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Base Catalyzed Microwave Assisted Synthesis, Characterization of 6-Bromo-Pyrazolo-[1,5-a]-Pyrimidine-3-Ethyl-Carboxylate & Its Biological Evaluation as CDKs Inhibitor

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In this work, 5-amino-1*H*-pyrazole-4-ethyl-carboxylate is synthesized by treating ethyl cyanoacetate and hydrazine hydrate in presence of different nanometal-oxide catalysts under solvent free microwave conditions. The yield quantity of the synthesized compounds varies for specific catalysts. 6-Bromopyrazolo-[1,5-a]-pyrimidine-3-ethyl-carboxylate was synthesized by reaction of 5-amino-1*H*-pyrazole-4-ethyl-carboxylate with 2-bromo-malonaldehyde using different bases. The rate of reactions were found to be influenced on the strength of bases. The synthesized compounds were confirmed by FT-IR, ^{1}H NMR and LC-MS spectra. The compound 6-bromo-pyrazolo[1,5-a]pyrimidine-3-ethyl-carboxylate was then tested for *in vitro* biological activity as CDKs inhibitor. It showed better IC_{50} values for CDK4 and CDK6 than roscovitine. Thus, the synthesized compound is a selective inhibitor or acceptor for abnormal cancer cell line (CDKs).

Keywords: Pyrazolo-pyrimidines, Nano metal-oxides, Cyclin dependent kinase.

INTRODUCTION

The researches in the medicinal chemistry are remarkable due to its essential role for human life. Pyrazolo-pyrimidines show a vast contribution towards drug chemistry by acting as an antagonist to several diseases of man. This work reported the synthesis of 6-bromo-pyrazolo-[1,5-a]-pyrimidine-3-ethyl-carboxylate (**5**) by changing bases for succesive trials under microwave conditions from 5-ammino-1*H*-pyrazole-4-carboxylate (**3**) [1-5]. Reaction time for a strong base like KOH completed at the least time. This inference emerged a new trend for the study of chemical kinetics of organic synthesis.

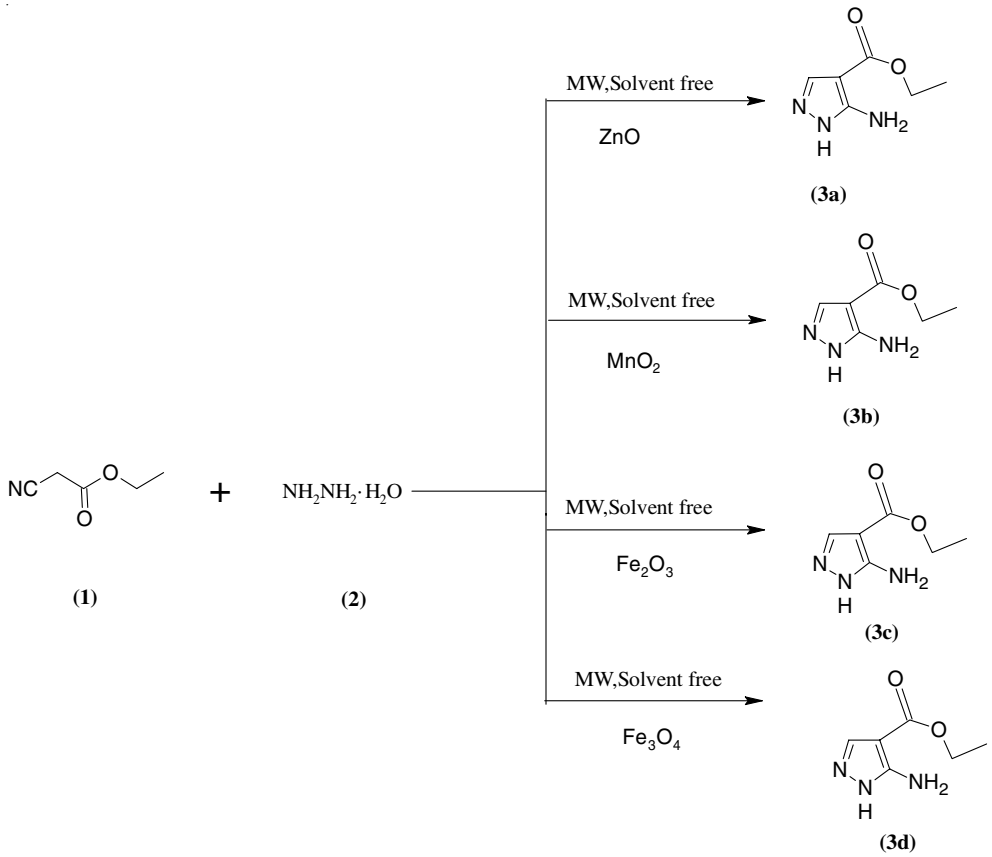
In this article, quantitative synthetic studies of 5-amino-1*H*-pyrazole-4-carboxylate (**3**) using different nano metal-oxide catalysts is reported [6]. α-Fe_3O_4 afforded highest yield among four different catalysts used. The compound **5** was screened for the biological activity as CDKs inhibitor with the reference compound roscovitine [7]. It proved as a better potent inhibitor for CDK4 & CDK6 (IC_{50}) than roscovitine.

EXPERIMENTAL

All the chemicals including nano-catalysts used were purchased from Sigma-Aldrich (AR) and Merck. Melting points were determined in open capillary tubes in Buchi B-540 melting point apparatus and are uncorrected. The reaction was monitored by thin layer chromatography using silica gel glass plates. NMR spectral data was determined on a BRUKER 400MHZ NMR spectrometer using TMS as internal reference. Mass spectra was recorded on ion trap mass spectrometer such as LC-MS, D-trap & XCT plus. FT-IR spectrometer (Bruker) was used for infrared analysis.

General synthetic procedure of 5-amino-1*H*-pyrazole-4-carboxylate (3) using ZnO nano-catalyst: To a solution of ethyl cyanoacetate **(1)** (10.0 g, 10.63 mL, 0.0884 mol), hydrazine hydrate (**2**) was added by dropwise under microwave condition at 80-100 ºC (**Scheme-I**). The solution turned brown in colour which was heated under microwave at 100 ºC. The solid precipitate was quenched with water and extracted to ethyl acetate. The organic phase was dried over sodium sulphite, filtered and concentrated to afford white solid 5-amino-1*H*-pyrazole-4-ethyl-carboxylate (**3a-d**). The compound was purified by washing with hot ethanol for several times. Thin layer chromatography and iodine chamber were used to monitor the reaction (**Scheme-I**). Similarly, same procedures and conditions were followed for different nano-metal oxides catalyst. The details of reaction conditions and yield using different nano-metal oxides are shown in Table-1.

5-Amino-1*H*-pyrazole-4-ethyl-carboxylate (3a): White solid; m.p.: 122.5 ºC, (8.9 g, yield: 89 %); Anal. Calcd. (%) for $C_6H_9N_3O_2$: C, 46.45; N, 27.08; H, 5.847. Found: C, 46.40; N, 27.31; H, 5.65. IR (KBr, ν_{max}, cm^{-1}): 3339.72 (N-H str.), 3230.43



Scheme-I: Synthesis of 5-amino-1*H*-pyrazole-4-ethyl carboxylate using different nanocatalysts

TABLE-1
REACTIONS CARRIED UNDER DIFFERENT NANO-CATALYSTS

Substrate	Reaction condition	Nano-catalyst	Product	Yield (%)
Ethyl cyanoacetate	MW, 2 min	ZnO	**3a**	84
Ethyl cyanoacetate	MW, 1 min	MnO_2	**3b**	89
Ethyl cyanoacetate	MW, 40 s	Fe_2O_3	**3c**	92
Ethyl cyanoacetate	MW, 30 s	Fe_3O_4	**3d**	94

(NH_2 str.), 2983.30 and 2908.67(-CH str.), 1717 (C=O ester); H^1 NMR (300 MHZ, DMSO): δ 1.266 (3H, t, CH_3),4.2 (2H, q, CH_2), 5.99 (1H, s, CH), 11.844 (1H, s, -NH), 7.4 (2H, s, -NH_2). LC-MS: (*m/z*) 273.0, 270.0; 225.9; 193.1, 192.1; 147.1.

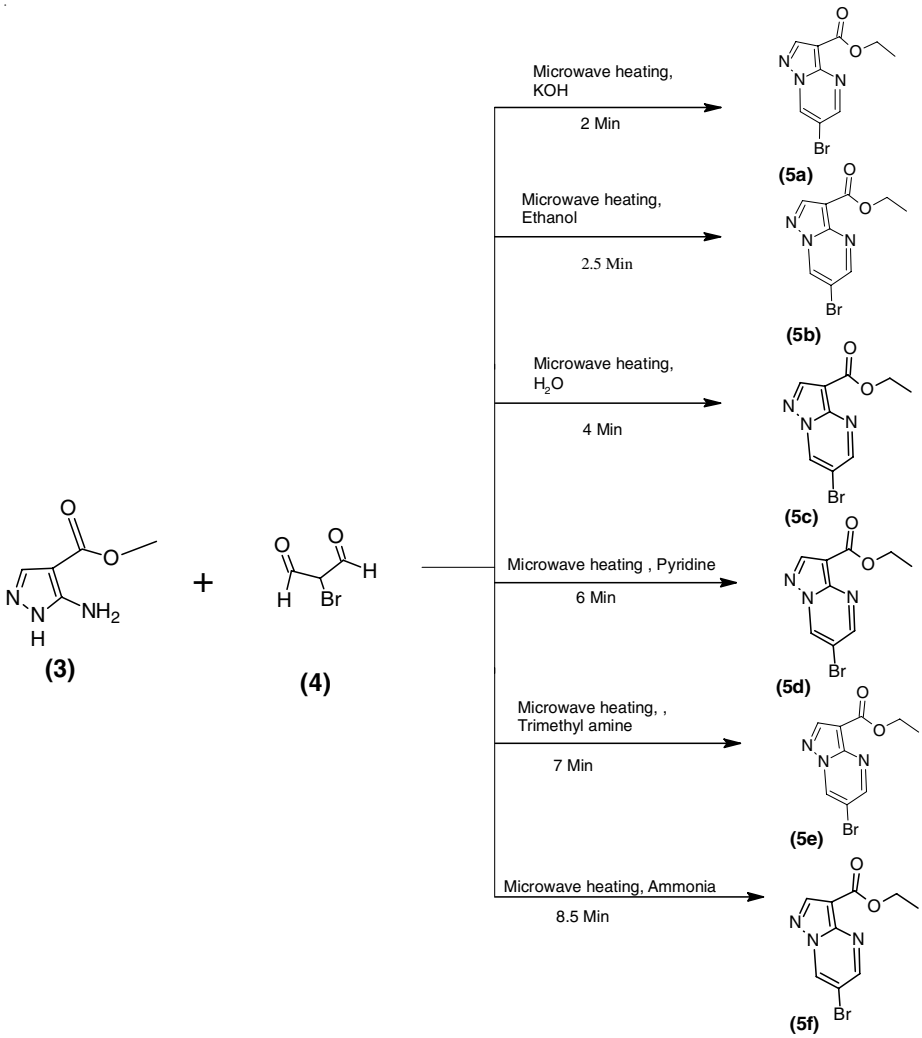
5-Amino-1H-pyrazole-4-ethyl-carboxylate (3b): White solid, m.p. 125 ºC, (8.9 g, yield: 89 %); Anal. calcd. (%) for $C_6H_9N_3O_2$: C, 46.45; N, 27.08; H, 5.847. Found: C, 46.45; N, 27.39; H, 5.45. IR (KBr, ν_{max}, cm^{-1}): 3356 (-NH, str.), 3230 (NH_2), 3060 (=C-H, str.), 1705 (C=O, ester), 1592 (C=C, weak), 1447 (CH_3, bend.). ^{1}H NMR (DMSO, 400 MHz): σ ppm 2.146 (NH_2), 2.508-2.526 (=CH-N, α to nitrogen), 3.323 (CH_2, α to ester), 6.522-7.85 (=NH, m);

5-Amino-1H-pyrazole-4-ethyl-carboxylate (3c): White solid, m.p. 122-125 ºC, (9.2 g, yield: 92 %). Anal. calcd. (%) for $C_6H_9N_3O_2$: C, 46.45; N, 27.08; H, 5.847. Found: C, 46.41; N, 27.63; H, 5.28. IR (KBr, ν_{max}, cm^{-1}): 3345 (-NH, str.), 3228 (NH_2, str.), 1732 (C=O, ester), 1598 (C=C, weak), 1528 (CH_2, bend.), 1350 (CH_3, bend.). ^{1}H NMR (DMSO, 400 MHz): 1.028-1.220 (NH_2), 2.086-2.499 (=CH, α to NH_2), 3.307 (CH_2, ester), 3.920 (OCH_3), 6.648-8.91 (=CH, α to -O-C=O).

5-Amino-1H-pyrazole-4-ethyl-carboxylate (3d): White solid, m.p. 119-123 ºC, (9.4 g, yield 94 %); Anal. calcd. (%) for $C_6H_9N_3O_2$: C, 46.45; N, 27.08; H, 5.847. Found: C, 46.22; N, 27.50; H, 5.72. IR (KBr, ν_{max}, cm^{-1}): 3363 (-NH, str.), 3212 (NH_2), 2968 (=C-H, str.), 2838 (-C-H, str.), 1648 (O-C=O, 1602 (C=C,

TABLE-2
REACTION CONDITIONS CARRIED BY DIFFERENT BASES

Substrate	Reaction condition	Bases	Time (min)	Purification	m.p. (°C) & colour	Product	Yield (%)
3	MW	KOH	2.0	Washed by ethanol & filtered	278; Yellow solid	**5a**	87
3	MW	EtOH	2.5	Washed by hot water & filtered	234; Yellow solid	**5b**	85
3	MW	H_2O	4.0	Washed by Ethanol & filtered	182-185; Yellow solid	**5c**	84
3	MW	Pyridine	6.0	Extracted by petroleum ether & washed by ethanol & filtered	166-168; Yellow solid	**5d**	80
3	MW	Triethyl amine	7.0	Solvent extract by petroleum ether & washed with ethanol & filtered	186; Yellow solid	**5e**	78
3	MW	Ammonia	8.5	Solvent extract by ethyl acetate & washed with ethanol & filtered	202; Yellow solid	**5f**	72



Scheme-II: Synthesis of 6-bromo-pyrazolo-[1,5-a]-pyrimidine-3-ethyl carboxylate using different base

weak), 1508 (CH_2, bend.), 1251 (C-O-C, strong). ^{1}H NMR (DMSO, 400 MHz): 2.481-2.499 (=CH, α to NH_2), 3.302 (CH_2, α to O-C=O), 3.817 (OCH_3),7.031-8.61 (=CH, α to O-C=O).

General synthetic procedure of 6-bromo-pyrazolo[1,5-a]-pyrimidine-3-ethyl-carboxylate (5) using KOH as base: To a compound **3**, 2-bromo-malonaldehyde (1.16 g, 0.0077 mol) was added in presence of KOH under microwave conditions at 110 ºC to afford 6-bromo-pyrazolo[1,5-a]pyrimidine-3-ethyl-carboxylate (**5**) (**Scheme-II**). The reaction was monitored by thin layer chromatography. The solid crude was washed with ethanol and filtered to afford 6-bromo-pyrazolo[1,5-a]-pyrimidine-3-ethyl-carboxylate as yellow solid. Similarly, same procedures were followed for EtOH (**5b**), H_2O (**5c**), pyridine (**5d**), triethylamine (**5e**) and ammonia (**5f**) as base solvent under the same conditions. The details of purification process and time consumed using different base are depicted in Table-2.

6-Bromo-pyrazolo[1,5-a]pyrimidine-3-ethyl-carboxylate (by KOH base) (5a): Yellow solid, m.p. 278 ºC, yield: 87 %; Anal. Calcd. (%) for $C_9H_9N_3O_2Br$: C, 39.57; N, 15.38; H, 3.33; Br, 30.02; Found: C, 39.83; N, 15.43; H, 3.39; Br, 29.71. IR (KBr, ν_{max}, cm^{-1}): 3018 (CH-), 2976, 2918 and 2864 (-CH str.), 1765 =O, ester), 683 (C-Br); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.410, 1.434 and 1.458 (m, 3H, CH_3), 4.423-4.494 (m, 2H, CH_2), 8.564 (s, 1H, C-C=H), 8.778, 8.786 (d, 1H, C=CH-), 8.917, 8.925 (d, 1H, C=CH-); LC-MS: *m/z* 273.0, 270.0, 225.9, 193.1, 192.1, 147.1.

6-Bromo-pyrazolo[1,5-a]pyrimidine-3-ethyl-carboxylate (by EtOH base) (5b): Yellow solid, m.p.: 234 °C, yield: 85 %); Anal. Calcd. (%) for $C_9H_9N_3O_2Br$: C, 39.57; N, 15.38; H, 3.33; Br, 30.02; Found: C, 39.74; N, 15.47; H, 3.29; Br, 29.93. IR (KBr, ν_{max}, cm^{-1}): 3040 (CH-), 2992, 2922 and 2854 (-CH stretch), 1775 (-C=O, ester), 693 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.77 (1H, d, *J* = 2.5 Hz), 6.96-6.94 (1H, ddd, *J* = 8.2, 1.5, 1.4 Hz), 7.28 (1H, ddd, *J* = 7.6, 1.5, 1.5 Hz), 7.4 (1H, td, *J* = 1.4, 0.5 Hz), 7.77 (1H, ddd, *J* = 8.2, 7.6, 0.5 Hz), 8.1 (1H, d, *J* = 2.5 Hz), 9.687 (=CH-N).

6-Bromo-pyrazolo[1,5-a]pyrimidine-3-ethyl-carboxylate (by H_2O base) (5c): Yellow solid, m.p.: 182-185 ºC, yield: 84 %); Anal. Calcd. (%) for $C_9H_9N_3O_2Br$: C, 39.57; N, 15.38; H, 3.33; Br, 30.02; Found: C, 39.89; N, 15.30; H, 3.41; Br, 29.48. IR (KBr, ν_{max}, cm^{-1}): 3022 (CH-), 2985, 2922 and 2854 (-CH str.), 1752 (-C=O, ester), 673 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.715-3.883 (2H, NH_2), 2.009-2.517 (-CH=N, str.), 6.82 (1H, d, *J* = 2.5 Hz), 7.04 (1H, td, *J* = 8.1, 1.1 Hz), 7.11 (1H, ddd, *J* = 8.3, 8.1, 1.4 Hz), 7.48 (1H, ddd, *J* = 8.3, 1.1, 0.5 Hz), 7.50-7.81 (2H, 7.48 (d, *J* = 2.5 Hz), 7.84 (ddd, *J* = 8.1, 1.4, 0.5 Hz), 8.64 (=CH-N).

6-Bromo-pyrazolo[1,5-a]pyrimidine-3-ethyl-carboxylate (by pyridine base) (5d): Yellow solid, m.p.: 166-168 ºC, yield: 80 %); Anal. Calcd. (%) for $C_9H_9N_3O_2Br$: C, 39.57; N, 15.38; H, 3.33; Br, 30.02; Found: C, 40.02; N, 15.07; H, 3.21; Br, 29.43. IR (KBr, ν_{max}, cm^{-1}): 3010 (CH-), 2982, 2928 and 2874 (-CH str.), 1762 (-C=O, ester), 662 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.503-3.317 (=CH-N), 7.18 (1H, d, *J* = 2.1 Hz), 7.33 (1H, ddd, *J* = 8.5, 8.1, 1.6 Hz), 7.49 (1H, d, *J* = 2.1 Hz), 7.62-7.75 (2H, 7.77 (ddd, *J* = 8.1, 7.5, 1.6 Hz), 7.8 (ddd, *J* = 7.5, 1.6, 0.5 Hz), 8.4 (1H, ddd, *J* = 8.5, 1.6, 0.5 Hz), 9.7 (1H, =CH-N).

6-Bromo-pyrazolo[1,5-a]pyrimidine-3-ethyl-carboxylate (by triethylamine base) (5e): Yellow solid, m.p.: 186 ºC, yield: 78 %); Anal. Calcd. (%) for $C_9H_9N_3O_2Br$: C, 39.57; N, 15.38; H, 3.33; Br, 30.02; Found: C, 40.17; N, 15.22; H, 3.37; Br, 29.97. IR (KBr, ν_{max}, cm^{-1}): 3016 (CH-), 2987, 2928 and 2874 (-CH str.), 1760 (- C=O, ester), 642 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.88 (3H, CH_3), 6.82 (1H, d, *J* = 2.4 Hz), 7.04 (1H, ddd, *J* = 8.2, 7.7, 1.4 Hz), 7.16 (1H, d, *J* = 2.4 Hz), 7.48-7.50 (2H, 7.77 (ddd, *J* = 7.7, 7.6, 1.3 Hz), 7.81 (ddd, *J* = 7.6, 1.4, 0.4 Hz), 7.86 (1H, ddd, *J* = 8.2, 1.3, 0.4 Hz), 8.64 (s, =CH-N).

6-Bromo-pyrazolo-[1,5-a]-pyrimidine-3-ethyl-carboxylate (by ammonia base) (5f): Yellow solid, m.p.: 202 ºC, yield: 72 %; Anal. Calcd. (%) for $C_9H_9N_3O_2Br$: C, 39.57; N, 15.38; H, 3.33; Br, 30.02; Found: C, 40.47; N, 15.01; H, 3.12; Br, 30.33. (KBr, ν_{max}, cm^{-1}): 3014 (CH-), 2987, 2929 and 2877 (-CH str.), 1761 (-C=O, ester), 644 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.503-3.317 (=CH-N), 7.18 (1H, d, *J* = 2.1 Hz), 7.33 (1H, ddd, *J* = 8.5, 8.1, 1.6 Hz), 7.49 (1H, d, *J* = 2.1 Hz), 7.62-7.75 (2H, 7.77 (ddd, *J* = 8.1, 7.5, 1.6 Hz), 7.8 (ddd, *J* = 7.5, 1.6, 0.5 Hz), 8.4 (1H, ddd, *J* = 8.5, 1.6, 0.5 Hz), 9.7 (1H, =CH-N).

RESULTS AND DISCUSSION

In order to study about the rate of reaction, both strong and weak bases were used for the trials. It was found that strong bases could afford the product much quicker than weaker bases. In this report, KOH reagent involved in the reaction completed at a shorter time and higher yield (Table-2). The rate of reaction from **5a** to **5f** was found slight different for microwave conditions because of small time difference between the trials [4,8]. Syntheses of 5-amino-1*H*-pyrazole-4-ethyl-carboxylate (**Scheme-I**) were carried by different nano metal-oxides & studied the inferences [9-11]. Quantitative yield from **3a** to **3d** was increased, which concluded that yield of the product could improvise by using more oxygen coordinated metal nano-catalysts. α-Fe_3O_4 possesses more oxygen atoms which have more active sites to adsorb. It facilitated starting materials to adsorb uniformly.

Biological activity: 6-Bromo-pyrazolo[1,5-a]pyrimidine-3-ethyl-carboxylate (**5**) was tested *in vitro* for anticancer activity and proved as potent inhibitor of CDKs substrates (CDK1, CDK2, CDK4, CDK5 and CDK6). The compound **5** showed more inhibition at IC_{50} values of 15.5 and 19.5 for CDK4 & CDK6, respectively (Table-3). This experiment showed it inhibits phosphorylation of CDK substrates, Rb and the RNA polymerase II C-terminal domain, cause down-regulation of cyclins A, E and D1 and results in cell cycle block in both the G2/M and S phases [12]. Hence, compound **5** proved as anti-proliferative agent for cancer cell lines or as potent selective CDK inhibitor for cancer patients compare to purine analogues of roscovitine [13,14].

TABLE-3
COMPARISON OF INHIBITION ACTIVITY (IC_{50}) OF 6-BROMO-PYRAZOLO-[1,5-a]-PYRIMIDINE-3-ETHYL-CARBOXYLATE (**5**) WITH ROSCOVITINE DRUG

Conc.	IC_{50} values		Roscovitine	Kinase
	Compd. **3**	Compd. **5**		
0.50	0.90	1.40	0.003	CDK1
0.05	0.10	0.07	0.003	CDK2
0.50	14.50	19.50	9.000	CDK4
0.50	0.02	0.26	0.003	CDK5
1.00	11.30	15.50	20.500	CDK6

Conclusion

A simple and affordable microwave assisted syntheses of pyrazolo-pyrimidines using inexpensive starting materials (bases and nano metal-oxides) is reported. The 6-bromo-pyrazolo-[1,5-a]-pyrimidine-3-ethyl-carboxylate (**5**) exhibited anticancer /anti proliferative activity as competent for the reference drug roscovitine.

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