Electronic Supplementary Information

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

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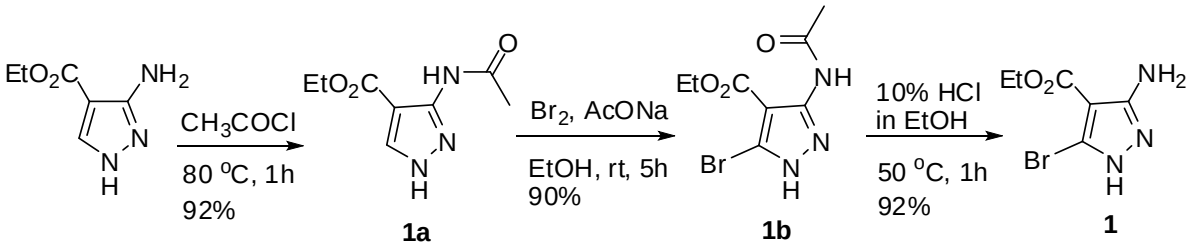
Experimental Section:

Chemistry

General methods: Unless stated otherwise, reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F_{254}), visualizing with ultraviolet light or iodine spray. Column chromatography was performed on silica gel (60-120 mesh) using distilled petroleum ether and ethyl acetate. ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$/DMSO-*d6* solution using 400 and 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.0) as internal standard and expressed in parts per million. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet) as well as b (broad). Coupling constants *(J)* are given in hertz. Infrared spectra were recorded on a FTIR spectrometer. Melting points were determined by using a Buchi melting point B-540 apparatus. MS spectra were obtained on a mass spectrometer. HRMS was determined using waters LCT premier XETOF ARE-047 apparatus.

General procedure for the preparation of ethyl 3-amino-5-bromo-1H-pyrazole-4-carboxylate (1)



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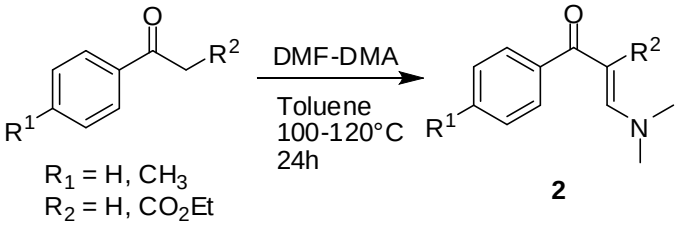
Step 1: 3-Amino-1H-pyrazole-4-carboxylic acid ethyl ester (10 g, 64.5 mmol) was added into acetyl chloride (50 mL) at room temperature. After stirring for 5 min, the mixture was heated at 80 °C for 1.0 h and cooled to 50 °C. The excess of acetyl chloride was evaporated off under reduced pressure, 10% sodium bi carbonate solution (100 mL) was added, and the resulting mixture was stirred for 1.0 h, the white solid precipitate was filtered off and dried to give ethyl 3-acetamido-1*H*-pyrazole-4-carboxylate (**1a**) (l1.7 g, 92%).

Step 2: To a mixture of ethyl 3-acetamido-1H-pyrazole-4-carboxylate **1a** (1 mmol), sodium acetate (8 mmol) in water (25 vol) and ethanol (25 vol) was added bromine (4 mmol) at room temperature. After stirring for 5.0 h, sodium bi carbonate solution (100 mL) was added, and the resulting mixture was stirred for 1.0 h, the white solid precipitate was filtered off and dried to give

ethyl 3-acetamido-5-bromo-1*H*-pyrazole-4-carboxylate **(1b**) (12.6 g, 90%).

Step 3: Ethyl 3-acetamido-5-bromo-1H-pyrazole-4-carboxylate (**1b**) was added in to 10% ethanol HCl (50 mL) at room temperature. After stirring for 5 min, the mixture was heated at 50 °C for 1.0 h. The excess of ethanol HCl was evaporated off under reduced pressure, diisopropyl ether was added (50 mL), and the resulting mixture was stirred for 1.0 h, the white solid was filtered off and dried to give ethyl 3-amino-5-bromo-1*H*-pyrazole-4-carboxylate (**1**) (Yield 92%).

General procedure for the preparation of 2-benzoyl-3-(dimethylamino) derivatives (2a-c)



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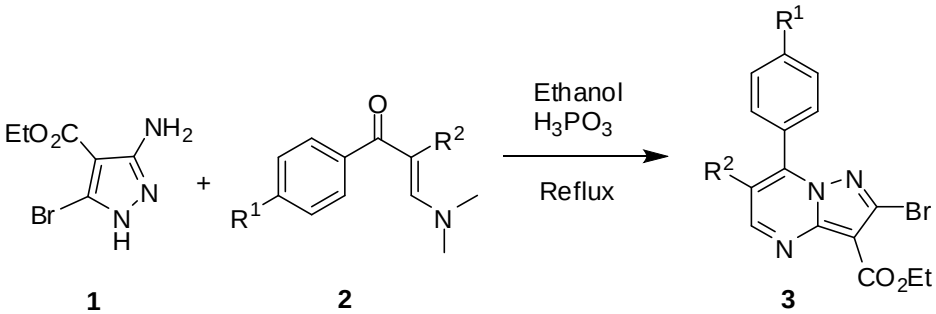
Appropriately substituted acetophenone (1 mmol) was added in to DMF-DMA (20 mL) at room temperature. After stirring for 5 min, the mixture was heated at 100-120 °C for 24.0 h and cooled to 50 °C. The excess of DMF-DMA was evaporated off under reduced pressure. The residue was triturated with diisopropyl ether; the solid precipitate was filtered and washed with diisopropyl ether to give the desired product.

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



1 **2** **3**

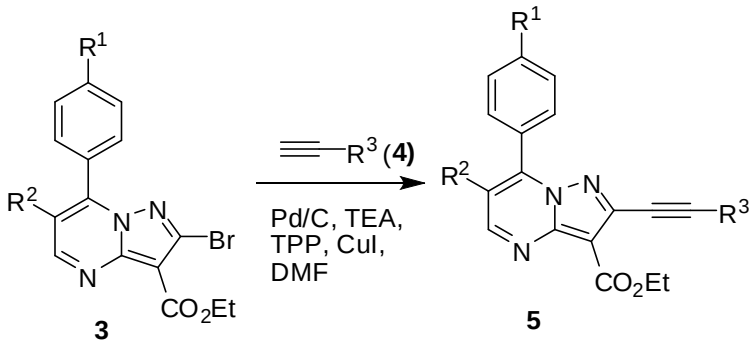
Entry	Ketone (**2**) R^1, R^2	Time (h)	Product (**3**)	%Yield[b]
1.	H, H (**2a**)	4.0	**3a**	75.0

2.	CH_3, H **(2b)**	4.0	**3b**	74.0
3.	H, CO_2Et (**2c**)	5.0	**3c**	70.0

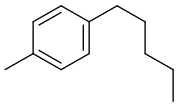
[a]All the reactions were carried out by using compound **1** (1.0 mmol), **2** (1.0 mmol) and H_3PO_3 (1.0 mmol) in ethanol at refluxing temperature. [b] Isolated yield.

A typical procedure: To a mixture of ethyl 3-amino-5-bromo-1*H*-pyrazole-4-carboxylate (**1**) (1 mmol) and 3-(dimethylamino)-1-phenylprop-2-en-1-one (**2a**) (1 mmol) in ethanol (100 mL) was added H_3PO_3 (1 mmol) at room temperature. After stirring for 5 min, the mixture was heated at reflux temperature for 6.0 h and cooled to 0-5°C. The solid precipitate was filtered and washed with diisopropyl ether to give the desired product.

Pd/C-mediated synthesis of 2-alkynyl pyrazolo[1,5-a]pyrimidines (5).[a]



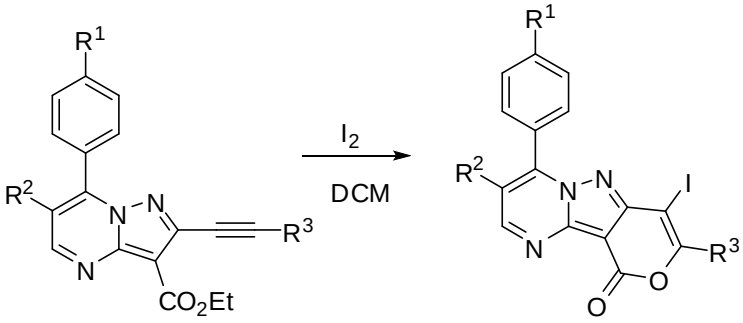
Entry	Bromo Pryazolo Pyrimidine (**3**)	Alkyne (**4**; R^3 =)	Time (h)	Product (**5**)	%yield[b]
1.	**3a**	**4a;** n-Hexyl	15.0	**5a**	70.0

2.	**3a**	**4b**; n-Pentyl	15.0	**5b**	65.0
3.	**3a**	**4c;** 	12.0	**5c**	65.0
4.	**3a**	**4d;** 	18.0	**5d**	68.0
5.	**3a**	**4e;** 	12.0	**5e**	60.0
6.	**3a**	**4f;** 	10.0	**5f**	70.0
7.	**3a**	**4g;** - CH_2CH_2OH	10.0	**5g**	67.0
8.	**3b**	**4a;** n-Hexyl	15.0	**5h**	70.0
9.	**3b**	**4e;** 	12.0	**5i**	65.0
10.	**3c**	**4a;** n-Hexyl	15.0	**5j**	60.0

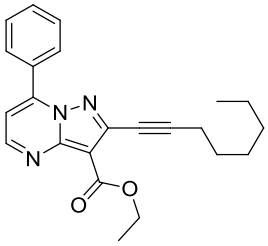
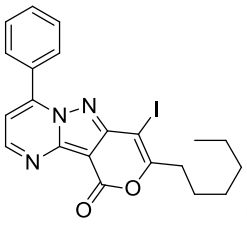
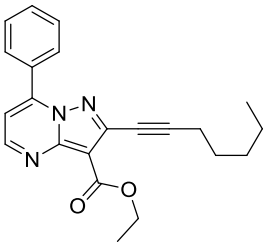
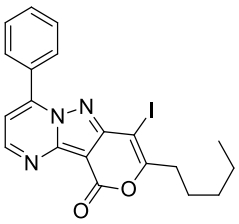
[a]All the reactions were carried out by using 3 (1.0 mmol), terminal alkyne (1.5 mmol), 1:4:2 ratio of Pd/C–PPh_3–CuI and TEA (3 equiv) in DMF at 120°C. [b] Isolated yield.

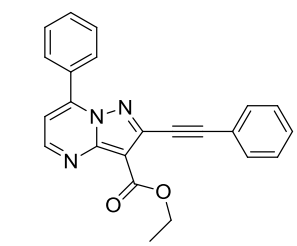
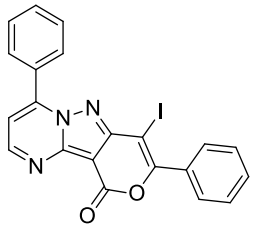
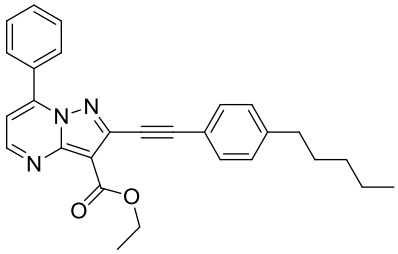
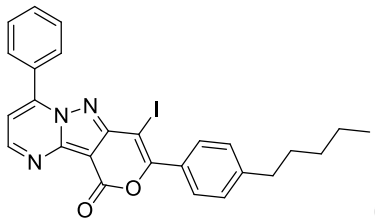
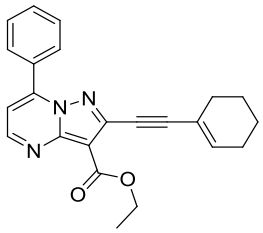
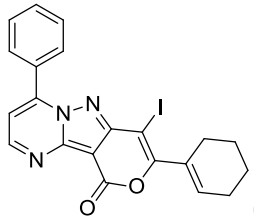
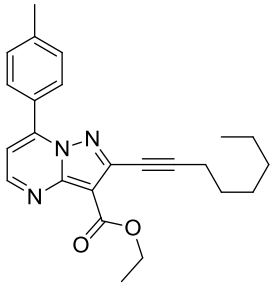
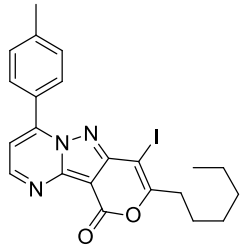
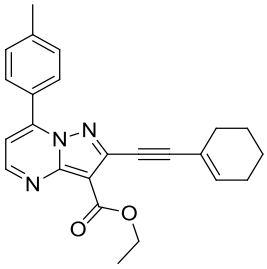
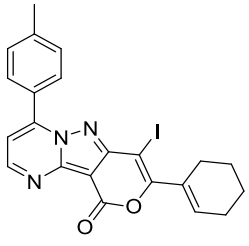
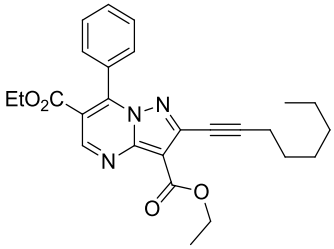
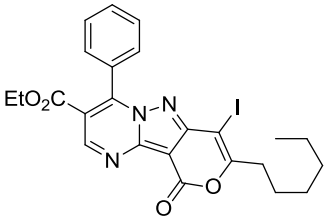
A typical procedure: A mixture of ethyl 2-bromo-7-phenylpyrazolo[1,5-a]pyrimidine-3-carboxylate (**3a**) (1 mmol), 10% Pd/C (0.01 mmol), PPh_3 (0.0.04 mmol), or $(PPh_3)_2PdCl_2$ (2 mol%), CuI (0.0.02 mmol) and triethylamine (4 mmol) in DMF (5 mL) was stirred at 25 °C for 30 min. To this mixture was added an appropriate terminal alkyne (1.5 mmol) slowly with stirring. The mixture was then heated to 120-130 °C for 15.0 h and cooled to room temperature. The reaction mixture was poured into ethyl acetate (25 mL) and extracted with brine solution (3 x 15 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography using petroleum ether-EtOAc to give the desired product.

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



5 **6**

Entry	2-Alkynyl pyrazolo[1,5-*a*] pyrimidine (**5**)	Time (h)	Product (**6**)	%yield[b]
1.	 **5a**	2.0	 **6a**	92
2.	 **5b**	2.0	 **6b**	91

3.	 **5c**	2.0	 **6c**	92
4.	 **5d**	2.0	 **6d**	90
5.	 **5e**	2.0	 **6e**	93
6.	 **5h**	2.0	 **6f**	88
7.	 **5i**	1.0	 **6g**	90
8.	 **5j**	2.0	 **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 2h. [b] Isolated yield.

General procedure for the preparation of 6: To a solution of compound **5** (1 mmol) in CH_2Cl_2 (10.0 mL) was added solution of iodine (2 mmol) in CH_2Cl_2 (5.0 mL) at room temperature. The mixture was stirred at 20-25 °C for 2 h. After completion of the reaction the mixture was washed with 10% sodium thiosulphate solution. The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated. The residue was triturated with ethanol; the solid precipitate was filtered and washed with diisopropyl ether to give the desired product.

Typical procedure for the preparation of compound 7a

A mixture of **6a** (1 mmol), 10% Pd/C (0.01 mmol), PPh_3 (0.04 mmol), CuI (0.02 mmol) and triethylamine (4 mmol) in DMF (5 mL) was stirred at 25 °C for 30 min. To this mixture was added appropriate terminal alkyne (1.2 mmol) slowly with stirring. The reaction mixture was then stirred at 80 °C for 3 h, cooled to room temperature. The reaction mixture was poured into ethyl acetate (25 mL) and washed with brine solution (3 x 15 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated. The residue was triturated with ethanol; filtered and washed with diisopropyl ether to give the desired product.

Compound **7b** was prepared from **6c** following a similar procedure as presented above.

Typical procedure for the preparation of compound 7c

A mixture of **6a** (1 mmol), $(PPh_3)_2PdCl_2$ (2 mol%), PPh_3 (0.15 mmol) and sodium carbonate (4 mmol) in DMF (5 mL) was stirred at 25 °C for 30 min. To this mixture was added appropriate boronic acid (1 mmol) at room temperature. The reaction mixture was then stirred at 80 °C for 2 h, cooled to room temperature, poured into ethyl acetate (25 mL) and washed with brine solution (3 x 15 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated. The residue was triturated with ethanol; filtered and washed with diisopropyl ether to give the desired product.

Compounds **7d, 7g, 7h** and **7i** were prepared from **6c** following a similar procedure as presented above.

Typical procedure for the preparation of compound 7e

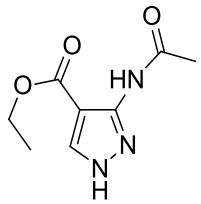
A mixture of **6a** (1 mmol), $(PPh_3)_2PdCl_2$ (2 mol%) and Et_3N (4 mmol) in DMF (5 mL) was stirred at 25 °C for 30 min. To this mixture was added appropriate alkene (2 mmol) at room temperature. The reaction mixture was then stirred at 80 °C for 2 h, cooled to room temperature, poured into

ethyl acetate (25 mL) and washed with brine solution (3 x 15 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated. The residue was triturated with ethanol; filtered and washed with diisopropyl ether to give the desired product.

Compound **7f** was prepared from **6a** following a similar procedure as presented above.

Spectral data:

Ethyl 3-acetamido-1*H*-pyrazole-4-carboxylate (1a)



Light brown solid, mp 129-131 °C.

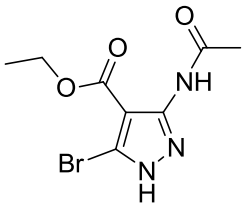
^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.90 (bs, 1H, NH), 9.59 (bs, 1H, NH), 7.77 (s, 1H, CH), 4.31 (q, *J*=7.3 Hz, 2H, OCH_2), 2.29 (s, 3H, CH_3), 1.38 (t, *J*=7.3 Hz, 3H, CH_3).

^{13}CNMR (DMSO-d_6, 100MHz): δ 169.2, 163.0, 140.9, 139.0, 99.0, 59.7, 23.1, 14.3.

IR (KBr): 3239, 2982, 1697, 1621 cm^{-1}

m/z (ES mass) 198.10 (M+1, 100%)

Ethyl 3-acetamido-5-bromo-1*H*-pyrazole-4-carboxylate (1b)



White solid, mp 197-199 °C.

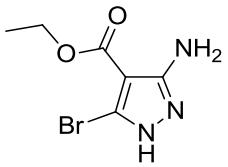
^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.56 (bs, 1H, NH), 9.99 (bs, 1H, NH), 4.27 (q, *J*=7.0 Hz, 2H, OCH_2), 2.19 (s, 3H, CH_3), 1.30 (t, *J*= 7.0 Hz, 3H, CH_3).

^{13}CNMR (DMSO-d_6, 50 MHz): δ 169.1, 161.6, 141.9, 126.2, 99.6, 60.1, 23.2, 14.1.

IR (KBr): 3220, 2980, 1692, 1605 cm^{-1}

HRMS (ESI): calcd for $C_8H_{11}N_3O_3Br$ $(M+H)^+$ 275.9984, found 275.9984.

Ethyl 3-amino-5-bromo-1*H*-pyrazole-4-carboxylate (1)



White solid, mp 142-145 °C.

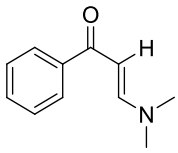
^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.0 (bs, 1H, NH), 6.08 (bs, 1H, NH_2), 4.27 (q, *J*=7.0 Hz, 2H, OCH_2), 1.26 (t, *J*=7.0 Hz, 3H, CH_3).

^{13}CNMR (DMSO-d_6, 100 MHz): δ 162.7, 152.6, 126.3, 92.7, 59.3, 14.6.

IR (KBr): 3445, 2992, 1683, 1510 cm^{-1}

HRMS (ESI): calcd for $C_6H_9N_3O_2Br$ $(M+H)^+$ 233.9878, found 233.9874.

(*E*)-3-(*N*,*N*-Dimethylamino)-1-phenyl-2-propen-1-one (2a)



Yellow solid, mp 91-92 °C.

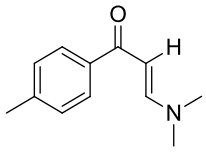
^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.91-7.88 (m, 2H, arom H), 7.82 (d, *J*=12.2 Hz, 1H, CH) , 7.47 – 7.38 (m, 3H, arom H), 5.73 (d, *J*=12.2 Hz, 1H, CH) , 3.13 (bs, 3H, NCH_3), 3.02 (bs, 3H, NCH_3).

^{13}CNMR (DMSO-d_6, 100 MHz): δ 185.7, 154.1, 140.2, 130.7, 128.1, 127.2, 90.9, 44.4, 37.1.

IR (KBr):3442, 1638, 1584, 1543 cm^{-1}

HRMS (ESI): calcd for $C_{11}H_{14}NO$ $(M+H)^+$ 176.1075, found 176.1078.

(*E*)-3-(*N*,*N*-Dimethylamino-1-(4-methylphenyl)-2-propen-1-one (2b)



Yellow solid, mp 90-92 °C.

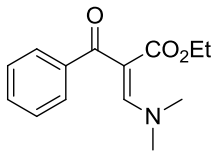
^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.82 (d, *J*=8.4 Hz, 2H, arom H), 7.80 (d, *J*=12.2 Hz, 1H, CH) , 7.22 (d, *J*=7.8 Hz, 2H, arom H), 5.73 (d, *J*=12.2 Hz, 1H, CH) , 3.11 (bs, 3H, CH_3), 2.93 (bs, 3H, NCH_3), 2.39 (s, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): δ 188.1, 153.9, 141.1, 137.6, 128.6, 127.4, 91.8, 44.5, 36.3, 21.3

IR (KBr):3441, 1645, 1581, 1539 cm^{-1}

HRMS (ESI): calcd for $C_{11}H_{16}NO$ $(M+H)^+$ 190.1232, found 190.1240.

(*E*)-Ethyl 2-(*N*,*N*-dimethylaminomethyliden) benzoylacetate (2c)



Yellow semi solid.

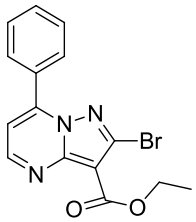
^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.80-7.72 (m, 2H, arom H), 7.49-7.37 (m, 3H, arom H), 3.96 (q, *J*=7.2 Hz, 1H, OCH_2), 2.90-3.20 (bs, 6H, NCH_3), 0.88 (t, *J*=7.2 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): δ 193.9, 168.5, 155.7, 140.9, 131.5, 128.9, 128.6, 128.3, 127.8, 99.5, 59.5, 46.1, 41.9, 13.9

IR (KBr):3443, 1640, 1706, 1592cm^{-1}

HRMS (ESI): calcd for $C_{14}H_{18}NO_3$ $(M+H)^+$ 248.1287, found 248.1286.

Ethyl 2-bromo-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate (3a)



White solid, mp 147-149 °C.

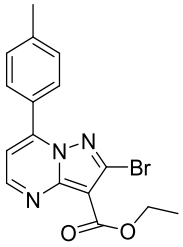
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.81 (d, *J*=4.9 Hz, 1H, arom H), 8.02-8.00 (m, 2H, arom H), 7.62-7.56 (m, 3H, arom H), 7.10 (d, *J*=4.9 Hz, 1H, arom H), 4.52 (q, *J*=6.9 Hz, 2H, OCH_2), 1.47 (t, *J*=6.9 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): δ 161.5, 152.6, 149.7, 147.1, 137.5, 131.8, 129.5, 128.8, 109.5, 102.6, 60.7, 14.4.

IR (KBr): 3388, 2983, 1712, 1610, 1543 cm^{-1}

HRMS (ESI): calcd for $C_{15}H_{13}N_3O_2Br$ $(M+H)^+$ 346.0191, found 346.0190.

Ethyl 2-bromo-7-(p-tolyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (3b)



White solid, mp 144-147 °C.

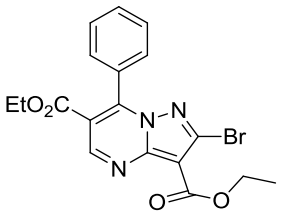
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.78 (d, *J*=4.8 Hz, 1H, arom H), 7.94 (d, *J*=7.8 Hz, 2H, arom H), 7.40 (d, *J*=7.8 Hz, 2H, arom H), 7.08 (d, *J*=4.8 Hz, 1H, arom H), 4.49 (q, *J*=6.8 Hz, 2H, OCH_2), 2.47 (s, 3H, CH_3), 1.46 (t, *J*=6.8 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): δ 161.6, 152.6, 149.8, 147.3, 142.6, 137.5, 129.6, 126.6, 109.1, 102.4, 60.7, 21.6, 14.4.

IR (KBr): 3422, 1707, 1604, 1542 cm^{-1}

HRMS (ESI): calcd for $C_{16}H_{15}N_3O_2Br$ $(M+H)^+$ 360.0348, found 360.0358.

Diethyl 2-bromo-7-phenylpyrazolo[1,5-*a*]pyrimidine-3,6-dicarboxylate (3c)



Light yellow solid, mp 152-154 °C.

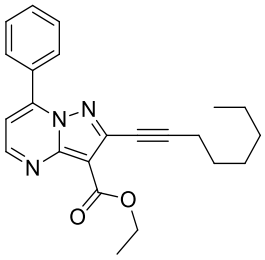
^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.23 (s, 1H, arom H), 7.61-7.48 (m, 5H, arom H), 4.51 (q, *J*=6.8 Hz, 2H, OCH_2), 4.17 (q, *J*=6.8 Hz, 2H, OCH_2), 1.46 (t, *J*=6.8 Hz, 3H, CH_3), 1.03 (t, *J*=6.8 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): δ 163.7, 161.2, 153.6, 149.4, 139.4, 131.0, 129.2, 128.7, 128.4, 114.1, 103.8, 62.0, 61.0, 14.4, 13.6.

IR (KBr): 3424, 2981, 1723, 1706, 1592, 1526 cm^{-1}

HRMS (ESI): calcd for $C_{18}H_{17}N_3O_4Br$ $(M+H)^+$ 418.0402, found 418.0415.

Ethyl 2-(oct-1-yn-1-yl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate (5a)



Light yellow solid, mp 82-84 °C.

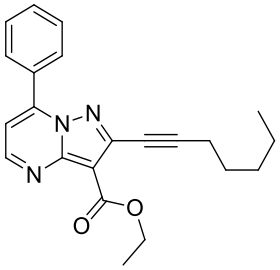
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.80 (d, *J*=4.4 Hz, 1H, arom H), 8.02-8.00 (m, 2H, arom H), 7.60-7.54 (m, 3H, arom H), 7.08 (d, *J*=4.4 Hz, 1H, arom H), 4.49 (q, *J*=7.0 Hz, 2H, OCH_2), 2.53 (t, *J*=7.3 Hz, 2H, CH_2), 1.72-1.43 (m, 8H, CH_2), 1.32 (t, *J*=7.3 Hz, 3H, CH_3), 0.92 (t, *J*=7.0 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): δ 162.2, 152.3, 149.2, 147.1, 142.1, 131.5, 130.0, 129.5, 128.8, 109.6, 104.2, 98.5, 72.8, 60.4, 31.3, 28.7, 28.2, 22.5, 19.8, 14.5, 14.0.

IR (KBr): 3448, 2928, 2230, 1716, 1615 cm^{-1}

HRMS (ESI): calcd for $C_{23}H_{26}N_3O_2$ $(M+H)^+$ 376.2025, found 376.2021.

Ethyl 2-(hept-1-yn-1-yl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate (5b)



Light yellow solid, mp 81-83 °C.

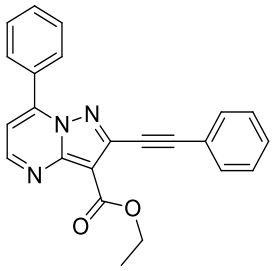
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.80 (d, *J*=4.4 Hz, 1H, arom H), 8.02-8.00 (m, 2H, arom H), 7.60-7.55 (m, 3H, arom H), 7.08 (d, *J*=4.4 Hz, 1H, arom H), 4.49 (q, *J*=7.3 Hz, 2H, OCH_2), 2.52 (t, *J*=7.4 Hz, 2H, CH_2), 1.72-1.35 (m, 9H, CH_2, CH_3), 0.92 (t, *J*=7.3 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): δ 162.2, 152.3, 149.2, 147.0, 142.1, 131.5, 129.9, 129.5, 128.8, 128.7, 109.6, 104.1, 98.4, 72.8, 60.4, 31.1, 27.9, 22.1, 19.8, 14.5, 13.9.

IR (KBr): 3449, 2955, 2230, 1713, 1612 cm^{-1}

HRMS (ESI): calcd for $C_{22}H_{24}N_3O_2$ $(M+H)^+$ 362.1869, found 362.1867.

Ethyl 7-phenyl-2-(phenylethynyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (5c)



Light yellow solid, mp 121-123 °C.

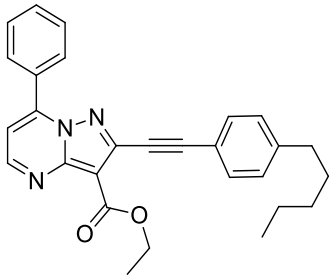
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.83 (d, *J*=4.4 Hz, 1H, arom H), 8.06-8.04 (m, 2H, arom H), 7.66-7.60 (m, 5H, arom H), 7.40-7.35 (m, 3H, arom H), 7.12 (d, *J*=4.4 Hz, 1H, arom H), 4.55 (q, *J*=7.0 Hz, 2H, OCH_2), 1.47 (t, *J*=7.0 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): 162.0, 152.5, 149.3, 147.2, 141.6, 132.0, 131.7, 129.9, 129.5, 129.2, 128.9, 128.4, 122.2, 109.8, 104.5, 95.9, 81.6, 60.6, 14.6.

IR (KBr): 3458, 2975, 2220, 1704, 1610 cm^{-1}

HRMS (ESI): calcd for $C_{23}H_{18}N_3O_2$ $(M+H)^+$ 368.1399, found 368.1400.

Ethyl 2-((4-pentylphenyl)ethynyl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate (5d)



Light yellow solid, mp 118-122 °C.

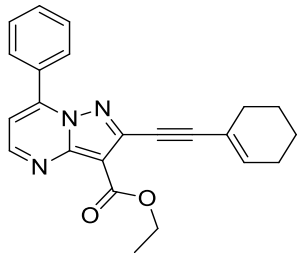
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.82 (d, *J*=4.4 Hz, 1H, arom H), 8.06-8.03 (m, 2H, arom H), 7.61-7.55 (m, 5H, arom H), 7.26-7.10 (m, 2H, arom H), 7.09 (d, *J*=4.4 Hz, 1H, arom H), 4.53 (q, *J*=6.8 Hz, 2H, OCH_2), 2.63 (t, *J*=7.9 Hz, 2H, CH_2), 1.66-1.30 (m, 9H, CH_2, CH_3), 0.89 (t, *J*=6.8 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): δ 162.1, 152.4, 149.3, 147.1, 144.5, 141.7, 132.0, 131.6, 129.9, 129.5, 128.8, 128.5, 119.3, 109.7, 104.4, 96.4, 81.0, 60.5, 35.9, 31.4, 30.8, 22.4, 14.6, 13.9.

IR (KBr): 2934, 2229, 1705, 1608 cm^{-1}

HRMS (ESI): calcd for $C_{28}H_{28}N_3O_2$ $(M+H)^+$ 438.2182, found 438.2169.

Ethyl 2-(cyclohex-1-en-1-ylethynyl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate (5e)



Light yellow solid, mp 120-122 °C.

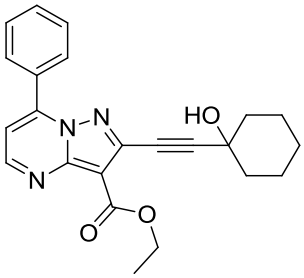
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.80 (d, *J*=4.4 Hz, 1H, arom H), 8.03-8.00 (m, 2H, arom H), 7.60-7.57 (m, 3H, arom H), 7.09 (d, *J*=4.4 Hz, 1H, arom H), 6.41-6.43 (m, 1H, CH), 4.50 (q, *J*=7.0 Hz, 2H, OCH_2), 2.30-1.63 (m, 8H, CH_2), 1.46 (t, *J*=7.00 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.1, 154.3, 151.3, 149.1, 144.0, 140.1, 133.6, 131.9, 131.5, 130.9, 130.8, 122.2, 111.6, 106.1, 100.1, 80.9, 62.4, 30.5, 27.9, 24.1, 23.3, 16.5.

IR (KBr): 3423, 2935, 2229, 1716, 1613 cm^{-1}

HRMS (ESI): calcd for $C_{23}H_{22}N_3O_2$ $(M+H)^+$ 372.1712, found 372.1696.

Ethyl 2-((1-hydroxycyclohexyl)ethynyl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate (5f)



Light yellow solid, mp 99-101 °C.

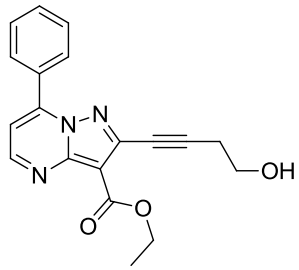
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.82 (d, *J*=4.4 Hz, 1H, arom H), 8.04-8.00 (m, 2H, arom H), 7.61-7.55 (m, 3H, arom H), 7.10 (d, *J*=4.4 Hz, 1H, arom H), 4.49 (q, *J*=7.0 Hz, 2H, OCH_2), 2.38 (bs, 1H, OH), 2.08 (t, *J*=7.0 Hz, 2H, CH_2), 1.80-1.49 (m, 8H, CH_2), 1.45 (t, *J*=7.0 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.0, 152.5, 149.2, 147.2, 141.2, 131.7, 129.8, 129.6, 128.8, 109.8, 104.5, 100.0, 69.1, 60.6, 39.5, 25.2, 23.0, 14.6.

IR (KBr): 3449, 2934, 2230, 1701, 1606 cm^{-1}

HRMS (ESI): calcd for $C_{23}H_{24}N_3O_3$ $(M+H)^+$ 390.1818, found 390.1812.

Ethyl 2-(4-hydroxybut-1-yn-1-yl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate (5g)



Light yellow solid, mp 115-117 °C.

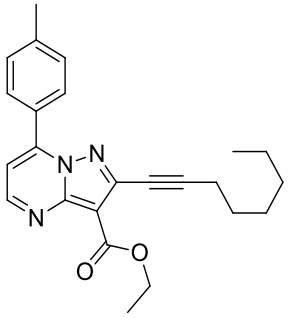
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.81 (d, *J*=4.4 Hz, 1H, arom H), 8.00-7.98 (m, 2H, arom H), 7.61-7.54 (m, 3H, arom H), 7.11 (d, *J*=4.4 Hz, 1H, arom H), 4.52 (q, *J*=7.0 Hz, 2H, OCH_2), 3.91 (t, *J*=5.9 Hz, 2H, OCH_2), 3.38 (bs, 1H, OH), 2.78 (t, *J*=5.9 Hz, 2H, OCH_2), 1.45 (t, *J*=7.0 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.5, 152.5, 148.8, 147.4, 142.2, 131.6, 129.8, 129.5, 128.8, 109.8, 104.5, 96.3, 75.1, 60.8, 60.6, 24.4, 14.5.

IR (KBr): 3440, 2874, 2235, 1708, 1606 cm^{-1}

HRMS (ESI): calcd for $C_{19}H_{18}N_3O_3$ $(M+H)^+$ 336.1348, found 336.1346.

Ethyl 2-(oct-1-yn-1-yl)-7-(p-tolyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (5h)



Light yellow solid, mp 84-86 °C.

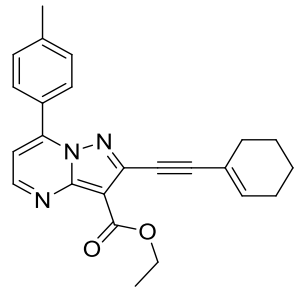
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.78 (d, *J*=4.4 Hz, 1H, arom H), 7.93 (d, *J*=7.9 Hz, 2H, arom H), 7.40-7.26 (m, 2H, arom H), 7.07 (d, *J*=4.4 Hz, 1H, arom H), 4.49 (q, *J*=6.4 Hz, 2H, OCH_2), 2.52 (t, *J*=7.4 Hz, 2H, CH_2), 2.46 (s, 3H, CH_3), 1.72-1.30 (m, 6H, CH_2), 1.32 (t, *J*=7.4 Hz, 2H, CH_3), 0.89 (t, *J*=6.8 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100 MHz): δ 162.2, 152.6, 152.2, 149.3, 147.2, 142.2, 129.5, 129.4, 127.0, 109.2, 104.0, 98.3, 72.8, 60.3, 31.3, 28.7, 28.2, 22.5, 21.5, 19.8, 14.4, 14.0.

IR (KBr): 3460, 2953, 2231, 1715, 1613 cm^{-1}

HRMS (ESI): calcd for $C_{24}H_{27}N_3O_2$ $(M+H)^+$ 375.1950, found 375.1954.

Ethyl 2-(cyclohex-1-en-1-ylethynyl)-7-(p-tolyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (5i)



Light yellow solid, mp 96-98 °C.

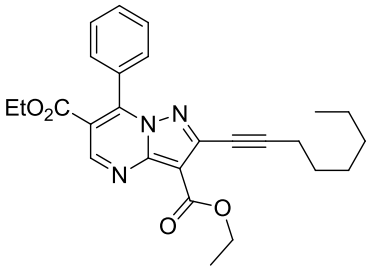
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.77 (d, *J*=4.4 Hz, 1H, arom H), 7.95 (d, *J*=8.3 Hz, 2H, arom H), 7.39-7.26 (m, 2H, arom H), 7.07 (d, *J*=4.4 Hz, 1H, arom H), 6.40-6.42 (m, 1H, CH), 4.48 (q, *J*=6.8 Hz, 2H, OCH_2), 2.46 (s, 3H, CH_3), 2.31-2.17 (m, 4H, CH_2), 1.70-1.57 (m, 4H, CH_2), 1.44 (t, *J*=6.8 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 162.2, 152.6, 152.2, 149.3, 147.1, 142.0, 137.9, 129.5, 129.4, 127.0, 120.2, 109.2, 103.9, 98.0, 79.1, 60.4, 28.6, 25.8, 22.1, 21.5, 21.3, 14.5.

IR (KBr): 3461, 2928, 2213, 1705, 1603 cm^{-1}

HRMS (ESI): calcd for $C_{24}H_{24}N_3O_2$ $(M+H)^+$ 386.1869, found 386.1884.

Diethyl 2-(oct-1-yn-1-yl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3,6-dicarboxylate (5j)



Light yellow solid, mp 109-111 °C.

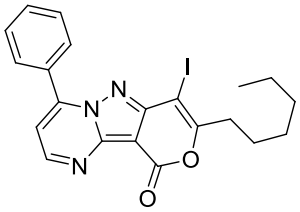
^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.21 (s, 1H, arom H), 7.58-7.48 (m, 6H, arom H), 4.50 (q, *J*=6.8 Hz, 2H, OCH_2), 4.15 (q, *J*=6.8 Hz, 2H, OCH_2), 2.47 (t, *J*=7.4 Hz, 2H, CH_2), 1.68-1.25 (m, 8H, CH_2), 1.02 (t, *J*=6.8 Hz, 2H, CH_3), 0.88 (t, *J*=6.8 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.0, 161.8, 153.3, 149.4, 149.0, 143.9, 130.8, 129.2, 129.1, 128.4, 114.1, 105.2, 99.8, 72.5, 61.9, 60.6, 31.2, 29.7, 28.7, 28.1, 22.5, 19.8, 14.4, 14.0, 13.6.

IR (KBr): 3440, 2928, 2249, 1716, 1607 cm^{-1}

HRMS (ESI): calcd for $C_{24}H_{28}N_3O_2$ $(M+H)^+$ 390.2182, found 390.2181.

8-Hexyl-7-iodo-4-phenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (6a)



Light yellow solid, mp 178-180 °C.

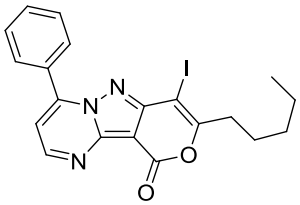
^{1}H NMR ($CDCl_3$, 400MHz): δ 8.91 (d, *J*=4.4 Hz, 1H, arom H), 8.23-8.20 (m, 2H, arom H), 7.65-7.61 (m, 3H, arom H), 7.35 (d, *J*=4.4 Hz, 1H, arom H), 2.92 (t, *J*=7.6 Hz, 2H, CH_2), 1.78 (p, *J*=7.6 Hz, 2H, CH_2), 1.43-1.32 (m, 6H, CH_2), 0.89 (t, *J*=6.7 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.0, 157.5, 156.1, 152.6, 149.7, 148.0, 132.1, 129.9, 129.6, 128.8, 110.9, 94.1, 63.0, 36.1, 31.4, 28.7, 27.2, 22.5, 14.0.

IR (KBr): 3440, 2935, 1750, 1618 cm^{-1}

HRMS (ESI): calcd for $C_{21}H_{21}N_3O_2I$ $(M+H)^+$ 474.0679, found 474.0664.

7-Iodo-8-pentyl-4-phenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (6b)



Light yellow solid, mp 166-168 °C.

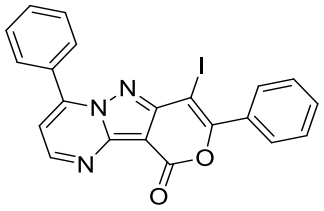
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.91 (d, *J*=4.8 Hz, 1H, arom H), 8.23-8.21 (m, 2H, arom H), 7.67-7.62 (m, 3H, arom H), 7.35 (d, *J*=4.8 Hz, 1H, arom H), 2.92 (t, *J*=7.6 Hz, 2H, CH_2), 1.75 (p, *J*=7.6 Hz, 2H, CH_2), 1.44-1.39 (m, 4H, CH_2), 0.92 (t, *J*=7.0 Hz, 3H, CH_3)

^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.0, 157.5, 156.2, 152.6, 149.8, 148.1, 132.1, 129.9, 129.6, 128.8, 110.9, 94.2, 63.0, 36.0, 31.2, 36.9, 22.4, 13.9.

IR (KBr): 3451, 2924, 1751, 1627 cm^{-1}

HRMS (ESI): calcd for $C_{20}H_{19}N_3O_2I$ $(M+H)^+$ 460.0522, found 460.0508.

7-Iodo-4,8-diphenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (6c)



Light yellow solid, mp 318-320 °C.

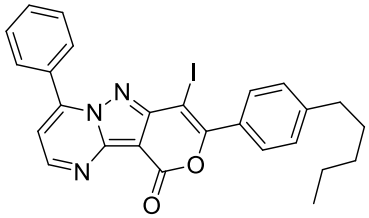
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.96 (d, *J*=4.8 Hz, 1H, arom H), 8.27-8.24 (m, 2H, arom H), 7.86-7.84 (m, 2H, arom H), 7.67-7.65 (m, 3H, arom H), 7.50-7.48 (m, 3H, arom H), 7.39 (d, *J*=4.8 Hz, 1H, arom H).

^{13}CNMR ($CDCl_3$, 100MHz): δ 159.6, 157.1, 156.8, 152.5, 149.3, 148.0, 132.0, 131.0, 129.9, 129.6, 128.9, 128.4, 111.0, 95.9, 62.2.

IR (KBr): 3424, 2933, 1752, 1620 cm^{-1}

HRMS (ESI): calcd for $C_{21}H_{13}N_3O_2I$ $(M+H)^+$ 476.0053, found 476.0060.

7-Iodo-8-(4-pentylphenyl)-4-phenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (6d)



Light yellow solid, mp 206-208 °C.

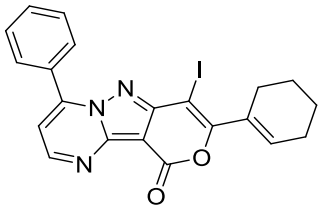
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.94 (d, *J*=4.8 Hz, 1H, arom H), 8.26-8.24 (m, 2H, arom H), 7.79-7.77 (m, 2H, arom H), 7.67-7.62 (m, 3H, arom H), 7.39 (d, *J*=4.8 Hz, 1H, arom H), 7.30-7.28 (m, 2H, arom H), 2.68 (t, *J*=7.6 Hz, 2H, CH_2), 1.67 (p, *J*=7.6 Hz, 2H, CH_2), 1.39-1.33 (m, 4H, CH_2), 0.91 (t, *J*=7.00 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.6, 157.1, 156.8, 152.7, 149.7, 148.0, 145.9, 132.1, 131.0, 129.9, 129.6, 128.9, 128.0, 111.0, 94.3, 62.2, 35.9, 31.5, 30.8, 22.5, 14.0.

IR (KBr): 3431, 2923, 1742, 1625 cm^{-1}

HRMS (ESI): calcd for $C_{26}H_{23}N_3O_2I$ $(M+H)^+$ 536.0835, found 536.0854.

8-(Cyclohex-1-en-1-yl)-7-iodo-4-phenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (6e)



Light yellow solid, mp 194-195 °C.

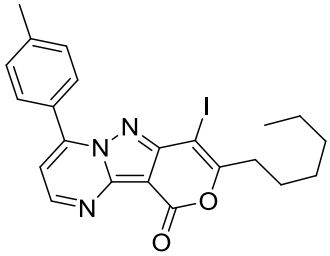
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.92 (d, *J*=4.8 Hz, 1H, arom H), 8.24-8.22 (m, 2H, arom H), 7.66-7.62 (m, 3H, arom H), 7.26 (d, *J*=4.8 Hz, 1H, arom H), 6.32-6.35 (m, 1H, CH), 2.40-2.24 (m, 4H, CH_2), 1.81-1.68 (m, 4H, CH_2).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.2, 157.3, 156.6, 152.6, 149.7, 147.9, 135.9, 132.5, 132.1, 129.9, 129.6, 128.8, 110.8, 94.3, 61.2, 26.3, 25.1, 22.2, 21.5.

IR (KBr): 3426, 2928, 1737, 1618 cm^{-1}

HRMS (ESI): calcd for $C_{21}H_{17}N_3O_2I$ $(M+H)^+$ 470.0366, found 470.0374.

8-Hexyl-7-iodo-4-(p-tolyl)-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (6f)



Light yellow solid, mp 162-164 °C.

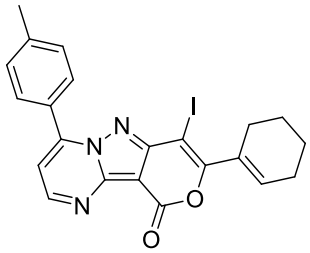
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.88 (d, *J*=4.8 Hz, 1H, arom H), 8.16 (d, *J*=8.0 Hz, 2H, arom H), 7.45 (d, *J*=8.3 Hz, 2H, arom H), 7.33 (d, *J*=4.8 Hz, 1H, arom H), 2.92 (t, *J*=7.6 Hz, 2H, CH_2), 2.50 (s, 3H, CH_3), 1.78 (p, *J*=7.6 Hz, 2H, CH_2), 1.45-1.32 (m, 6H, CH_2), 0.89 (t, *J*=7.0 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.9, 157.5, 156.1, 152.5, 149.8, 148.2, 142.9, 129.9, 129.6, 126.7, 110.5, 94.0, 63.1, 36.1, 31.5, 28.7, 27.2, 22.5, 21.7, 14.0.

IR (KBr): 3425, 2922, 1756, 1623 cm^{-1}

HRMS (ESI): calcd for $C_{22}H_{23}N_3O_2I$ $(M+H)^+$ 488.0835, found 488.0836.

8-(Cyclohex-1-en-1-yl)-7-iodo-4-(p-tolyl)-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (6g)



Light yellow solid, mp 219-221 °C.

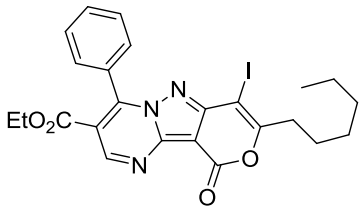
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.89 (d, *J*=4.4Hz, 1H, arom H), 8.17 (d, *J*=8.0 Hz, 2H, arom H), 7.45 (d, *J*=8.0 Hz, 2H, arom H), 7.34 (d, *J*=4.4 Hz, 1H, arom H), 6.32-6.34 (m, 1H, CH), 2.50 (s, 3H, CH_3), 2.40-2.25 (m, 4H, CH_2), 1.79-1.58 (m, 4H, CH_2).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.1, 157.4, 156.6, 152.5, 149.7, 148.0, 142.9, 135.9, 132.6, 129.9, 129.5, 126.7, 110.5, 94.2, 61.3, 26.3, 25.1, 22.2, 21.7, 21.5.

IR (KBr): 3425, 2928, 1754, 1620 cm^{-1}

HRMS (ESI): calcd for $C_{22}H_{19}N_3O_2I$ $(M+H)^+$ 484.0522, found 484.0546.

Ethyl 8-hexyl-7-iodo-10-oxo-4-phenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (6h):



Light yellow solid, mp 130-132 °C.

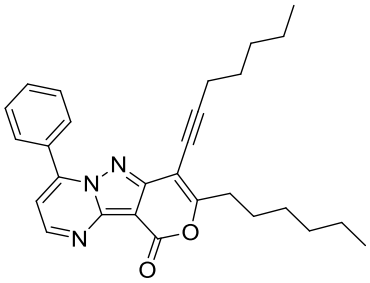
^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.29 (s, 1H, arom H), 7.66-7.57 (m, 5H, arom H), 4.19 (q, *J*=6.8 Hz, 2H, OCH_2), 2.90 (t, *J*=7.4 Hz, 2H, CH_2), 1.75 (p, *J*=7.4 Hz, 2H, CH_2), 1.42-1.30 (m, 6H, CH_2), 1.07 (t, *J*=7.4 Hz, 3H, CH_3), 0.89 (t, *J*=6.8 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.4, 163.8, 157.2, 157.1, 153.7, 150.1, 149.6, 131.2, 129.6, 128.7, 128.3, 115.9, 95.0, 62.8, 62.2, 36.1, 31.4, 28.7, 27.2, 22.4, 14.0, 13.6.

IR (KBr): 3462, 2927, 1742, 1621 cm^{-1}

HRMS (ESI): calcd for $C_{24}H_{25}N_3O_4I$ $(M+H)^+$ 546.0890, found 546.0877.

7-(Hept-1-yn-1-yl)-8-hexyl-4-phenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (7a)



Light brown solid, mp 163-165 °C.

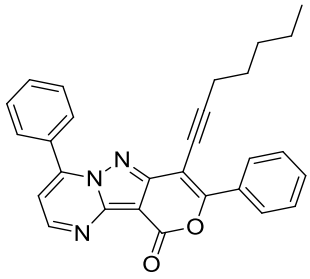
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.89 (d, *J*=4.8 Hz, 1H, arom H), 8.20-8.17 (m, 2H, arom H), 7.63-7.60 (m, 3H, arom H), 7.31 (d, *J*=4.8 Hz, 1H, arom H), 2.86-2.84 (t, *J*=7.3 Hz, 2H, CH_2), 2.49 (t, *J*=7.3 Hz, 2H, CH_2), 1.80-1.61 (m, 4H, CH_2), 1.52-1.25 (m, 10H, CH_2), 0.91 (m, *J*=7.3 Hz, 6H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.0, 157.2, 155.6, 152.3, 149.1, 148.0, 131.9, 129.9, 128.7, 110.8, 98.2, 95.4, 94.4, 70.6, 32.6, 31.4, 30.9, 28.7, 28.3, 22.5, 22.2, 19.7, 14.0, 13.9.

IR (KBr): 3454, 2926, 1733, 1625 cm^{-1}

HRMS (ESI): calcd for $C_{28}H_{32}N_3O_2(M+H)^+$ 442.2495, found 442.2493.

7-(Hept-1-yn-1-yl)-4,8-diphenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (7b)



White solid, mp 196-199 °C.

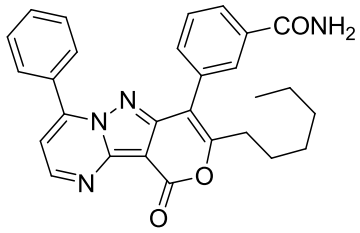
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.92 (d, *J*=4.4 Hz, 1H, arom H), 8.29-8.22 (m, 4H, arom H), 7.65-7.60 (m, 3H, arom H), 7.48-7.46 (m, 3H, arom H), 7.35 (d, *J*=4.4 Hz, 1H, arom H), 2.49 (t, *J*=7.3 Hz, 2H, CH_2), 1.68-1.25 (m, 6H, CH_2), 0.88 (t, *J*=7.3 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100MHz): δ 160.7, 156.5, 152.4, 149.0, 147.9, 132.2, 131.9, 130.5, 129.9, 129.8, 128.8, 128.7, 128.6, 128.0, 126.0, 110.9, 99.6, 94.8, 94.6, 71.6, 31.0, 28.0, 22.2, 19.9, 13.9.

IR (KBr): 3454, 2927, 1756, 1624 cm^{-1}

HRMS (ESI): calcd for $C_{28}H_{24}N_3O_2(M+H)^+$ 434.1869, found 434.1855.

3-(8-Hexyl-10-oxo-4-phenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-7-yl)benzamide (7c)



Light yellow solid, mp 234-236 °C.

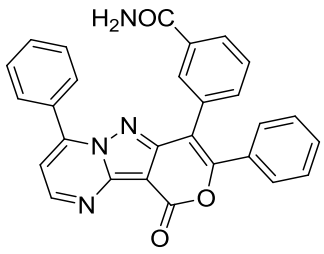
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.90 (d, *J*=4.4 Hz, 1H, arom H), 8.08-8.04 (m, 3H, arom H), 7.85-7.83 (m, 1H, arom H), 7.62-7.48 (m, 5H, arom H), 7.30 (d, *J*=4.4 Hz, 1H, arom H), 6.00 (bs, 1H, NH), 5.60 (bs, 1H, NH), 2.64 (t, *J*=7.3 Hz, 2H, CH_2), 1.78 (p, *J*=7.3 Hz, 2H, CH_2), 1.33-1.19 (m, 6H, CH_2, CH_3), 0.84 (t, *J*=6.8 Hz, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.5, 160.2, 156.5, 155.0, 153.2, 148.4, 147.0, 134.2, 133.0, 132.1, 131.7, 130.0, 129.7, 128.5, 128.3, 127.1, 111.7, 110.2, 93.7, 30.7, 30.3, 28.0, 27.0, 21.9, 13.9.

IR (KBr): 3434, 2927, 1740, 1687, 1631 cm^{-1}

HRMS (ESI): calcd for $C_{28}H_{27}N_4O_3(M+H)^+$ 467.2083, found 467.2082.

3-(10-Oxo-4,8-diphenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-7-yl)benzamide (7d)



Light yellow solid, mp 298-300 °C.

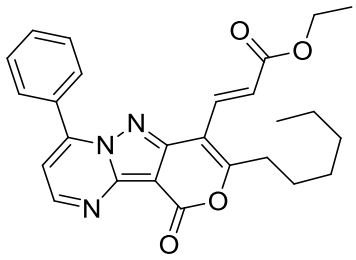
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.92 (d, *J*=4.7 Hz, 1H, arom H), 8.14-8.08 (m, 2H, arom H), 8.08 (s, 1H, arom H), 7.80 (d, *J*=6.9 Hz, 1H, arom H), 7.59-7.26 (m, 11H, arom H), 5.95 (bs, 1H, NH), 5.42 (bs, 1H, NH).

^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.3, 155.9, 155.7, 155.0, 153.3, 148.3, 146.9, 134.2, 133.4, 132.4, 132.1, 131.7, 130.2, 130.0, 129.8, 129.5, 129.4, 128.4, 128.3, 128.1, 126.9, 111.7, 110.4, 94.0.

IR (KBr): 3429, 3182, 1746, 1682, 1624 cm^{-1}

HRMS (ESI): calcd for $C_{28}H_{19}N_4O_3(M+H)^+$ 459.1457, found 459.1449.

Ethyl 3-(8-hexyl-10-oxo-4-phenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-7-yl)acrylate (7e)



White solid, mp 192-194 °C.

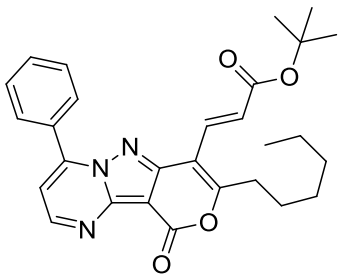
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.95 (d, *J*=4.9 Hz, 1H, arom H), 8.21-8.18 (m, 2H, arom H), 7.78 (d, *J*=16.2 Hz, 1H, CH), 7.68-7.63 (m, 3H, arom H), 7.78 (d, *J*=15.7 Hz, 1H, CH), 7.37 (d, *J*=4.9 Hz, 1H, arom H), 4.28 (q, *J*=6.8 Hz, 2H, CH_2), 2.87 (t, *J*=7.8 Hz, 2H, CH_2), 1.80 (p, *J*=7.8 Hz, 2H, CH_2), 1.43-1.31(m, 9H, CH_2, CH_3), 0.89 (t, *J*=6.8Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.5, 167.3, 156.7, 154.2, 152.9, 148.3, 147.9, 134.1, 132.2, 129.8, 129.7, 128.8, 122.4, 110.8, 105.9, 60.4, 31.4, 28.8, 28.2, 22.5, 14.3, 14.0

IR (KBr): 3428, 2931, 1749, 1714, 1618cm^{-1}

HRMS (ESI): calcd for $C_{26}H_{28}N_3O_4(M+H)^+$ 446.2080, found 446.2082.

tert-Butyl 3-(8-hexyl-10-oxo-4-phenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-7-yl)acrylate (7f)



White solid, mp 219-221 °C

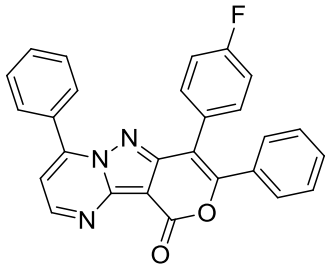
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.95 (d, *J*=4.9 Hz, 1H, arom H), 8.21-8.18 (m, 2H, arom H), 7.71 (d, *J*=15.7 Hz, 1H, CH), 7.66-7.62 (m, 3H, arom H), 7.44 (d, *J*=15.7 Hz, 1H, CH), 7.37 (d, *J*=4.9 Hz, 1H, arom H), 2.86 (t, *J*=7.8 Hz, 2H, CH_2), 1.80 (p, *J*=7.8 Hz, 2H, CH_2), 1.58 (s, 9H, CH_3), 1.43-1.30 (m, 6H, CH_2), 0.88 (t, *J*=6.8 Hz, 3H, CH_3).

^{13}CNMR ($CDCl_3$, 100MHz): δ 166.9, 166.8, 156.8, 154.2, 152.8, 148.4, 147.9, 133.0, 132.0, 129.8, 129.7, 128.8, 124.2, 110.7, 105.9, 95.1, 80.2, 31.4, 31.3, 28.8, 28.2, 22.5, 14.0.

IR (KBr): 3466, 2930, 1749, 1715, 1618 cm^{-1}

HRMS (ESI): calcd for $C_{28}H_{32}N_3O_4(M+H)^+$ 474.2393, found 474.2368.

7-(4-Fluorophenyl)-4,8-diphenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-a]pyrimidin-10-one (7g)



White color solid, mp 270-272°C.

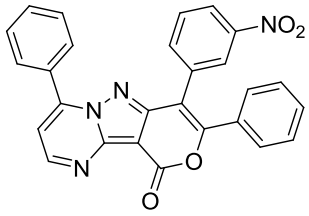
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.92 (d, *J*=4.4 Hz, 1H, arom H), 8.12-8.10 (d, *J*=7.9 Hz, 2H, arom H), 7.62-7.53 (m, 3H, arom H), 7.48-7.46 (m, 2H, arom H), 7.41-7.38 (m, 2H, arom H), 7.35-7.26 (m, 4H, arom H), 7.04-7.00 (m, 2H, arom H),

^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.6, 161.1, 157.4, 156.5, 156.3, 152.4, 149.0, 147.7, 132.7, 132.6, 132.5, 131.9, 129.8, 129.8, 129.7, 129.0, 128.7, 128.3, 128.0, 115.5, 115.3, 110.7, 110.3, 95.4.

IR (KBr): 3440, 3071, 1739, 1623, 1545 cm^{-1}

m/z (ES mass) 434.20 (M+1, 100%)

7-(3-Nitrophenyl)-4,8-diphenyl-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (7h)



Light yellow color solid, mp 253-256°C.

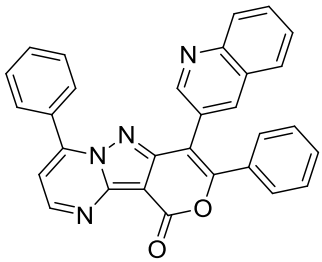
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.97 (d, *J*=4.4 Hz, 1H, arom H), 8.73 (s, 1H, arom H), 8.16-8.14 (m, 3H, arom H), 7.67-7.55 (m, 3H, arom H), 7.50-7.48 (m, 3H, arom H), 7.39-7.26 (m, 5H, arom H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ 157.8, 157.0, 155.4, 152.8, 152.4, 149.0, 148.2, 148.0, 137.1, 134.2, 132.2, 131.9, 130.3, 130.0, 129.8, 129.6, 129.4, 129.1, 128.9, 128.4, 126.1, 125.8, 122.8, 111.0, 109.1, 95.2

IR (KBr): 3461, 3063, 1741, 1624, 1543 cm^{-1}

m/z (ES mass) 461.20 (M+1, 100%)

4,8-Diphenyl-7-(quinolin-3-yl)-10*H*-pyrano[4',3':3,4]pyrazolo[1,5-*a*]pyrimidin-10-one (7i)



Light yellow color solid, mp 270-272°C.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.96-8.95 (m, 1H, arom H), 8.82 (s, 1H, arom H), 8.39 (s, 1H, arom H), 8.12-8.08 (m, 3H, arom H), 7.76-7.72 (m, 2H, arom H), 7.58-7.50 (m, 6H, arom H), 7.38-7.24 (m, 4H, arom H),

^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.5, 147.6, 133.8, 131.9, 130.7, 130.6, 130.0, 129.7, 129.6, 129.4, 129.0, 128.9, 128.4, 128.1, 127.9, 127.4, 125.9, 111.0, 94.6

IR (KBr): 3423, 3042, 1746, 1634, 1574 cm^{-1}

m/z (ES mass) 467.20 (M+1, 100%)

Single crystal X-ray data for compounds 6e and 5d.

Single crystals suitable for X-ray diffraction of **5d** and **6e** were grown from methanol Ethyl acetate. The crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data was collected at room temperature on Bruker's KAPPA APEX II CCD Duo with graphite monochromated Mo-Kα radiation (0.71073 Å). The crystals were glued to a thin glass fibre using FOMBLIN immersion oil and mounted on the diffractometer. The intensity data were processed using Bruker's suite of data processing programs (SAINT), and absorption corrections were applied using SADABS.[1] The crystal structure was solved by direct methods using SHELXS-97 and the data was refined by full matrix least-squares refinement on F^2 with anisotropic displacement parameters for non-H atoms, using SHELXL-97.[2]

Crystal data of 5d: Molecular formula = $C_{28}H_{27}N_3O_2$, Formula weight = 437.53, Crystal system = Monoclinic, space group = $P2_1/C$, 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}, 14662 reflections measured, 3278 independent reflections, 2433 observed reflections [I > 2.0 σ (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.

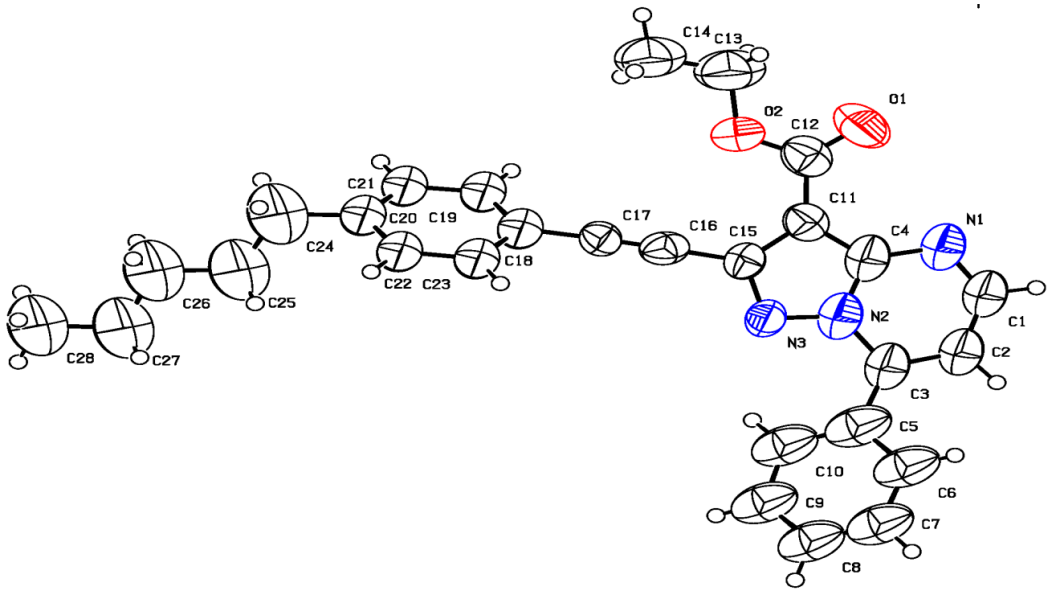


Figure 1. X-ray crystal structure of **5d** (ORTEP diagram). Thermal ellipsoids are drawn at 50% probability level.

Crystal data of 6e: Molecular formula = $C_{21}H_{16}IN_3O_2$, Formula weight = 469.27, Crystal system = Monoclinic, space group = $P2_1/C$, 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},15729 reflections measured, 4043 independent reflections, 3497 observed reflections [I > 2.0 σ (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.

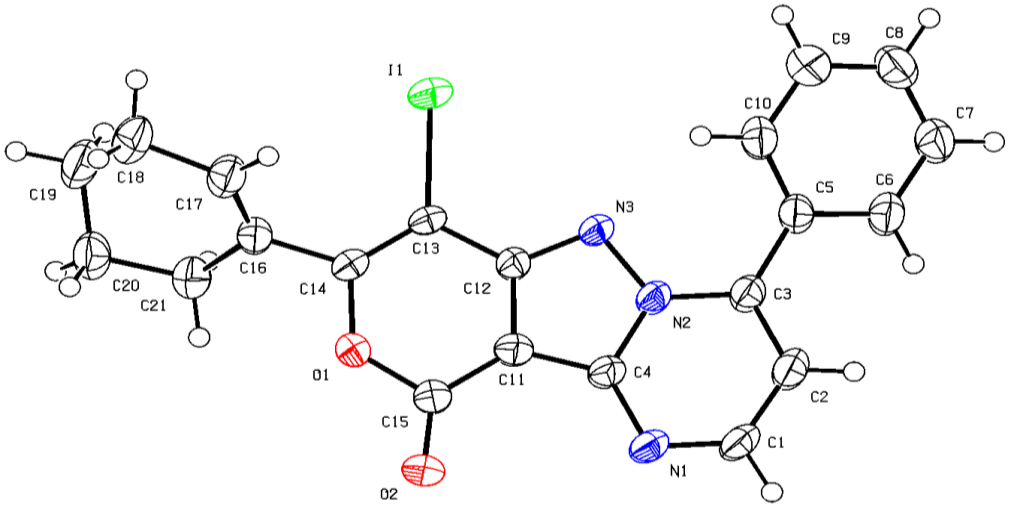


Figure 2. X-ray crystal structure of **6e** (ORTEP diagram). Thermal ellipsoids are drawn at 50% probability level.

Reference

1. Bruker SADABS V2008-1, Bruker AXS.: Madison, WI, USA, **2008**.
2. Sheldrick, G. M.; SHELX-97, Program for Crystal Structure Determination, University of Göttingen, **1997**.

Pharmacology

Materials and Methods

Cells and Reagents

HEK 293 and Sf9 cells were obtained from ATCC (Washington D.C., USA). HEK 293 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Invitrogen Inc., San Diego, CA, USA). Sf9 cells were routinely maintained in Grace's supplemented medium (Invitrogen) with 10% FBS. RAW 264.7 cells (murine macrophage cell line) were obtained from ATCC and routinely cultured in RPMI 1640 medium with 10% fetal bovine serum (Invitrogen Inc.). cAMP was purchased from SISCO Research Laboratories (Mumbai, India). PDElight HTS cAMP phosphodiesterase assay kit was procured from Lonza (Basel, Switzerland). PDE4B1, PDE4A4 and PDE4D3 cDNA clones were from OriGene Technologies (Rockville, MD, USA). PDE4D2 enzyme was purchased from BPS Bioscience (San Diego, CA, USA). Lipopolysaccharide (LPS) was from *Escherichia coli* strain 0127:B8 obtained from Sigma (St. Louis, MO, USA). Mouse TNF-α ELISA kit was procured from R&D Systems (Minneapolis, MN, USA).

Evaluation of PDE4 inhibitory potential by cell based cAMP reporter assay

One day prior to transfection, HEK 293 cells were seeded in p60 cell culture dish (Tarsons Inc.) and were transfected using Lipofectamine 2000, as per the manufacturer's instructions with 2.4 µg of PDE4B1 or PDE4A4 or PDE4D3 expression plasmid and 4.0 µg of pCRELuc plasmid. After 5 h of transfection, medium was aspirated, cells were trypsinized and seeded in 96 well plates at a density of 60000 cells/well. Plates were incubated overnight in a CO_2 incubator set to 37^{o}C and 5% CO_2. Twenty four hours post transfection, cells were pre-treated with various concentrations (0.001 to 30 µM) of compounds for 30 minutes, followed by stimulation with 5 µM forskolin for 4 h. Subsequently medium was removed and cells were lysed in reporter lysis buffer (Promega Inc.) for 15 min with gentle rocking at RT. Luciferase activity in the lysates was measured by a Multilabel plate reader (Perklin Elmer 1420 Multilabel counter). Percentage of cAMP elevation is calculated using the following formulae; EC_{50} values were determined using GraphPad Prism (San Diego, U.S.A) and EC_{50} values are expressed as mean ± SD.

$$Fold\ activation = \frac{(RLU\ of\ compound - RLU\ of\ vehicle\ control)}{(RLU\ of\ forskolin - RLU\ of\ vehicle\ control)}$$

$$\%\ of\ cAMP\ elevation = \left(\frac{Fold\ activation\ of\ test\ dose\ of\ compound}{Fold\ activation\ of\ saturating\ dose\ of\ compound}\right) \times 100$$

PDE4B protein production and purification

PDE4B1 cDNA was sub-cloned into pFAST Bac HTB vector (Invitrogen) and transformed into DH10Bac (Invitrogen) competent cells. Recombinant bacmids were tested for integration by PCR analysis. Sf9 cells were transfected with bacmid using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Subsequently, P3 viral titer was amplified, cells were infected and 48 h post infection cells were lysed in lysis buffer (50 mM Tris-HCl pH 8.5, 10 mM 2-Mercaptoethanol, 1 % protease inhibitor cocktail (Roche), 1 % NP40). Recombinant His-tagged PDE4B protein was purified as previously described elsewhere (Wang et al., 1997). Briefly, lysate was centrifuged at 10,000 rpm for 10 min at 4˚C and supernatant was collected. Supernatant was mixed with Ni-NTA resin (GE Life Sciences) in a ratio of 4:1 (v/v) and equilibrated with binding buffer (20 mM Tris-HCl pH 8.0, 500 mM-KCl, 5 mM imidazole, 10 mM 2-mercaptoethanol and 10 % glycerol) in a ratio of 2:1 (v/v) and mixed gently on rotary shaker for 1 hour at 4˚C. After incubation, lysate-Ni-NTA mixture was centrifuged at 4,500 rpm for 5 min at 4˚C and the supernatant was collected as the flow-through fraction. Resin was washed twice with wash buffer (20 mM Tris-HCl pH 8.5, 1 M KCl, 10 mM 2-Mercaptoethanol and 10% glycerol). Protein was eluted sequentially twice using elution buffers (Buffer I: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 250 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol, Buffer II: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 500 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol). Eluates were collected in four fractions and analyzed by SDS-PAGE. Eluates containing PDE4B protein were pooled and stored at -80˚C in 50% glycerol until further use.

PDE4 enzymatic assay

The inhibition of PDE4 enzyme was measured using PDElight HTS cAMP phosphodiesterase assay kit (Lonza) according to manufacturer's recommendations. Briefly, 10 ng of in house purified PDE4B1 or 0.5 ng commercially procured PDE4D2 enzyme was pre-incubated either with DMSO (vehicle control) or compound for 15 min before incubation with the substrate cAMP (5 µM) for 1 hour. The reaction was halted with stop solution and reaction mix was incubated with detection reagent for 10 minutes in dark. Dose response studies were performed at 13 different concentrations ranging from 200 µM to 0.001 µM. Luminescence values (RLUs) were measured by a Multilabel plate reader (Perklin Elmer 1420 Multilabel counter).The percentage of inhibition was calculated using the following formula and the IC_{50} values were determined by a nonlinear

regression analysis from dose response curve using Graphpad Prism software (San Diego, U.S.A). IC_{50} values are expressed as mean ± SD.

$$\%\ inhibition = \frac{(RLU\ of\ vehicle\ control - RLU\ of\ inhibitior)}{RLU\ of\ vehicle\ control} X\ 100$$

Reference:

1. Wang P., Myers J.G., Wu P., Cheewatrakoolpong B., Egan R.W., Billah M.M., 1997. Expression, purification, and characterization of human cAMP-specific phosphodiesterase (PDE4) subtypes A, B, C, and D. Biochem. Biophys. Res. Commun 19, 320-324.

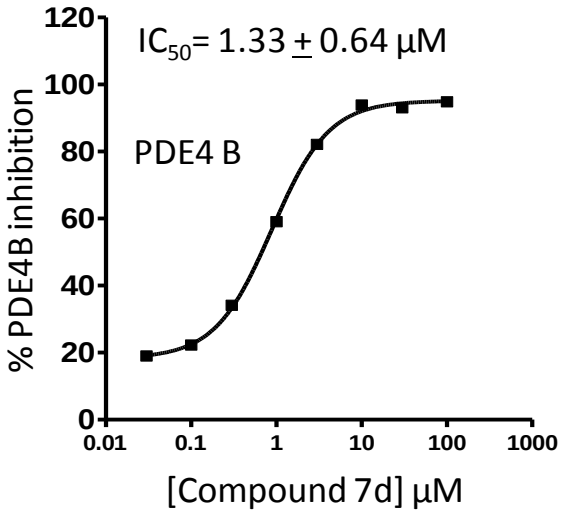
TNF-α production assay

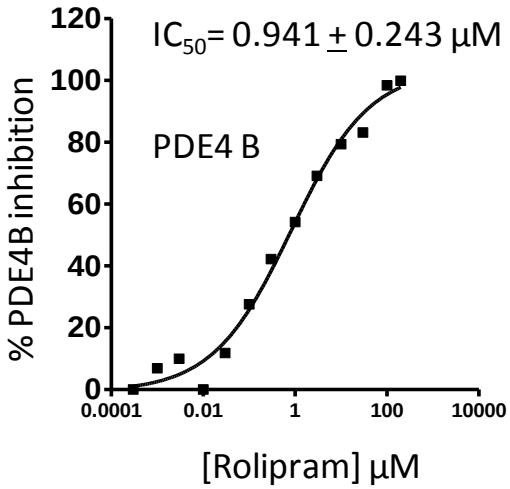
The production of TNF-α is measured following a procedure described previously after few modifications.[1] Briefly, RAW 264.7 cells were pre-incubated either with DMSO (vehicle control) or compound for 30 minutes and then stimulated with 1 µg/ml of LPS overnight. Dose response studies were carried out at eight different concentrations (30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 µM). Post-stimulation, cell supernatants were harvested, centrifuged to clear cell debris and the amount of TNF-α in the supernatants was measured using mouse TNF-α DuoSet ELISA kit from R&D Systems according to manufacturer's recommendations. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{inhibition} = 100 - \left[\frac{(\ \text{LPS stimulated}_{\text{compound}} - \text{unstimulated})}{(\text{LPS stimulated}_{\text{DMSO}} - \text{unstimulated})}\ \text{X}\ 100\right]$$

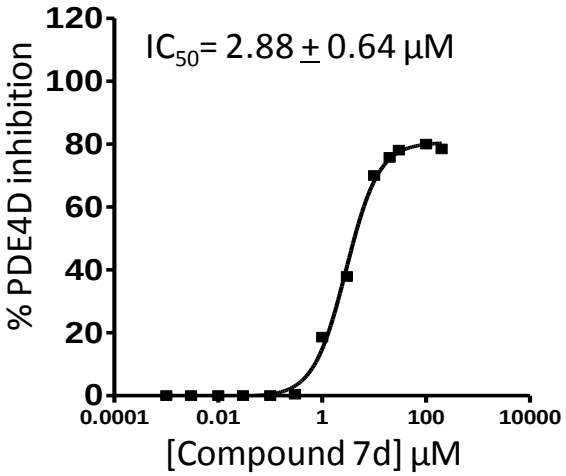
The IC_{50} values were determined by a nonlinear regression analysis from dose response curve using Graphpad Prism software (San Diego, U.S.A). IC50 values are expressed as mean ± SD.

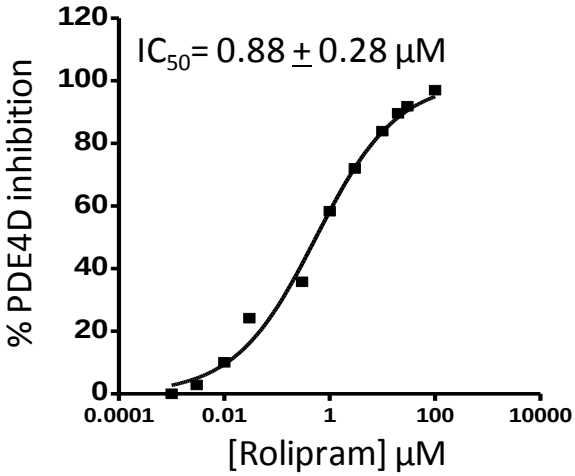
1) PDE4B enzyme assay



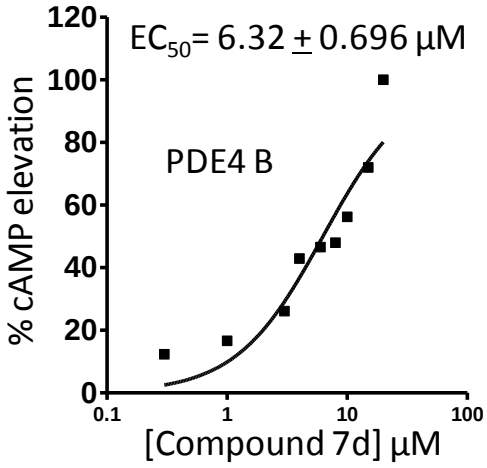


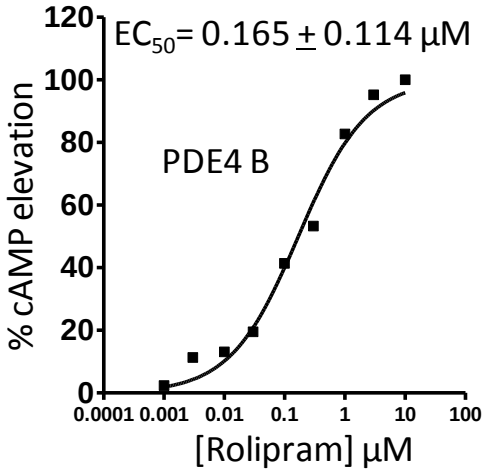
2) PDE4D enzyme assay



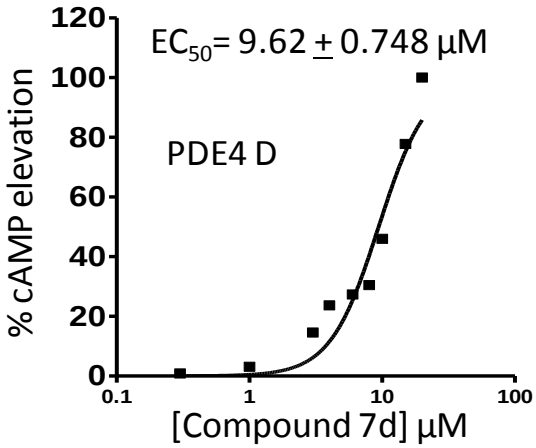


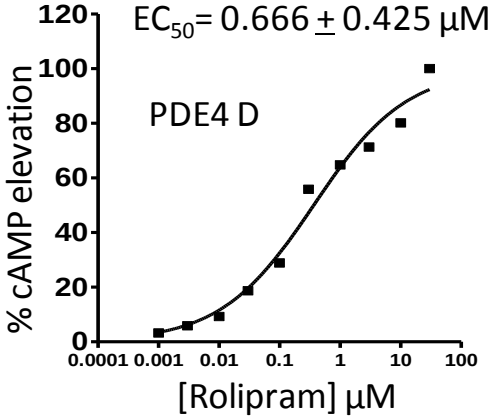
3) PDE4B cell based reporter assay



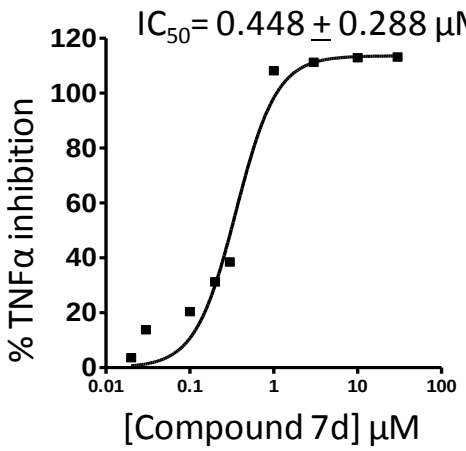


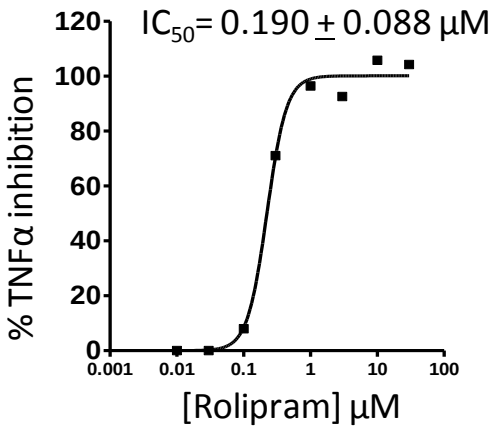
4) PDE4D cell based reporter assay





5) TNF alpha inhibition assay





Reference:

1. Parsa, K. V.; Ganesan, L. P.; Rajaram, M. V.; Gavrilin, M. A.; Balagopal, A.; Mohapatra, N. P.; Wewers, M. D.; Schlesinger, L. S.; Gunn, J. S. Tridandapani, S. *PLoS Pathog.* 2006, 2, 681-690.

Evaluation of compound toxicity in zebrafish embryo model

Experimental animals

Adult zebrafish (indigenous wild type strains) were obtained from local commercial distributor (Vikrant Aquaculture, Mumbai, India). They were maintained in 5L tanks in a recirculation system using purified ELIX (Millipore) grade water, under optimal conditions of 14h light and 10h dark cycle and temperature (26-29^{o}C). Males and females were separated for four days before they were allowed to spawn. On day five, males and females in a ratio of 2:1 were housed in a separate tank for spawning under optimal conditions. Embryos were collected and grown in E3 embryo medium (Westerfield, 2007) for seven days at 26-29^{o}C.

Chemicals and preparation of solutions

Rolipram, Terfenadine and Dimethyl Sulphoxide (DMSO) were procured from Sigma Aldrich, India. Ingredients of the medium for embryo culture viz. Sodium Chloride (NaCl), Calcium Chloride (CaCl2), Potassium Chloride (KCl) and Magnesium Sulphate (MgSO4) were procured

from Himedia. Stocks of compounds were prepared in 100% DMSO. The stocks were diluted in E3 embryo medium to obtain the concentration of 100µM Terfenadine, 10 & 20 µM of Rolipram and 10 & 20 µM of 7d in 0.1% DMSO.

Drug exposure and observations

Seven day old embryos were distributed in order of three embryos per well in twelve randomized wells of a 24-well plate. Embryos were treated with 300µl of one of the six different solutions viz. Control (0.1% DMSO), 10 µM Rolipram, 20µM Rolipram, 10 µM **7d**, 20µM **7d** and 100µM Terfenadine with two wells per treatment. 100µM Terfenadine was considered as the positive control. At different time points (1, 4, 6, 8, 24 h.) embryos were examined for mortality or any apparent changes in morphology or locomotor activity. Evaluation of heart rate was carried out by manual counting of heart beats under a compound microscope at 24h.

Zebrafish embryo toxicity model

Each embryo was assigned a lesion score (5-0) based on the morphological score assessment guideline suggested by Panzica-Kelly et.al. The score guidelines used are shown in the following table.

Score	Morphological Assessment
5	Anatomical structure is entirely normal for developmental stage
4	Slight variation in morphology suggestive of a developmental delay or anomaly that is potentially recoverable
3	Structure has a mild abnormality, typically associated with only 1 abnormality
2	Structure has moderate malformation(s), typically with two or more abnormalities
1	Structure has severe malformation(s), typically with multiple abnormalities
0.5	Structure is not evident by gross morphology assessment
0	Embryo is dead

References

1. 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 B Dev Reprod Toxicol.* 2010, **89**, 382.
2. M. Westerfield The zebrafish book, 2007 5th ed. Eugene: University of Oregon Press.