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Poly (ethylene glycol) (PEG-400): A green approach towards synthesis of novel pyrazolo [3,4-d] pyrimidin-6-amines derivatives and their antimicrobial screening

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ABSTRACT

A simple and convenient route is described for the synthesis of novel pyrazolo [3, 4-d] pyrimidin-6-amines derivatives by using recyclable PEG-400 as an alternative reaction solvent. The reaction is clean with excellent yield, shorter reaction time and reduces the use of volatile organic compounds (VOCs).The chemical structure of the newly synthesized compounds was confirmed by IR,^{1}H NMR,^{13}C NMR and Mass spectral data. Furthermore, these newly synthesized compounds were screened for their antimicrobial activity against Escherichia coli (MTCC2939) St – Salmonella typhi (MTCC 98), Sa – Staphylococcus aureus (MTCC 96),and Bacillus subtilis (MTCC 441). The antifungal activity was evaluated against Aspergillus niger (MTCC 281), Candida albicans(MTCC183 and Trichoderma viridae (MTCC 167). The result revealed that most of the compounds showed good to moderate Antimicrobial screening. The major advantages of this protocol are high yields, operational simplicity, and short reaction times.

Keywords: pyrazolo [3,4-d] pyrimidin-6-amines, Guanidine hydrochloride, Pathogens.

INTRODUCTION

The organic compound containing pyrazole nucleus has wide applications in medicinal chemistry as well as considerable interest in the chemotherapeutic importance. Pyrazolopyrimidine and related fused heterocycles are of interest as potential bioactive molecules. Pyrazole and its synthetic analogues have been found to exhibit industrial, agricultural and some biological application [1-5].They are known to exibit pharmacological activities such as neuroleptic[6] and tuberculostatic[7]. Pyrazolo [3,4-d] pyrimidines were identified as a general class of adenosine receptors[8,9]. There is not much difference in the basic structure of Pyrazolopyrimidines and purines analogues. Some alkyl, aryl substituted Pyrazolopyrimidines have been documented as adenosine antagonist [10-12], and known to possess antibacterial [13], antifungal [14], and antitumor activity [15-16]. Due to importance of ring system in biological processes, many therapeutic agents contain pyrazole moiety as basics. Pharmacologically active purine analogues include tricyclic structure such as imidazolo [2,1-i] purinones (e.g. PSB-11 an Apotent A3 adenosine receptors antagonist) ,pyrimido [4,5-b] indoles (e.g. APEPI,a potent A_1 adenosine receptors antagonist), imidazo[2,1-A] and purine (e.g. tricyclic ganciclovir analogas) which are potent antiviral agents [17]. Present study reports synthesis of novel Pyrazolo [3, 4-d] pyrimidin-6-amines derivatives using green chemistry principles.

Reducing or eliminating the use of volatile organic solvents can minimize the generation of waste, which is a requirement of one of the principles of green chemistry [18, 19]. Recently, polyethylene glycol (PEG) has been

found to be an interesting solvent system. In continuation of own work on bioactive molecules as precursors in the synthesis of various heterocycles [20], we designed and synthesized a series of novel hetero pyrazolo [3, 4-d] pyrimidin-6-amines derivatives by applying the principles of green chemistry, using PEG-400 as an alternative reaction medium. PEG is an environmentally benign reaction solvent; it is non-toxic, inexpensive, potentially recyclable and water soluble, which facilitates its removal from the reaction Product.

MATERIALS AND METHODS

Synthesis of 4-(4-substituted phenyl)-3-methyl-1-phenyl-1*H*-pyrazolo [3, 4-d] pyrimidin-6-amine (3a)

As per scheme 1, A mixture of 4-(4-substituted benzylidene)-3-methyl-1-phenyl-1H-pyrazol-5-ones 2a (0.27 gm, 0.001 mole), guanidine hydrochloride (0.12 gm, 0.0015 mole) and 1-2 pallets of solid sodium hydroxide was stirred in PEG-400 (15 mL) at 40 °C for 2 hour. After completion of the reaction (monitored by TLC), the crude mixture was worked up in ice-cold water (100 mL). The product which separated out was filtered. The filtrate was evaporated to remove water leaving PEG behind. The same PEG was utilized to synthesize further derivates.

Analytical Procedures

Melting points were uncorrected and determined in open capillary tubes. Table 1 represents Physical-chemical data of substituted pyrazolo [3, 4-d] pyrimidin-6-amine derivatives.

The purity of the products was checked by thin layer chromatography (TLC) on precoated sheets of silica gel-G of 0.25 mm thickness. IR spectra were recorded (in KBr palates) on FTIR Schimadju spectrometer. 1H NMR spectra were recorded in DMSO-d6 in Avance 300 MHz spectrometer using TMS as an internal standard. The mass spectra were recorded on Ei-Shimadju-GC-MS mass spectrometer. Elemental analyses were performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer.

Spectroscopic data of selected compounds:

3a. 4-(2-butyl-4-chloro-1H-imidazol-5-yl)-3-methyl-1-phenyl-1H-pyrazolo [3,4-d] pyrimidine-6-amine.

IIIa: M.P. 129°C; Yield, 78%; IR(KBr): 718(C-Cl),1599(-C=N), 3338(-NH)cm^{-1}; ^{1}H NMR(DMSO-d_6, 300 MHz): δ0.93(t, 3H, -CH_3), δ1.31(m, 2H, -CH_2-), δ1.65(m, 2H, -CH_2-), δ2.76(t, 2H, -CH_2), δ1.93(t,3H,-$CH_{3pyra.}$), δ4.7(brs, 2H, -NH_2),δ6.89-7.56(m, 5H, Ar-H), δ8.19(s, 1H, -NH, D_2O exchangeable), ppm; ^{13}C NMR (100 MHz, DMSO-*d*6, TMS): δ 14 (CH_3),15(CH_3),22(CH_2),29(CH_2),30(CH_2),116,118 (2×C), 128(2×C), 129(2×C),130,132,133, 140,148, 164. EIMS(*m/z*): 381[M$^+$], Anal. Calcd. For $C_{19}H_{20}N_7Cl$: C, 59.76, H, 5.28; N, 25.68%.Found: C, 59.66; H, 5.18; N, 25.39%

3b.4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-methyl-1-phenyl-1H-pyrazolo[3,4-d] pyrimidine-6-amine.

IIIb: M.P. 135°C; Yield, 76%; IR(KBr): 1580(-C=N), 1550(-C=C),746(C-Cl)cm^{-1}; ^{1}H NMR(DMSO-d_6, 300 MHz): δ2.1(t, 3H, -CH_3), δ1.87 (t, 3H, -CH_3-), δ4.7(brs, 2H, -NH_2), δ6.96-7.58(m, 10H, Ar-H), ppm; ^{13}C NMR (100 MHz, DMSO-*d*6, TMS):δ14 (CH_3),15(CH_3), 114,118 (2×C),124, 128, 129(2×C),132,133,140,143,148,149,164.ppm; EIMS(*m/z*): 415[M$^+$], Anal. Calcd. For $C_{22}H_{18}N_7Cl$: C, 63.54, H, 4.36; N, 23.58%.Found: C, 63.46; H, 4.28; N, 23.24%

3c.4-(3-(4nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine-6-amine.

IIIc: M.P. 140°C; Yield, 89%; IR (KBr): 1598(-C=N), 1558(-C=C)cm^{-1}; ^{1}H NMR(DMSO-d_6, 300 MHz): δ2.06(t, 3H, -CH_3), δ4.8(brs, 2H, -NH_2), δ6.92-7.56(m, 14H, Ar-H), ppm; ^{13}CNMR(100MHz,DMSO-*d*6,TMS): δ15(CH_3), 113,118(2×C),124(2×C),124(2×C), 126(2×C),129(2×C),132.138,139,140,143,147,165ppm;; EIMS(*m/z*): 488[M$^+$], Anal. Calcd. For $C_{27}H_{20}N_8O_2$: C, 66.38, H, 4.13; N, 22.9%.Found: C, 66.23; H, 3.98; N, 22.85%

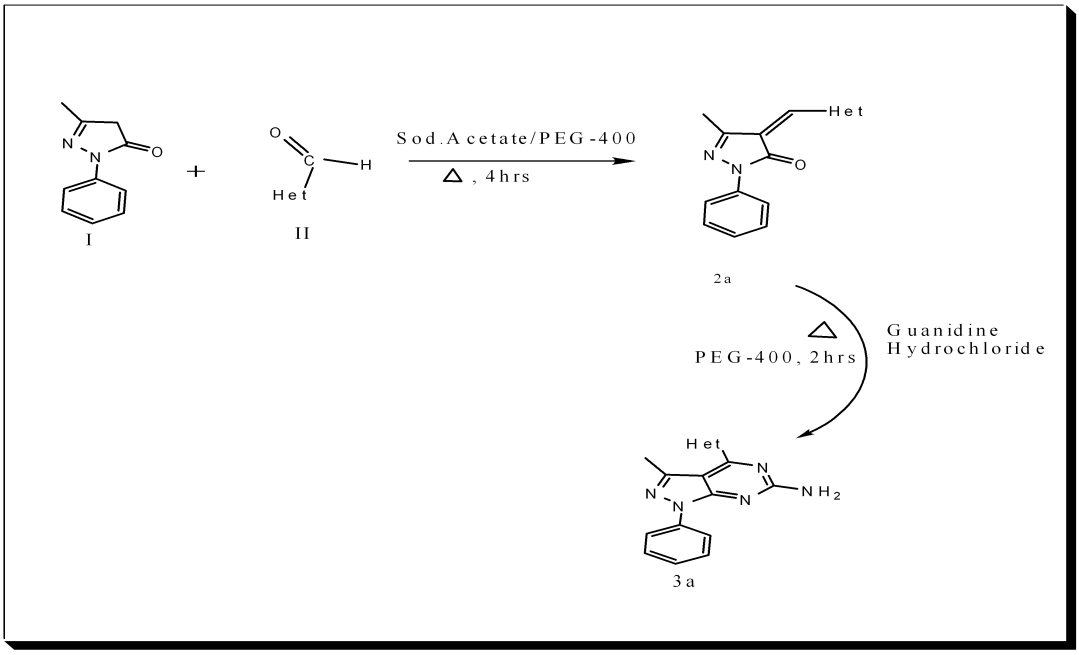
3d.4-(3-(4chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine-6-amine.

IIId: M.P. 156°C; Yield, 81%; IR(KBr): 1590(-C=N),1549(-C=C)cm^{-1}; ^{1}H NMR(DMSO-d_6, 300 MHz): δ2.1(t, 3H, -CH_3), δ5.1(brs, 2H, -NH_2), δ6.96-7.78(m, 14H, Ar-H), ppm; ^{13}CNMR(100MHz, DMSO*d*6, TMS): δ15(CH_3),113,118,124,128(2×C),129(2×C),131,132,134,139(2×C),140,143,147,165ppm;EIMS(*m/z*): 477[M$^+$], Anal. Calcd. For $C_{27}H_{20}N_7Cl$: C, 67.85, H, 4.22; N, 20.51%.Found: C, 67.78; H, 4.12; N, 20.43%

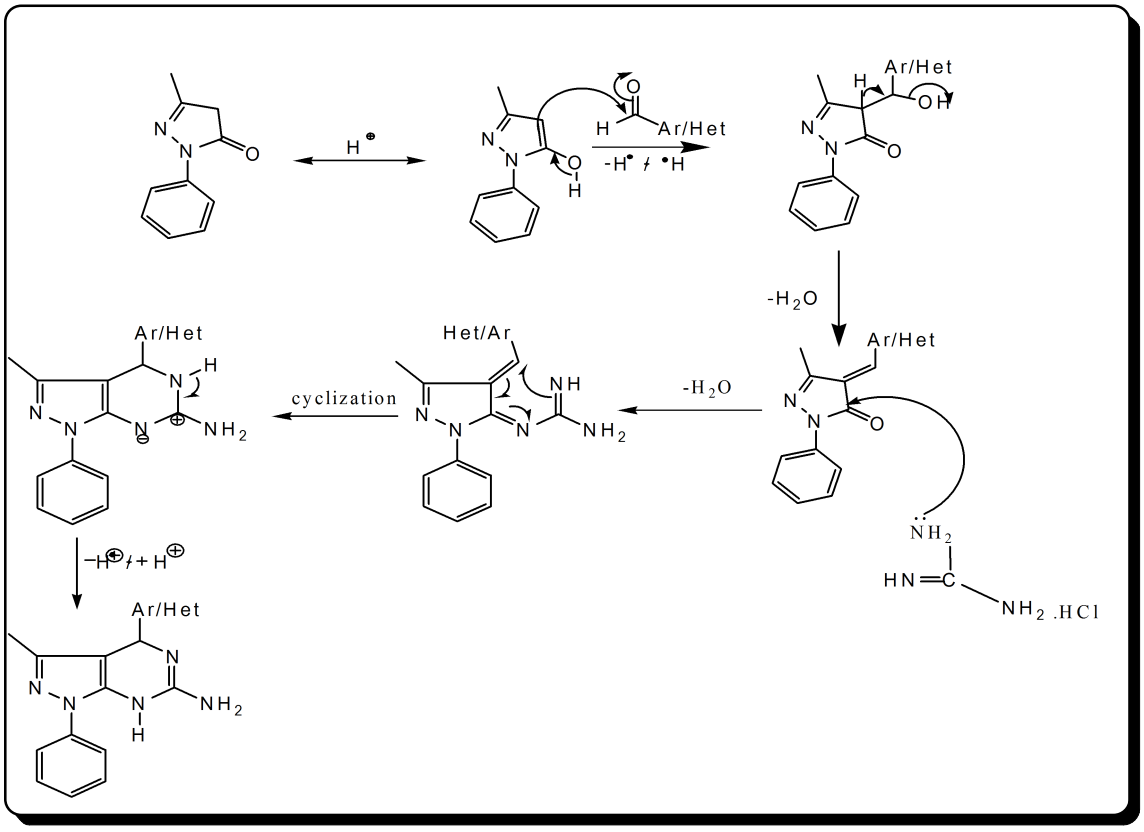
3e.4-(3-(4methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-3-methyl-1-phenyl-1H- pyrazolo[3,4-d]pyrimidine-6-amine.

IIIe: M.P. 125°C; Yield, 83%; IR(KBr): 1594(-C=N),1545(-C=C), 3345(-NH)cm^{-1}; ^{1}H NMR(DMSO-d_6, 300 MHz): δ2.1(t, 3H, -CH_3), δ3.76(t, 3H, -OCH_3-), δ4.9(brs, 2H, -NH_2), δ6.96-7.78(m, 14H, Ar-H), ppm;^{13}C NMR (100 MHz, DMSO-*d*6, TMS):δ15(CH_3), 56 (OCH_3)113, 115(2×C), 118(2×C), 124(2×C), 125, 129(2×C), 132.138, 139, 140,

143, 147, 160,165 ppm;; EIMS(*m/z*): 473[M^+], Anal. Calcd. For $C_{28}H_{23}N_7O$: C, 71.01, H, 4.90; N, 20.71%.Found: C, 69.89; H, 4.78; N, 20.54%

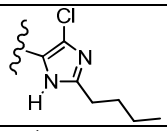
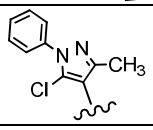
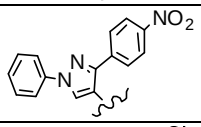
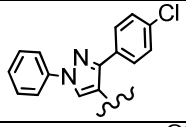
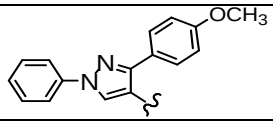
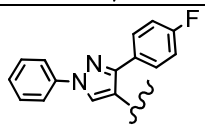
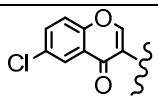
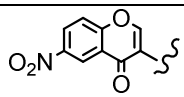
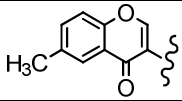
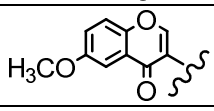


Scheme 1: Synthesis of 4-(4-substituted phenyl)-3-methyl-1-phenyl-1*H*-pyrazolo [3, 4-d] pyrimidin-6-amine (3a)



Tentative Mechanism of proposed Scheme 1 :

Table 1- Physical-chemical data of substituted pyrazolo [3, 4-d] pyrimidin-6-amine derivatives

ENTRY	PRODUCT	HET	YIELDS (%)	TIME (min)	M.P (°C)
1	3a		78	87	129
2	3b		76	85	135
3	3c		83	110	125
4	3d		81	106	156
5	3e		89	87	140
6	3f		86	94	153
7	3g		82	120	164
8	3h		93	98	125
9	3I		89	85	147
10	3J		90	89	153

Antimicrobial Screening:

The antimicrobial activities of the synthesized compounds **3(a-j)** were determined by agar well diffusion method [21]. The compounds were evaluated for antibacterial activity against, *Escherichia coli* (*MTCC2939) St – Salmonella typhi (MTCC 98), Sa – Staphylococcus aureus (MTCC 96),*and *Bacillus subtilis (MTCC 441).* The antifungal activity was evaluated against *Aspergillus niger* (*MTCC 281*), *Candida albicans(MTCC183* and *Trichoderma viridae (MTCC 167),*were procured from Institute of Microbial technology (IMTech), Chandigarh, India. The antibiotic penicillin (25µg/mL) and nystatin (25µg/mL) was used as reference drug for antibacterial and antifungal activity, respectively. Dimethyl sulphoxide (1%, DMSO) was used a control without compound. The results of antimicrobial data are summarized in Table-2. In comparison with standard antibacterial penicillin, compounds **3b, 3e, 3g** and **3h** found to be active against *Escherichia coli* (*MTCC2939).* Compounds **3c, 3d** were also found to be active against *Staphylococcus aureus (MTCC 96)* Compounds **3a, 3b** and **3j** showed good activity comparatively active against *Bacillus subtilis (MTCC 441).* As compared with standard antibacterial compounds 3a, 3b and 3gwere observed as active against Salmonella *typhi (MTCC98).* On the other hand, compound **3a, 3c** and **3f** were found to be reduced growth growth activity against *Aspergillus niger* (*MTCC 281*). Compounds **3d**, **3f, 3g, 3i** and **3j** were observed no fungal growth against *Candida albicans (MTCC 183).* Compounds **3c, 3e, 3h, 3i** and **3j** found to be reduced growth against *Trichoderma viridae (MTCC 167).*

Comp. No.	(zone of inhibition, mm)						
	Antibacterial activity				Antifungal activity		
					(Growth)		
	E. coli	*S. typhi*	*S.Aureus*	*B. subtilis*	*A. niger*	*C.albicans*	*T. viridae*
3a	13	12	09	14	RD	+ve	+ve
3b	14	13	10	13	+ve	RD	-ve
3c	09	08	13	11	RD	-ve	RD
3d	14	12	13	08	+ve	-ve	+ve
3e	14	10	08	13	+ve	RD	RD
3f	10	08	12	12	RD	-ve	-ve
3g	15	11	12	13	+ve	-ve	-ve
3h	14	09	08	11	-ve	+ve	RD
3i	11	11	10	13	+ve	-ve	RD
3j	12	10	12	12	+ve	-ve	RD
Penicillin	16	14	16	15	NA	NA	NA
Nystatin	NA	NA	NA	NA	-ve	-ve	-ve

Solvents: DMSO, water, Escherichia coli (MTCC2939) St – Salmonella typhi (MTCC 98), Sa –Staphylococcus aureus (MTCC 96),Bs – Bacillus subtilis (MTCC 441), An – Aspergillus niger (MTCC 281) Trichoderma viridae (MTCC 167) and Candida albicans (MTCC 183). –ve -No growth; +ve -Growth of fungi;RD-Reduced growth; NA-Not Applicable

RESULTS AND DISCUSSION

Prompted by the above mentioned biological properties of pyrazolo moieties, it was contemplated to synthesize some new series of pyrazolo-pyrimidine derivatives under the frame of 'green chemistry'. In recent years, poly(ethylene glycol) prompted reactions have attracted the attention of organic chemists due to their solvating ability and aptitude to act as a phase transfer catalyst, negligible vapor pressure, easy recyclability, ease of work-up, eco-friendly nature and economical cost. PEG is non-toxic, non-halogenated, inexpensive potentially recyclable and water soluble which facilitate its removal from reaction product. As part of our research programme, and in continuation of our work herein we report an efficient synthesis of substituted Pyrazoles-pyrimidine derivatives. Since the isolation of pyrimidine derivatives, considerable attention has been developed to their chemistry and biological screening. In recent year, there has been increasing interest in the synthesis of pyrimidine derivatives, and there are some methods used to synthesize pyrimidine ring, allowing access to a large no, of malfunctionised pyrimidine derivatives.

Treatment of substituted-3-methyl-4-methylene-1-phenyl-1H-pyrazol-5(4H)-one with guanidine hydrochloride with sodium hydroxide neutralizes it in presence of PEG-400 as a reaction solvent stirred 2 hrs to afford the corresponding (3a-j). The IR spectra of these compounds indicates disappearance of (>C=O) group. For eg.the spectrum of compound (3a) showed characteristics absorption band at 3338 cm^{-1} (NH), 1599(-C=N) and 718(C-Cl), The mass spectrum showed that the molecular ion peak is co-agreement with molecular weight of that corresponding compound.i.e.eg.(3a) EIMS(*m/z*): 381. The ^{1}H NMR spectrum of compound for eg.(3a) revealed signals at δ0.93 as triplet for $–CH_3$, δ1.31 as multiplet for $-CH_2$, δ1.6 multiplet for $-CH_2$,δ2.76 triplet for $-CH_2$, The basically characteristics of $–NH_2$ appears broad signals at 4.5-5.05 in range and all aromatic proton appear at δ6.96-7.78 in aromatic region.As like other compound shows signal showing in spectral analysis.

Compounds **3b, 3e, 3g** and **3h** found to be active against *Escherichia coli*(*MTCC2939)*. Compounds **3c, 3d** were also found to be active against *Staphylococcus aureus (MTCC 96)* Compounds **3a, 3b** and **3j** showed good activity comparatively active against *Bacillus subtilis (MTCC 441)*. As compared with standard antibacterial compounds **3a, 3b** and **3g**were observed as active against *Salmonella typhi (MTCC98).* compound **3a, 3c** and **3f** were found to be reduced growth growth activity against *Aspergillus niger* (*MTCC 281*). Compounds **3d**, **3f, 3g, 3i** and **3j** were observed no fungal growth against *Candida albicans (MTCC 183).* Compounds **3c, 3e, 3h, 3i** and **3j** found to be reduced growth against *Trichoderma viridae (MTCC 167*

CONCLUSION

In conclusion, our protocol is a practical approach which uses PEG-400 as a commercially available, low-cost, easily available solvent. In most cases, the reaction proceeded smoothly to produce the corresponding pyrazolo [3, 4-d] pyrimidin-6-amine derivatives. The reaction was clean and the products were obtained in excellent yields without formation of any side products. Most of the compounds showed good to moderate antibacterial and antifungal activity.

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