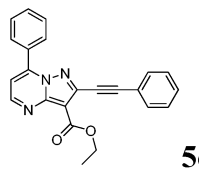
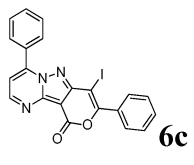
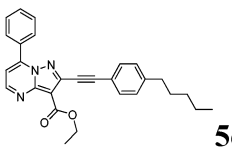
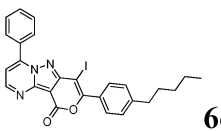
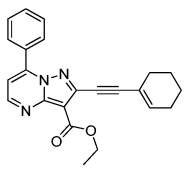
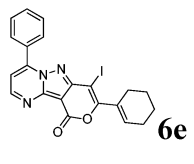
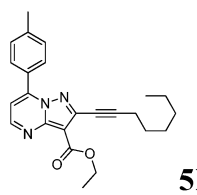
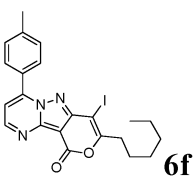
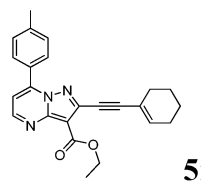
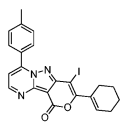
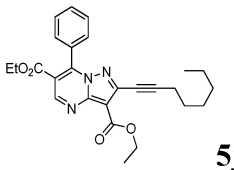
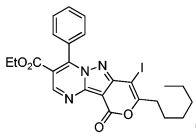
Entry	2-Alkynyl pyrazolo[1,5-*a*]-pyrimidine (**5**)	Product (**6**)	Yield[b] (%)
1	 **5a**	 **6a**	92
2	 **5b**	 **6b**	91
3	 **5c**	 **6c**	92
4	 **5d**	 **6d**	90
5	 **5e**	 **6e**	93
6	 **5h**	 **6f**	88
7	 **5i**	 **6g**	90

Table 3 (*Contd.*)

Entry	2-Alkynyl pyrazolo[1,5-*a*]-pyrimidine (**5**)	Product (**6**)	Yield[b] (%)
8	 **5j**	 **6h**	85

[a] All the reactions were carried out by using 5 (1.0 mmol), iodine (2.0 mmol) in DCM at 25 °C for 2 h. [b] Isolated yield.

(Scheme 2).[14a] Other reactants **2** were prepared from appropriate aryl ketones following a known procedure (Scheme 3).[14b] 2-Bromopyrazolo[1,5-*a*]pyrimidines[14c] (**3**) prepared *via* a new H_3PO_3-mediated[14d] condensation reaction of **1** and **2** (Table 1) were reacted with a variety of terminal alkynes (**4**) in the presence of Pd/C under Sonogashira conditions (Table 2).[15] A variety of 2-alkynyl pyrazolopyrimidines (**5**) were prepared and the molecular structure of a representative compound **5d** was determined unambiguously by X-ray crystallographic analysis (Fig. 2).[17a] Intramolecular regioselective iodocyclization[15,16] of alkyne **5** yielded corresponding 7-iodo derivatives (**6**, Table 3). The molecular structure of **6e** (**6**; R^1, R^2 = H, R^3 = 1-cyclohexenyl) was determined unambiguously by X-ray crystallographic analysis (Fig. 3).[17b]

The iodo derivatives (**6**) were used for further structural elaboration under Sonogashira or Suzuki or Heck reaction conditions to give 7-alkynyl, aryl and alkenyl derivatives (**7**) (Table 4).

Most of the compounds (**7**) synthesized were evaluated for their PDE4 inhibitory properties *in vitro*. The report[11] that PDE4D inhibition by allosteric inhibitors (maximum inhibition, I_{max} 80–90%) did not cause emetic side effects has raised a possibility that PDE4B inhibitors with partial but not complete inhibition of PDE4D (I_{max} of ~60 to 80%) could be developed to treat COPD and asthma without causing emetic side effects. Thus our goal was to identify inhibitors possessing such a balanced selectivity towards PDE4B. Initially, PDE4B inhibitory potential was assessed by using PDE4B enzyme isolated

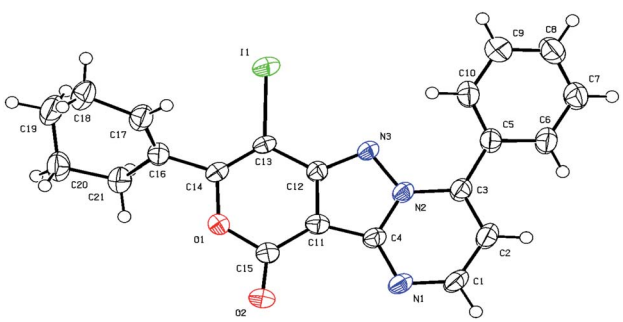
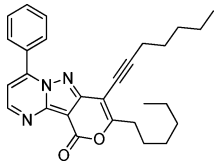
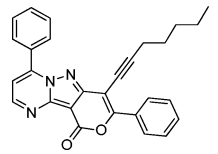
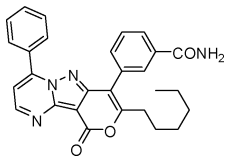
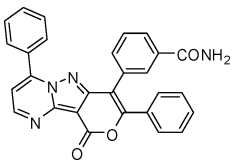
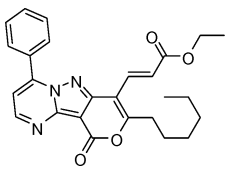
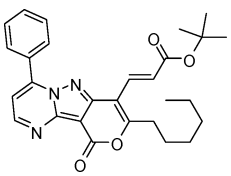
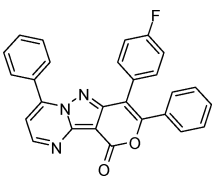
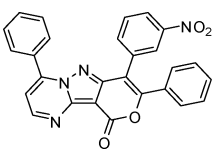
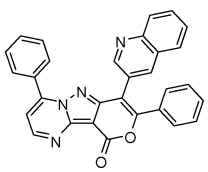


Fig. 3 X-ray crystal structure of **6e** (ORTEP diagram). Thermal ellipsoids are drawn at 50% probability level.

Table 4 Synthesis of 7-substituted 10*H*-pyrano[4′,3′:3,4] pyrazolo[1,5-*a*] pyrimidin-10-ones (**7**) from compounds **6**

Entry	Product (**7**)	Compound **6**	Time (h)	Yield[a] (%)
1	 **7a**	**6a**	3.0	93
2	 **7b**	**6c**	3.0	92
3	 **7c**	**6a**	2.0	91
4	 **7d**	**6c**	2.0	91
5	 **7e**	**6a**	2.0	91
6	 **7f**	**6a**	2.0	92
7	 **7g**	**6c**	2.0	86
8	 **7h**	**6c**	2.0	82
9	 **7i**	**6c**	2.0	80

[a] Isolated yield.

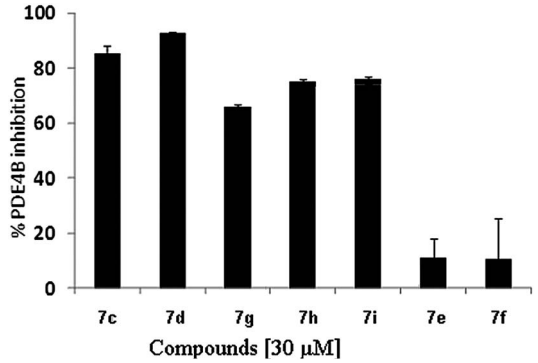


Fig. 4 Inhibition of PDE4B by compounds **7** (enzyme assay).

from *Sf9* cells.[18a] Among all the compounds tested **7c**, **7d**, **7g**, **7h** and **7i** showed significant inhibition of PDE4B (*i.e.* 85, 93, 66, 74 and 74% respectively) at 30 μM (Fig. 4) whereas other compounds *e.g.* **7e** and **7f** were inactive. The compounds **7a** and **7b** showed ~50% inhibition of PDE4B at 30 μM. These observations suggested that the presence of an aryl group at C-3 of the pyranone ring was important and a $-CONH_2$ group attached to this aryl ring (*e.g.* **7c** and **7d**) played a key role in PDE4B inhibition. Notably, both aryl and alkyl groups were tolerated at C-2 of the pyranone ring (*e.g.* **7c** *vs.* **7d**). Compound **7d** was then tested in the cell based cAMP reporter assay in HEK 293 cells and its TNF-α inhibitory activity[19] was measured in lipopolysaccharide (LPS) stimulated RAW 264.7 cells.[18b] Rolipram, a well known inhibitor of PDE4 was used as a reference compound (Table 5). Based on the data generated (Table 5 and Fig. 5–7, see ESI† also) compound **7d** was identified as a promising inhibitor of PDE4.

Preliminary safety evaluation of compound **7d** was carried out in a zebrafish embryo model. Zebrafish embryos were examined for mortality, scored for phenotypic effects and locomotor activity. All embryos in the control group survived the entire duration of the study and did not show any apparent effects on phenotype or locomotor activity. One embryo was found dead in both rolipram groups (10 and 20 μM) at 1 and 24 h time points, respectively. All the other embryos in both the rolipram exposed groups exhibited lordosis (inward curvature of the vertebrae), and mild to moderate effects on locomotor activity during the course of treatment. No mortality was observed at any time point in the embryos exposed to compound **7d**; though mild effects on locomotor activity were noticed in **7d** groups (10 and 20 μM) at 6 and 24 h, respectively. All the embryos were found dead in the 100 μM Terfenadine (positive control) showing severe defects in

Table 5 *In vitro* data of compound **7d**

		Compounds	
Assay	PDE4 subtype	Rolipram	**7d**
Enzymatic assay (IC_{50}, μM)	PDE4B1	0.94 ± 0.24	1.33 ± 0.64
	PDE4D2	0.88 ± 0.28	2.84 ± 0.64
Reporter assay (EC_{50}, μM)	PDE4A4	2.62 ± 1.29	11.66 ± 1.10
	PDE4B1	0.16 ± 0.11	6.32 ± 0.40
	PDE4D3	0.67 ± 0.42	9.62 ± 0.75
TNF-α assay (IC_{50}, μM)		0.19 ± 0.09	0.45 ± 0.29

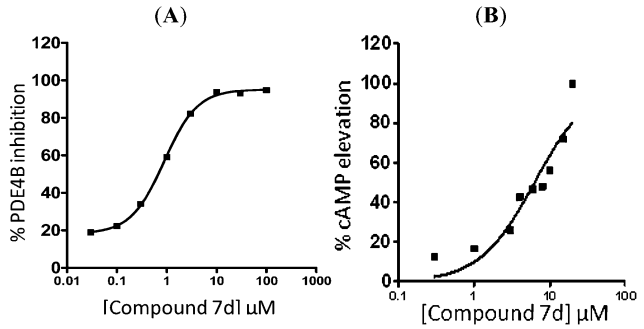


Fig. 5 PDE4B inhibition by **7d**: (A) enzyme assay and (B) cell based reporter assay.

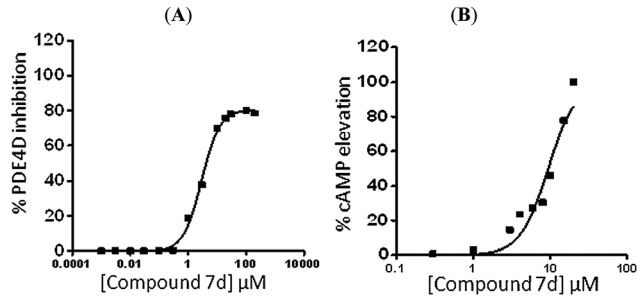


Fig. 6 PDE4D inhibition by **7d**: (A) enzyme assay and (B) cell based reporter assay.

locomotor activity at initial time points validating the test procedure. Embryos exposed to either rolipram or compound **7d** did not display any cardiac arrhythmias under basal conditions (data not shown).

Each embryo was assigned a lesion score (5-0) based on the morphological score assessment guideline suggested by Panzica-Kelly *et al.*[20] and the results are shown in Fig. 8. Statistical analysis of 24 h time point data was performed by the Kruskal–Wallis one-way analysis of variance by ranks followed by Dunn's *post hoc* test. Incubation of embryos with rolipram (at 10 or 20 µM) but not with compound **7d** for 24 h resulted in statistically significant increase in the abnormal lesions of embryos ($p < 0.002$). Collectively, the data obtained demonstrate that compound **7d** was less toxic and was better tolerated than rolipram in zebrafish embryos under the experimental conditions tested.

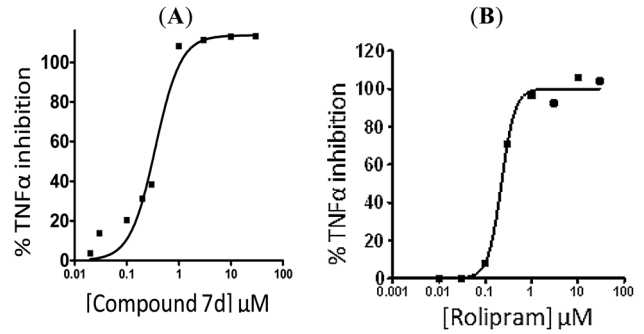


Fig. 7 TNF-α inhibition by (A) compound **7d** and (B) rolipram.

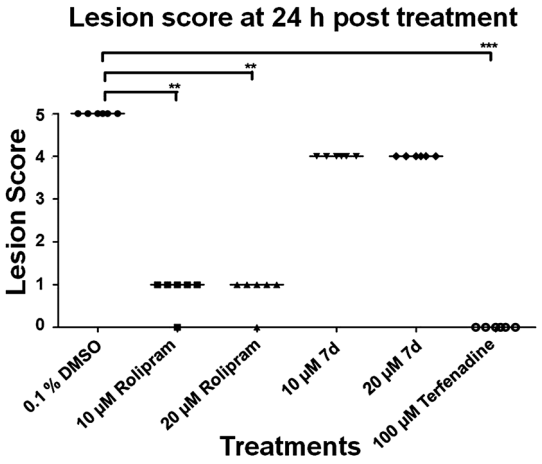


Fig. 8 Evaluation of **7d** and rolipram in zebrafish embryo toxicity model. See ESI† for scoring guidelines. Kruskal–Wallis, Dunn's multiple comparison *post hoc* test. $*p < 0.05$, $**p < 0.002$, $***p < 0.0001$.

In summary, a new chemistry strategy *i.e.* (Pd/C-mediated) coupling–iodocyclization–coupling has been explored to discover PDE4 inhibitors. This effort resulted in a new H_3PO_3 mediated facile synthesis of pyrazolopyrimidine under mild and microwave free conditions. A pyranone ring fused with a pyrazolopyrimidine moiety has been investigated and exemplified as a potential template for PDE4 inhibitors. The lead compound was found to be less toxic and was better tolerated than rolipram in zebrafish embryos. Overall, a novel and seemingly safer PDE4B inhibitor has been discovered for the potential treatment of COPD and asthma.

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Notes and references

1 A. Kodimuthali, S. Jabaris and M. Pal, *J. Med. Chem.*, 2008, **51**, 5471.
2 M. D. Houslay, P. Schafer and K. Y. J. Zhang, *Drug Discovery Today*, 2005, **10**, 1503.
3 P. J. Barnes and R. A. Stockley, *Eur. Respir. J.*, 2005, **25**, 1084.
4 F. J. Soto and N. A. Hanania, *Curr. Opin. Pulm. Med.*, 2005, **11**, 129.
5 B. J. Lipworth, *Lancet*, 2005, **365**, 167.
6 J. O. Odingo, *Expert Opin. Ther. Pat.*, 2005, **15**, 773.
7 V. B. Smith and D. Spina, *Curr. Opin. Invest. Drugs*, 2005, **6**, 1136.
8 Y. J. Rao and L. Xi, *Acta Pharmacol. Sin.*, 2009, **30**, 1.
9 D. Ma, P. Wu, R. W. Egan, M. M. Billah and P. Wang, *Mol. Pharmacol.*, 1999, **55**, 50.
10 S. L. Jin, L. Lan, M. Zoudilova and M. Conti, *J. Immunol.*, 2005, **175**, 1523.
11 A. B. Burgin, O. T. Magnusson, J. Singh, P. Witte, B. L. Staker, J. M. Bjornsson, M. Thorsteinsdottir, S. Hrafnsdottir, T. Hagen, A. S. Kiselyov, L. J. Stewart and M. E. Gurney, *Nat. Biotechnol.*, 2010, **28**, 63.
12 L. C. Gibson, S. F. Hastings, I. McPhee, R. A. Clayton, C. E. Darroch, A. Mackenzie, F. L. Mackenzie, M. Nagasawa, P. A. Stevens and S. J. Mackenzie, *Eur. J. Pharmacol.*, 2006, **538**, 39.
13 (*a*) A. Kodimuthali, R. Gupta, K. V. L. Parsa, P. L. Prasunamba and M. Pal, *Lett. Drug Des. Discovery*, 2010, **7**, 402; (*b*) G. R. Reddy, T. R. Reddy, S. C. Joseph, K. S. Reddy, L. S. Reddy, P. M. Kumar, G. R. Krishna, C. M. Reddy, D. Rambabu, R. Kapavarapu, C. Lakshmi, T. Meda, K. K. Priya, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2011, **47**, 7779; (*c*) S. Pal, S. Durgadas, S. B. Nallapati, K. Mukkanti, R. Kapavarapu, C. L. T. Meda, K. V. L. Parsa and

M. Pal, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 6573; (*d*) P. M. Kumar, K. S. Kumar, P. K. Mohakhud, K. Mukkanti, R. Kapavarapu, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2012, **48**, 431.

14 (*a*) P. Toto, J. Chenault, A. E. Hakmaoui, M. Akssira and G. Guillaumet, *Synth. Commun.*, 2008, **38**, 674; (*b*) R. SanMartin, E. M. de Marigorta and E. A. Dominguez, *Tetrahedron*, 1994, **50**, 2255; (*c*) L. Ming, W. Shuwen, W. Lirong, Y. Huazheng and Z. Xiuli, *J. Heterocycl. Chem.*, 2005, **42**, 925; (*d*) To the best of our knowledge this is the first example of H_3PO_3-mediated synthesis of pyrazolo[1,5-*a*]pyrimidines.

15 (*a*) D. K. Barange, V. R. Batchu, D. Gorja, V. R. Pattabiraman, L. K. Tatini, J. M. Babu and M. Pal, *Tetrahedron*, 2007, **63**, 1775; (*b*) M. Pal, *Synlett*, 2009, 2896.

16 For recent and leading examples, see: (*a*) R. Mancuso, S. Mehta, B. Gabriele, G. Salerno, W. S. Jenks and R. C. Larock, *J. Org. Chem.*, 2010, **75**, 897; (*b*) A. K. Verma, T. Aggarwal, V. Rustagi and R. C. Larock, *Chem. Commun.*, 2010, **46**, 4064; (*c*) S. Mehta and R. C. Larock, *J. Org. Chem.*, 2010, **75**, 1652; (*d*) C.-H. Cho, B. Neuenswander and R. C. Larock, *J. Comb. Chem.*, 2010, **12**, 278.

17 (*a*) Crystal data of **5d**: molecular formula = $C_{28}H_{27}N_3O_2$, formula weight = 437.53, crystal system = monoclinic, space group = *P*21/*C*, a = 15.2480 (13) Å, b = 12.5719(10) Å, c = 14.0243 (12) Å, V = 2423.6 (4) Å^3, T = 296 K, Z = 2, D_c = 0.10 Mg m^{-3}, μ(Mo-Kα) = 0.08 mm^{-1}, 14 662 reflections measured, 3278 independent reflections, 2433 observed reflections [$I > 2.0\sigma(I)$], R_1_obs = 0.042, goodness of fit = 2.591. Crystallographic data (excluding structure factors) for **5d** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 832934; (*b*) Crystal data of **6e**: molecular formula = $C_{21}H_{16}IN_3O_2$, formula weight = 469.27, crystal system = monoclinic, space group = *P*21/*C*, a = 7.3912 (4) Å, b =12.3586(6) Å, c = 20.4375 (10) Å, V = 1861.53 (9) Å^3, T = 296 K, Z = 4, D_c = 1.264 Mg m^{-3}, μ(Mo-Kα) = 1.74 mm^{-1}, 15 729 reflections measured, 4043 independent reflections, 3497 observed reflections [$I > 2.0\sigma(I)$], R_1_obs = 0.024, goodness of fit = 0.834. Crystallographic data (excluding structure factors) for **6e** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 832935.

18 (*a*) P. Wang, J. G. Myers, P. Wu, B. Cheewatrakoolpong, R. W. Egan and M. M. Billah, *Biochem. Biophys. Res. Commun.*, 1997, **19**, 320; (*b*) K. V. Parsa, L. P. Ganesan, M. V. Rajaram, M. A. Gavrilin, A. Balagopal, N. P. Mohapatra, M. D. Wewers, L. S. Schlesinger, J. S. Gunn and S. Tridandapani, *PLoS Pathog.*, 2006, **2**, 681.

19 For our effort on identification of TNF-α inhibitors, see: (*a*) K. S. Kumar, P. M. Kumar, K. A. Kumar, M. Sreenivasulu, A. A. Jafar, D. Rambabu, G. R. Krishna, C. M. Reddy, R. Kapavarapu, K. Shivakumar, K. K. Priya, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2011, **47**, 5010; (*b*) K. S. Kumar, P. M. Kumar, M. A. Reddy, Md. Ferozuddin, M. Sreenivasulu, A. A. Jafar, G. R. Krishna, C. M. Reddy, D. Rambabu, K. S. Kumar, S. Pal and M. Pal, *Chem. Commun.*, 2011, **47**, 10263.

20 J. M. Panzica-Kelly, C. X. Zhang, T. L. Danberry, A. Flood, J. W. DeLan, K. C. Brannen and K. A. Augustine-Rauch, *Birth Defects Res., Part B*, 2010, **89**, 382.

