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Ultrasound assisted synthesis of pyrazolo[1,5-a]pyrimidine-antipyrine hybrids and their anti-inflammatory and anti-cancer activities

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RESEARCH ARTICLE

KEYWORDS

ABSTRACT

A series of antipyrinyl-pyrazolo[1,5-a]pyrimidines have been synthesized by reactions of aminopyrazole (4) with various formylated active proton compounds in the presence of $KHSO_4$ (aqueous media), under ultrasound irradiation. The structures of the compounds have been established with the help of spectral and analytical data. N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-7-phenylpyrazolo[1,5-a]pyrimidine-3-carboxamide (6a) was further subjected to X-ray crystallographic studies to avoid any ambiguity of the derived structures. Crystal data for compound 6a, $C_{51}H_{46}N_{12}O_5$ (M =907.00 g/mol): triclinic, space group P-1 (no. 2), a = 9.9554(3) Å, b = 14.0875(4) Å, c = 17.4572(4) Å, α = 79.676(2)°, β = 85.283(2)°, γ = 72.647(2)°, V = 2297.97(11) Å^3, Z = 2, T = 296.15 K, μ(MoKα) = 0.088 mm^{-1}, $Dcalc$ = 1.311 g/cm^3, 29732 reflections measured (4.174° ≤ 2Θ ≤ 57.068°), 10681 unique (R_{int} = 0.0400, R_{sigma} = 0.0533) which were used in all calculations. The final R_1 was 0.0566 (I > 2σ(I)) and wR_2 was 0.1663 (all data). The novel compounds were also screened for their biological activities.

1. Introduction

Pyrazolo[1,5-a]pyrimidine, an important pharmacophore and an adjective structure in medicinal chemistry, is well known for its innumerable biological and pharmacological properties [1,2]; like antitumor [3-5], antileukemic [3], antineoplastic [6], CNS stimulant [7], antihypertensive [8], adenosine receptors [9], tuberculostatic [10], antibacterial and antifungal [11], anti-inflammatory [12], anti-atherosclerotic [12,13] antischistosomal [14], and antileishmanial activities [15]. A well-known drug Zaleplon [16] which has found a positive role as a sedative for the treatment of insomnia without the risk of dependence consists of the core pyrazolo [1,5-a]pyrimidine ring. Also, 4-aminoantipyrine, containing the functional parent antipyrine has been reported to show myriads of biological activities such as anti-inflammatory [17], analgesic [18], antipyretic [19], antimicrobial [20], and anti-cancer activities [21]. 4-Aminoantipyrine has been used for the protection against oxidative stress as well as prophylactic of some diseases including cancer, and these are found to be

important directions in medical applications [22]. In view of these information and in continuation with our research work [23-25] on synthesis of pharmacologically important pyrazolo [1,5-a]pyrimidines, it was found worthy to synthesize novel pyrazolo[1,5-a]pyrimidines incorporating the antipyrine moiety to study their biological properties. Ultrasound (US) irradiation has helped researchers tremendously in accomplishing the green goals of chemistry by shortening the reaction time with easy work-up, resulting in high yields of the products and use of readily available solvents and catalysts [26-28]. Ultrasound tool has been advantageously employed to achieve the desired synthesis.

2. Experimental

2.1. Chemistry

2.1.1. Material and methods
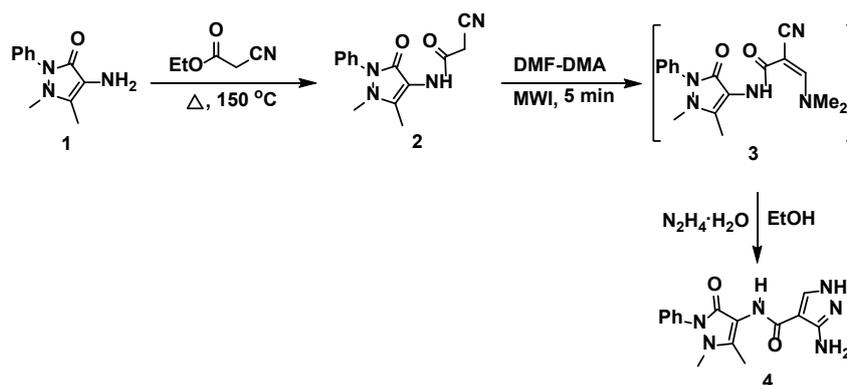
Scheme 1

Melting points were recorded by open capillary method and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 983 spectrometer. High-resolution ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were measured on a DRX-400 Varian spectrometer and Bruker spectrometer, respectively, and CDCl$_3$ and DMSO-d_6 was used as the solvent. The chemical shifts (δ, ppm) and the coupling constants (Hz) are reported in the standard fashion with reference to tetramethylsilane (TMS) as internal reference. In the NMR spectral data, the abbreviations s = singlet, t = triplet, m = multiplet, d = doublet, dd = double-doublet are used. The X-ray diffraction data were collected at 296 K with MoKα radiation (λ = 0.71073 Å) using a Bruker Nonius SMART APEX II CCD diffractometer equipped with a graphite monochromator. The structure was solved by direct methods (SHELXS97) and refined by full-matrix least squares based on F^2. All calculations were carried out using WinGX system version 1.80.05 [29]. All the non-H atoms were refined in the anisotropic approximation: H-atoms were located at calculated positions. The electron spray mass spectra were recorded on a THERMO Finnigan LCQ Advantage max ion trap mass spectrometer. Elemental analysis was performed on a Vario-EL III instrument. Ultrasound irradiation was carried out in an EQUITRON Digital Ultrasonic Cleaner-2.5 L, model 8425.025.424 at 170 Watt and 50 Hz. Microwave irradiation was carried out in a CEM Discover Benchmate microwave digester at 850 Watt in an open reaction vessel. Formylated active proton compounds were synthesized by our previously reported procedure [30,31]. The aminopyrazole **4** used as a synthon for reactions with formylated active proton compounds were obtained as shown in Scheme 1. Compound **2** was obtained following the procedure reported by Fadda *et al.* [32] which was then formylated with DMF-DMA using microwave (850 Watt, 5 minutes). The reaction mixture was then without further purification, reacted with hydrazine hydrate in refluxing ethanol [33], yielding the aminopyrazole (**4**).

2.1.2. Synthesis

2.1.2.1. Synthesis of antipyrinyl-7-arylpyrazolo[1,5-a] pyrimidines (6a-e)

Aminopyrazole (**4**) (1 mmol), enaminones **5** (1 mmol), and KHSO$_4$ (2 mmol) were suspended in 5 mL of ethanol:water (1:1, *v:v*) system and the resulting mixture was irradiated under the influence of ultrasound waves for 3-6 minutes resulting in the formation of a precipitated product (Scheme 2). After the completion of reaction monitored by thin layer chromatography (TLC), the precipitate was collected by filtration, washed repeatedly with water to ensure complete removal of acid and dried to give practically pure pyrazolo

pyrimidines (**6**) in 93-96 % yields. Further, purification was achieved by column chromatography using silica gel and 100 % EtOAc.

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**6a**): Color: Yellow solid. Yield: 95 %. M.p.: 191-192 °C. FT-IR (KBr, ν, cm^{-1}): 3448 (NH), 1645 (CO), 1637 (CO). ^{1}H NMR (400 MHz, CDCl$_3$, δ, ppm): 2.43 (s, 3H, CH$_3$), 3.17 (s, 3H, NCH$_3$), 7.10 (d, 1H, C6-pyrimidine, *J* = 4.5 Hz), 7.30-7.34 (m, 1H, ArH), 7.45-7.50 (m, 4H, ArH), 7.57-7.62 (m, 3H, ArH), 8.03-8.06 (m, 2H, ArH), 8.72 (d, 1H, C5-pyrimidine, *J* = 4.5 Hz), 8.75 (s, 1H, C2-pyrazole), 9.57 (s, 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$, δ, ppm): 12.7, 36.0, 105.5, 108.8, 108.9, 124.4, 127.1, 128.9, 129.3, 129.5, 130.0, 131.8, 134.5, 146.7, 147.5, 148.4, 149.0, 151.1, 160.9, 161.1. MS (EI, *m/z* (%)): 425 (MH)$^+$. Anal. calcd. for C$_{24}$H$_{20}$N$_6$O$_2$: C, 67.91; H, 4.75; N, 19.80. Found: C, 67.98; H, 4.72; N, 19.75 %.

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-7-(*p*-tolyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**6b**): Color: Yellow solid. Yield: 94 %. M.p.: 252-253 °C. FT-IR (KBr, ν, cm^{-1}): 3448 (NH), 1675 (CO), 1624 (CO). ^{1}H NMR (400 MHz, CDCl$_3$, δ, ppm): 2.42 (s, 3H, CH$_3$), 2.47 (s, 3H, CH$_3$), 3.17 (s, 3H, NCH$_3$), 7.07 (d, 1H, C$_6$-pyrimidine, *J* = 4.5 Hz), 7.28-7.32 (m, 1H, ArH), 7.39 (d, 2H, ArH, *J* = 7.8 Hz), 7.44-7.49 (m, 4H, ArH), 7.96 (d, 2H, ArH, *J* = 7.8 Hz), 8.68 (d, 1H, C5-pyrimidine, *J* = 4.5 Hz), 8.73 (s, 1H, C$_2$-pyrazole), 9.56 (s, 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$, δ, ppm):12.8, 21.7, 36.4, 105.4, 108.4, 109.1, 124.2, 126.9, 127.1, 129.2, 129.5, 129.6, 134.7, 142.5, 146.6, 147.5, 148.4, 149.1, 151.0, 160.9, 161.3. MS (EI, *m/z* (%)): 439 (MH)$^+$. Anal. calcd. for C$_{25}$H$_{22}$N$_6$O$_2$: C, 68.48; H, 5.06; N, 19.17. Found: C, 68.59; H, 5.04; N, 19.12 %.

7-(4-Chlorophenyl)-*N*-(1, 5-dimethyl-3-oxo-2-phenyl-2, 3-dihydro-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine-3-carbox amide (**6c**): Color: Yellow solid. Yield: 95 %. M.p.: 261-262 °C. FT-IR (KBr, ν, cm^{-1}): 3448 (NH), 1675 (CO), 1624 (CO). ^{1}H NMR (400 MHz, CDCl$_3$, δ, ppm): 2.42 (s, 3H, CH$_3$), 3.16 (s, 3H, CH$_3$), 7.09 (d, 1H, C6-pyrimidine, *J* = 4.5 Hz), 7.30-7.33 (m, 1H, ArH), 7.44-7.49 (m, 4H, ArH), 7.57 (d, 2H, ArH, *J* = 8.7 Hz), 8.03 (d, 2H, ArH, *J* = 8.7 Hz), 8.71-8.75 (m, 2H, C2-pyrazole, C5-pyrimidine), 9.51 (s, 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$, δ, ppm): 12.8, 36.4, 105.7, 108.6, 109.0, 124.2, 127.0, 128.3, 129.2, 130.9, 134.2, 134.6, 138.1, 146.7, 147.0, 147.3, 149.1, 151.1, 160.7, 161.3. MS [ESI]: m/z 459 (MH)$^+$. Anal. calcd. for C$_{24}$H$_{19}$ClN$_6$O$_2$: C, 62.82; H, 4.17; N, 18.31. Found: C, 62.70; H, 4.15; N, 18.35 %.

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-7-(4-methoxyphenyl)pyrazolo[1, 5-*a*]pyrimidine-3-carbox amide (**6d**): Color: Light yellow solid. Yield: 93 %. M.p.: 247-248 °C. FT-IR (KBr, ν, cm^{-1}): 3449 (NH), 1655 (CO), 1643 (CO). ^{1}H NMR (400 MHz, CDCl$_3$, δ, ppm): 2.41 (s, 3H, CH$_3$), 3.12 (s, 3H, NCH$_3$), 3.90 (s, 3H, OCH$_3$), 7.07-7.10 (m, 3H, C6-pyrimi
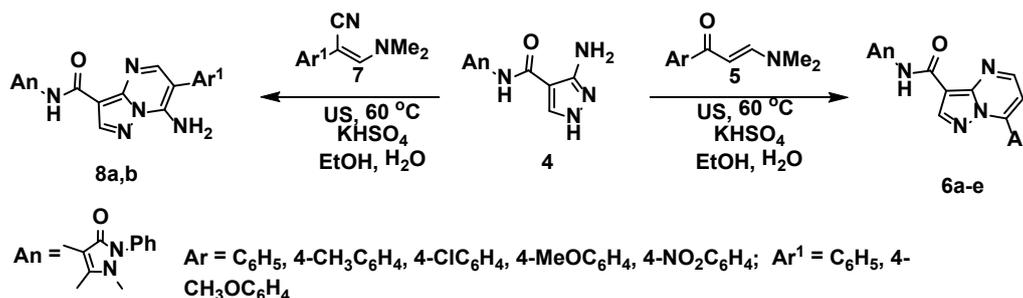
Scheme 2

dine, ArH), 7.26-7.28 (m, 1H, ArH), 7.45-7.47 (m, 4H, ArH), 8.09 (d, 2H, ArH, J = 8.7 Hz), 8.65 (d, 1H, C5-pyrimidine, J = 4.5 Hz), 8.73 (s, 1H, C2-pyrazole), 9.53 (s, 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$, δ, ppm): 12.9, 36.6, 55.6, 87.2, 105.4, 107.8, 109.5, 114.3, 122.1, 123.8, 126.5, 129.2, 131.4, 135.1, 146.6, 147.6, 148.0, 149.4, 150.9, 160.8, 162.1. MS (EI, *m/z* (%)): 455.2 (MH)$^+$. Anal. calcd. for C$_{25}$H$_{22}$N$_6$O$_3$: C, 66.07; H, 4.88; N, 18.49. Found: C, 66.14; H, 4.84; N, 18.44 %.

N-(1,5-Dimethyl-3-oxo-2-phenyl-2, 3-dihydro-1*H*-pyrazol-4-yl)-7-(4-nitrophenyl)pyrazolo[1,5-*a*]pyrimidine-3-carbox amide (**6e**): Color: Yellow solid. Yield: 96 %. M.p.: 223-224 °C. FT-IR (KBr, ν, cm^{-1}): 3418 (NH), 1656 (CO), 1624 (CO). ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ, ppm): 2.69 (s, 3H, CH$_3$), 3.53 (s, 3H, CH$_3$), 7.74-7.83 (m, 3H, ArH), 7.92-7.96 (t, 2H, ArH), 8.06 (d, 1H, C6-pyrimidine, J = 4.5 Hz), 8.82 (d, 2H, ArH, J = 9.1 Hz), 8.89 (d, 2H, ArH, J = 9.1 Hz), 9.16 (s, 1H, C2-pyrazole), 9.39 (d, 1H, C5-pyrimidine, J = 4.5 Hz), 9.56 (s, 1H, NH). MS (EI, *m/z* (%)): 470.3 (MH)$^+$. Anal. calcd. for C$_{24}$H$_{19}$N$_7$O$_4$: C, 61.40; H, 4.08; N, 20.89. Found: C, 61.47; H, 4.10; N, 20.93 %.

2.1.2.2. Synthesis of 7-amino-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-6-arylpyrazolo[1,5-a] pyrimidine-3-carboxamide (8a,b)

A mixture of aminopyrazole (**4**) (1 mmol) and enamino nitriles **7** (1 mmol) in the presence of KHSO$_4$ (2 mmol) in 5 mL of ethanol:water (1:1, *v:v*) was US irradiated for 3-10 minutes to give a precipitate (Scheme 2). After the completion of reaction (monitored by TLC), the precipitate was collected by filtration, washed repeatedly with ethanol-water (1:1) ensuring complete removal of acid, and then dried to give practically pure pyrazolopyrimidines (**8**) in 87-92 % yields. Further, purification was carried out by column chromate-graphy (silica gel, 100 % EtOAc-hexane).

7-Amino-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-6-phenylpyrazolo[1, 5-*a*]pyrimidine-3-carbox amide (**8a**): Color: Pale White. Yield: 87 %. M.p.: 184-186 °C. FT-IR (KBr, ν, cm^{-1}): 3640 (NH$_2$), 3199 (NH), 1659 (CO), 1635 (CO). ^{1}H NMR (400 MHz, CDCl$_3$, δ, ppm): 2.35 (s, 3H, CH$_3$), 3.11 (s, 3H, NCH$_3$), 6.90 (s, 2H, NH$_2$), 7.38-7.49 (m, 10H, ArH), 8.49-8.52 (m, 1H, C5-pyrimidine), 9.19 (s, 1H, C2-pyrazole), 9.35 (s, 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$, δ, ppm): 11.0, 35.8, 85.0, 106.3, 117.2, 123.6, 123.8, 126.5, 127.0, 127.8, 129.1, 129.5, 129.6, 130.4, 132.7, 135.0, 139.4, 152.9, 162.0, 163.4. MS (EI, *m/z* (%)): 440.4 (MH)$^+$. Anal. calcd. for C$_{24}$H$_{21}$N$_7$O$_2$: C, 65.59; H, 4.82; N, 22.31. Found: C, 65.65; H, 4.78; N, 22.26 %.

7-Amino-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-6-(4-methoxyphenyl)pyrazolo[1,5-*a*]pyrimidine-3- carboxamide (**8b**): Color: Pale White. Yield: 92 %. M.p.: 275-276 °C. FT-IR (KBr, ν, cm^{-1}): 3394 (NH$_2$), 3297 (NH), 1678 (CO), 1662. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ, ppm): 2.36 (s, 3H, CH$_3$), 3.11 (s, 3H, NCH$_3$), 3.85 (s, 3H, OCH$_3$), 7.11 (m, 2H, NH$_2$), 7.26-7.46 (m, 9H, ArH), 8.22 (s, 1H, C5-pyrimidine), 8.53 (s, 1H, C2-pyrazole), 9.31 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-

d$_6$, δ, ppm): 11.0, 35.8, 55.2, 102.6, 104.4, 107.3, 114.6, 115.0, 123.5, 125.0, 126.4, 129.1, 130.8, 135.0, 145.1, 146.1, 146.6, 151.4, 159.0, 160.5, 161.7. MS (EI, *m/z* (%)): 532.1 (M+Na+ K+H)$^+$. Anal. calcd. for C$_{25}$H$_{23}$N$_7$O$_3$: C, 63.96; H, 4.94; N, 20.88. Found: C, 63.79; H, 4.90; N, 20.75 %.

2.1.2.3. Synthesis of antipyrinyl-7-hetarylpyrazolo[1,5-a] pyrimidines (9,10)

Aminopyrazole (**4**) (1 mmol), enaminones **I/II** (1 mmol), and KHSO$_4$ (2 mmol) were suspended in 5 mL of ethanol:water (1:1, *v:v*) and the mixture was irradiated under the influence of US waves for 5-6 minutes giving a precipitated product (Scheme 3). After the completion of reaction monitored by TLC, the precipitate was collected by filtration, washed repeatedly with water ensuring complete removal of acid and dried to produce practically pure pyrazolopyrimidines (**9**, **10**) in 90-94 % yields. Further, purification was achieved by column chromatography using silica gel and 100 % EtOAc.

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-7-(pyridin-4-yl)pyrazolo[1, 5-*a*]pyrimidine-3-carbox amide (**9**): Color: Yellow solid. Yield: 94 %. M.p.: 212-213 °C. FT-IR (KBr, ν, cm^{-1}): 3414 (NH), 1664 (CO), 1638 (CO). ^{1}H NMR (400 MHz, CDCl$_3$, δ, ppm): 2.40 (s, 3H, CH$_3$), 3.14 (s, 3H, NCH$_3$), 7.21 (d, 1H, C6-pyrimidine, J = 4.12 Hz), 7.30-7.32 (m, 1H, ArH), 7.46-7.47 (m, 4H, ArH) 8.02 (d, 2H, 3',5'-H, J = 5.5 Hz), 8.74 (s, 1H, C2-pyrazole), 8.78 (d, 1H, C5-pyrimidine, J = 4.12 Hz), 8.89 (d, 2H, 2',6'-H, J = 5.5 Hz), 9.36 (s, 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$, δ, ppm): 12.8, 36.5, 106.3, 108.9, 109.2, 123.4, 124.0, 126.8, 129.2, 134.9, 138.0, 145.1, 146.9, 147.1, 149.5, 150.1, 151.2, 160.4, 162.0. MS (EI, *m/z* (%)): 425.4 (M)$^+$. Anal. calcd. for C$_{23}$H$_{19}$N$_7$O$_2$: C, 64.93; H, 4.50; N, 23.05. Found: C, 64.98; H, 4.51; N, 23.00 %.

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-7-(pyridin-3-yl)pyrazolo[1, 5-*a*]pyrimidine-3-carbox amide (**10**): Color: Yellow solid. Yield: 90 %. M.p.: 238-239 °C. FT-IR (KBr, ν, cm^{-1}): 3356 (NH), 1679 (CO), 1613 (CO). ^{1}H NMR (400 MHz, CDCl$_3$, δ, ppm): 2.41 (s, 3H, CH$_3$), 3.13 (s, 3H, NCH$_3$), 7.16 (d, 1H, C6-pyrimidine, J = 4.12 Hz), 7.27-7.28 (m, 1H, ArH), 7.46-7.47 (m, 4H, ArH), 7.55-7.56 (m, 1H, 5'-H), 8.55 (d, 1H, 4'-H), 8.75-8.76 (m, 2H, 6'H, C5-pyrimidine), 8.84 (d, 1H, 2'-H, J = 5.0 Hz), 9.18 (s, 1H, C2-pyrazole), 9.42 (s, 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$, δ, ppm): 12.8, 36.5, 87.2, 106.2, 108.6, 109.2, 123.9, 126.4, 126.6, 129.2, 135.0, 137.2, 145.2, 146.9, 147.2, 149.5, 149.8, 151.1, 152.4, 160.5, 162.0. MS (EI, *m/z* (%)): 426.2 (MH)$^+$. Anal. calcd. for C$_{23}$H$_{19}$N$_7$O$_2$: C, 64.93; H, 4.50; N, 23.05. Found: C, 65.01.; H, 4.49; N, 23.00 %.

2.1.2.4. Synthesis of 6-acetyl/carboalkoxy-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-7-methyl pyrazolo[1,5-a]pyrimidine-3-carboxamide (13a, b)
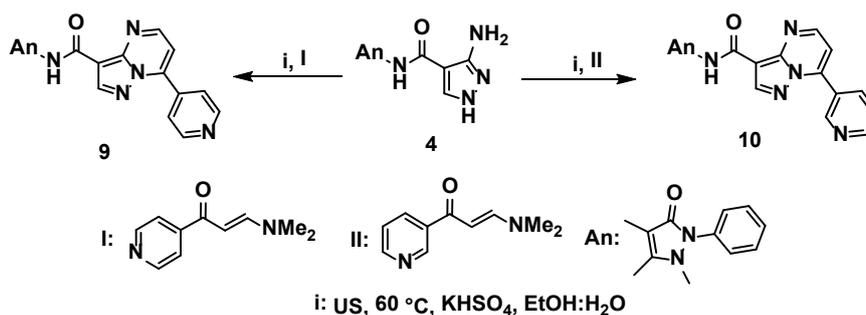
Scheme 3
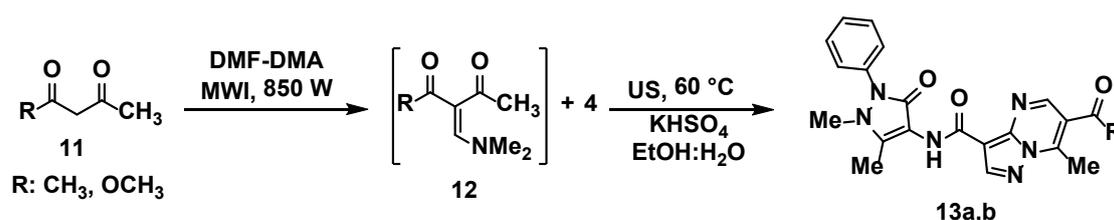
Scheme 4

In order to synthesize the target pyrazolo[1,5-*a*] pyrimidine (**13**), formylated active proton compounds of type **12** were required. This was accomplished by the microwave irradiation of acyclic active proton compounds (**11**) (1 mmol) with DMF-DMA in a microwave digester for 5 minutes. The reaction mixture (monitored by TLC) was evaporated to dryness under reduced pressure. To the resulting residue, aminopyrazole (**4**) (1 mmol) was added, and the content was dissolved in 5 mL of ethanol:water mixture (1:1, *v*:*v*). KHSO₄ (2 mmol) was then added, and the solution was subjected to ultrasound (US) irradiation for 4-6 min giving a precipitate (Scheme 4). After the completion of reaction (monitored by TLC), the precipitate was collected by filtration, washed repeatedly with ethanol: water (1:1, *v*:*v*), and dried over anhydrous CaCl₂ to give practically pure products (**13a**, **b**) in 92-96 % yields. Further, purification was achieved by column chromatography (silica gel, 100 % EtOAc).

6-Acetyl-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-7-methylpyrazolo[1, 5-*a*]pyrimidine-3-carbox amide (**13a**): Color: Light yellow solid. Yield: 92 %. M.p.: 211-212 °C. FT-IR (KBr, ν, cm⁻¹): 3328 (NH), 1692 (CO), 1680 (CO). ¹H NMR (400 MHz, CDCl₃, δ, ppm): 2.41 (s, 3H, CH₃), 2.72 (s, 3H, CH₃), 3.12 (s, 3H, NCH₃), 3.21 (s, 3H, CH₃), 7.27-7.29 (m, 1H, ArH), 7.45-7.46 (m, 4H, ArH), 8.80 (s, 1H, C5-pyrimidine), 9.03 (s, 1H, C2-pyrazole), 9.30 (s, 1H, NH). ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 12.9, 15.4, 30.0, 36.6, 106.6, 109.2, 119.0, 123.9, 126.6, 129.2, 135.0, 145.8, 148.6, 149.2, 150.9, 152.2, 160.1, 161.9, 195.9. MS (EI, *m/z* (%)): 405.3 (MH)⁺. Anal. calcd. for C₂₁H₂₀N₆O₃: C, 62.37; H, 4.98; N, 20.78. Found: C, 62.44; H, 4.95; N, 20.73 %.

Methyl 3-((1, 5-dimethyl-3-oxo-2-phenyl-2, 3-dihydro-1*H*-pyrazol-4-yl)carbamoyl)-7-methylpyrazolo[1, 5-*a*]pyrimidine-6-carboxylate (**13b**): Color: Light yellow solid. Yield: 96 %. M.p.: 214-215 °C. FT-IR (KBr, ν, cm⁻¹): 3313 (NH), 1736 (CO), 1690 (CO). ¹H NMR (400 MHz, CDCl₃, δ, ppm): 2.39 (s, 3H, CH₃), 3.12 (s, 3H, NCH₃), 3.28 (s, 3H, CH₃), 4.00 (s, 3H, OCH₃), 7.27-7.28 (m, 1H, ArH), 7.45-7.46 (m, 4H, ArH), 8.79 (s, 1H, C5-pyrimidine), 9.10 (s, 1H, C2-pyrazole), 9.25 (s, 1H, NH). ¹³C NMR (400 MHz, CDCl₃, δ, ppm): 12.8, 15.3, 36.5, 52.9, 106.8, 109.0, 112.0, 123.9, 126.6, 129.2, 135.0, 146.2, 148.3, 149.6, 151.8, 153.2, 160.2, 161.9, 164.5. MS (EI, *m/z* (%)): 422.0 (M)⁺. Anal. calcd. for C₂₁H₂₀N₆O₄: C, 59.99; H, 4.79; N, 19.99. Found: C, 59.81; H, 4.81; N, 19.94 %.

2.2. Biology

2.2.1. Material and methods

2.2.1.1. Anti-inflammatory test

Griess reagent system (naphthylethylenediamine dihydro chloride, sulphanilamide and nitrite standard) from Promega (USA), Freund's Complete Adjuvant (FCA) from GeNei (India), Wright stain, sodium chloride, disodium hydrogen phosphate dihydrate, potassium dihydrogen orthophosphate, dimethyl sulfoxide (DMSO) were procured from HiMEDIA (India), methanol was purchased from MERCK (India).

Swiss Albino mice of both sexes were purchased from Pasteur institute, Shillong. The animals were kept in a tempe-rature controlled room with a 12 hours light and dark cycle. Cell lines CHO K1 (Chinese Hamster Ovary) were procured from National Centre for Cell Science (NCCS), Pune.

2.2.1.1.1. Measurement of paw edema

This experiment was performed according to Lai *et al.* [34] with certain modifications. Swiss Albino male and female mice aged between 10-12 weeks (3 per group) were injected with about 50 μL of FCA into left hind paw of the mice to induce inflammation. The test compounds (50 mg/kg body weight in DMSO) were then administered 1 hour after FCA injection and the diameter of paw edema was then measured at different time intervals such as 0, 1, 2, 3 and 24 hours using a caliper.

After 24 hours, blood was collected by retro-orbital bleeding and stored for nitric oxide assay and differential WBC count. The paws were then excised, weighed and kept in ice-cold normal saline. These were then homogenized in 10 % ice-cold normal saline, centrifuged at 12,000 rpm and the supernatant was collected and stored at -20 ºC for nitric oxide assay.

2.2.1.1.2. Nitric oxide assay

The amount of nitric oxide produced was calculated using Griess reaction. The sulfanilamide and naphthylethylene diamine dihyrochloride (NED) solutions were made to equilibrate to room temperature for 15 to 30 minutes.

Table 1. Optimization of reaction conditions.
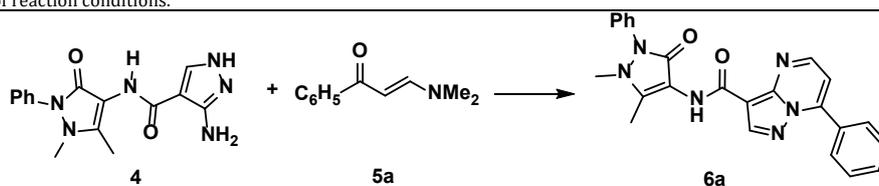
Entry	Mode	Temperature (°C)	Solvent	Reaction time (min)	Yield (%)
1	Silent	25	Water	80	51
2	Silent	25	Water-Ethanol	65	60
3	Silent	60	Water	60	40
4	Silent	60	Water-Ethanol	50	50
5	Sonication	25	Water	6	58
6	Sonication	25	Water-Ethanol	5	67
7	Sonication	60	Water	5	62
8	Sonication	60	Water-Ethanol	4	94

To a 96 well microtiter plate, 50 μL of the sample was added in triplicate. To each of these wells, 50 μL of the sulfanilamide solution was then added and incubated for 5 to 10 minutes at room temperature protected from light. After the incubation period, 50 μL of the NED solution was added to each of the wells and incubated again in dark at room temperature. A purple colour developed which was further quantified at 520 nm.

Three columns in the 96 wells plate were used for the nitrite standard reference curve in which a six serial fold dilution of 100 μM nitrite solution (50 μL/well) in triplicate was performed to generate the nitrite standard reference curve.

Differential WBCs count. Differential WBCs counts were performed according to the method described by Houwen [35]. In brief, blood film was prepared on glass slides till it dried. The film was fixed in absolute methanol for 30 seconds. The slides were then stained with Wright's stain for 2 minutes in a horizontal position after which Sorensen's buffer (KH_2PO_4, Na_2HPO_4, pH = 6.4) was added and mixed. This was allowed to stand for 3 minutes and then rinsed with distilled water and dried. The slides were then observed under a microscope and differential WBC counting was done.

2.2.1.2. Anti-cancer assay

CHO K1 cell lines were procured from NCCS (Pune), Dulbecco's modified Eagle medium/Nutrient mixture F-12, dimethyl sulfoxide, fetal bovine serum (FBS), sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, trypsin phosphate versene glucose (TPVG) solution, 3-[4,5-dimethylthiazoyl-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT) were purchased from HiMEDIA. Antibiotic antimycotic solution was purchased from Sigma. The cytotoxicity of the test compounds was assessed using MTT assay, according to Freshney [36] with certain modify-cations.

CHO K1 cells at a concentration of 0.5 × 10 to 103 in 100 μL growth medium (Dulbecco's modified Eagle medium/ Nutrient mixture F-12 with 10 % FBS) per well were seeded in a 96 well-microtiter plate. The cells were then incubated in the CO_2 incubator at 37 °C. After 24 hours, the medium was removed and 80 μL of the fresh medium was added. To six wells, 20 μL (10 mg/mL in 10 % DMSO) of test compounds (in triplicate) was added, while three wells were loaded with 20 μL of 10 % DMSO to serve as control. The plate was again incubated for 24 hours and at the end of incubation period, the spent medium was replaced with 100 μL of the fresh medium and 20 μL of MTT (5 mg/mL) reagent prepared in phosphate buffer saline (PBS) was added to all the wells. The plates were then wrapped in aluminium foil and returned to the incubator

for 2 hours until an insoluble purple formazan product was formed. After 2 hours, the medium was removed and 200 μL of 10 % DMSO was added to all the wells to dissolve the formazan product. Contents were mixed and the absorbance was recorded at 570 nm.

3. Results and discussion

3.1. Chemistry

To start with, 4-aminoantipyrine was fused with ethyl cyanoacetate at 150 °C for the formation of compound **2** [32]. This was then formylated with DMF-DMA using microwave (850 W, 5 min). The reaction mixture was subsequently treated with hydrazine hydrate in refluxing ethanol [33], yielding practically pure aminopyrazole **4** (Scheme 1). The aminopyrazole **4** thus obtained was used as a synthon for subsequent reactions without further purification.

In order to optimize the conditions for the reaction between pyrazole **4** and enaminones, the reaction of compounds **4** and **5a** was taken as a model experiment and a series of experiments was conducted under various conditions as presented in Table 1. Sonication at 60 °C in water-ethanol gave the desired product **6a** within 4 minutes in 94 % yield. The most remarkable aspect of this condition is that the product precipitated out and could be isolated by simple filtration in practically pure form. Therefore, it was decided to carry out the rest of the reactions of the series in water-ethanol at 60 °C under US irradiation.

Thus, 3-Aminopyrazole **4** was irradiated for 3-4 minutes with enaminones **5** and $KHSO_4$ in an aqueous medium in an ultrasonic bath at 60 °C (Scheme 2) to give products **6a-e** in 93-96 % yields. The structures of these compounds were well established on the basis of their spectral and analytical data. These reaction conditions could well be applied for the reactions of **4** with enaminonitriles (**7**) giving the 7-amino-pyrazolopyrimidines **8a**, **b** in 87-92 % overall yields (Scheme 2).

The synthetic protocol was applied for the synthesis of antipyrinyl-pyrazolo[1,5-*a*]pyrimidines with hetaryl group at C-7 position of the pyrimidine ring. This was achieved by ultrasound irradiation of 3-aminopyrazole **4** with hetarylen aminones **I** or **II** and $KHSO_4$ in water-ethanol mixture 60 °C (Scheme 3). The products were obtained in 90-96 % yields in 5-6 minutes. Further, the reaction of aminopyrazole **4** with formylated active proton compounds **12** (Scheme 4) derived from 1,3-diketones and prepared in situ was carried out under similar reaction conditions. The reaction proceeded smoothly with the product **13a**, **b** precipitating out in 92-96 % yields within 4-6 minutes.

Table 2. Synthesis of antipyrinyl-pyrazolo[1,5-*a*]pyrimidines.

Pyrazolo[1,5-*a*]pyrimidines	Isolated yield (%)	Time (min)	Melting point (°C)
6a	95	3	191-192
6b	94	4.5	252-253
6c	95	4	261-262
6d	93	4	247-248
6e	96	4	223-224
8a	87	10	184-186
8b	92	3	275-276
9	94	6	212-213
10	90	5	238-239
13a	92	6	211-212
13b	96	4	214-215
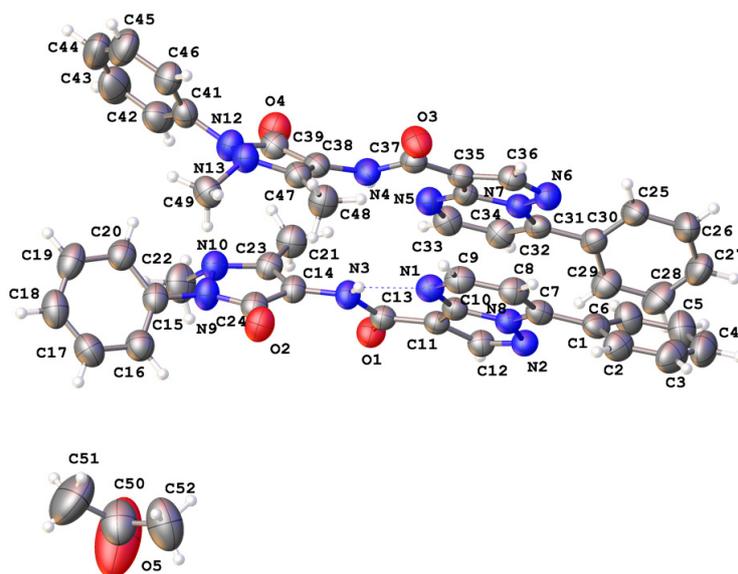
Figure 1. The molecular structure of compound **6a**.

The infrared spectra of compounds **6a-e** and **8a**, **b** showed NH stretching peak in the region 3199-3449 cm^{-1} and the NH$_2$ spectra of compounds **8a,b** gave peaks at around δ 3394-3640 ppm. The carbonyl groups gave characteristic bands at around 1645-1678 and 1624-1662 cm^{-1}. In the ^{1}H NMR spectra of compounds **6a-d**, the methyl group gave sharp singlet at around δ 2.42 ppm and in compounds **8a,b** it showed at δ 23.5 and 23.6 ppm, respectively. The *N*-methyl group gave singlet at about δ 3.16 ppm for compounds **6a-c**, and at δ 3.12 and 3.11 ppm for compound **6d** and compounds **8a,b**, respectively. The C6-H and C5-H protons of the pyrimidine ring for compounds **6a**, **6b** resonated as doublets (*J* = 4.5 Hz) at about δ 7.09 and 8.70 ppm, respectively, whereas for compound **6c** the C5-H and C2-H protons signals overlapped and appeared as multiplet in the range δ 8.71-8.75 ppm. In compound **6d** the C6-H proton signal was obscured by the aromatic protons while the C5-H signal appeared as doublet at δ 8.65 ppm. The C2-H protons for compound **6a** appeared as singlet at δ 8.75 ppm and for compound **6b** and **6d** it resonated as singlet at δ 8.73 ppm. The methyl groups of compound **6e** resonated as singlet at δ 2.69 and 3.53 ppm. The C6-H and C5-H protons of the pyrimidine ring of compound **6e** resonated as doublet (*J* = 4.5 Hz) at δ 8.06 and 9.39 ppm, respectively, while the C2-H proton appeared as singlet at δ 9.16 ppm. The NH proton for compounds **6a-e** gave sharp singlet at around δ 9.55 ppm. The methoxy group protons of compound **8b** resonated as singlet in their usual range at δ 3.85 ppm. The amino protons appeared as singlet at around δ 6.90-7.11 ppm. The C5-H proton of compound **8a** appeared as multiplet at δ 8.49-8.52 ppm, while that in compound **8b** resonated as singlet at δ 8.22 ppm. The C2-H proton gave signal at δ 9.19 ppm in compound **8a** and at δ 8.53 ppm in compound **8b**. The -NH proton

resonated as singlet at around δ 9.35 ppm. The spectral data of compounds **9**, **10**, **13a** and **13b** (experimental section) were in full agreement with the assigned structures.

The ^{13}C NMR spectra, of all the compounds showed signals at about δ 160.4-160.8 and δ 161.3-162.0 ppm indicating the presence of carbonyl groups. Mass spectrometry results were in full support of the assigned structures. The synthesized antipyrinyl-pyrazolo[1,5-*a*]pyrimidines are mentioned in Table 2.

3.2. X-ray crystallography

The structure of *N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**6a**) was further supported by using X-ray crystallography. The crystal data and structure refinement [37] values of **6a** are mentioned in Table 3. The molecular structure of compound **6a** is depicted in Figure 1. The details of the structure of compound **6a** have been deposited with the Cambridge Crystallographic Data Centre No. CCDC-1401935. The bond length and angles are within the normal ranges [38]. Selected bond lengths and bond angles are given in Tables 4 and 5.

The compound exists as a dimer in an asymmetric unit arranged in an opposite manner, with a molecule of acetone trapped. This could be probably due to the existence of short contacts between N5 and H21A at a distance of 2.744 Å, C42 and H20 at a distance of 2.828 Å and C34 and C11 at a distance of 3.391 Å which are definitely shorter than the sum of the corresponding van der Waal radii of N (1.55 Å) and H (1.2 Å); C (1.7 Å) and H (1.2 Å); and C (1.7 Å) and C (1.7 Å) respectively.

Table 3. Crystal data and structure refinement for *N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**6a**).

Parameter	Value
Empirical formula	$C_{51}H_{46}N_{12}O_5$
Formula weight	907.00
Temperature (K)	296.15
Crystal system	triclinic
Space group	P-1
a (Å)	9.9554(3)
b (Å)	14.0875(4)
c (Å)	17.4572(4)
α (°)	79.676(2)
β (°)	85.283(2)
γ (°)	72.647(2)
Volume (Å^3)	2297.97(11)
Z	2
ρ_{calc} (g/cm^3)	1.311
μ (mm^{-1})	0.088
F(000)	952.0
Crystal size (mm^3)	0.30 × 0.14 × 0.08
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection (°)	4.174 to 57.068
Index ranges	-13 ≤ *h* ≤ 13, -18 ≤ *k* ≤ 18, -23 ≤ *l* ≤ 22
Reflections collected	29732
Independent reflections	10681 [R_{int} = 0.0400, R_{sigma} = 0.0533]
Data/restraints/parameters	10681/0/627
Goodness-of-fit on F^2	1.058
Final R indexes [I≥2σ (I)]	R_1 = 0.0566, wR_2 = 0.1433
Final R indexes [all data]	R_1 = 0.1032, wR_2 = 0.1663
Largest diff. peak/hole / (e Å^{-3})	0.47/-0.30

Table 4. Selected bond lengths for compound **6a** (Å).

Atom	Atom	Length	Atom	Atom	Length	Atom	Atom	Length
N3	C14	1.408(2)	C1	C2	1.394(3)	C35	C36	1.388(3)
N3	C13	1.366(2)	C1	C6	1.387(3)	C35	C37	1.470(2)
N1	C10	1.353(2)	C2	C3	1.381(3)	C37	O3	1.223(2)
N1	C9	1.320(2)	C6	C5	1.385(3)	C30	C29	1.390(3)
N2	N8	1.3713(19)	C3	C4	1.371(3)	C30	C25	1.397(3)
N2	C12	1.329(2)	C4	C5	1.371(3)	C38	C39	1.431(3)
N5	C34	1.351(2)	N10	N9	1.418(2)	C38	C47	1.355(2)
N5	C33	1.321(2)	N10	C23	1.397(2)	C32	C33	1.390(3)
N6	N7	1.374(2)	N10	C22	1.463(3)	C39	O4	1.232(2)
N6	C36	1.327(2)	N9	C24	1.381(2)	C39	N12	1.391(2)
N4	C37	1.362(2)	N9	C15	1.417(2)	C41	C46	1.381(3)
N4	C38	1.407(2)	C24	O2	1.231(2)	C41	C42	1.379(3)
N7	C34	1.392(2)	C23	C21	1.488(3)	C41	N12	1.426(2)
N7	C31	1.373(2)	C15	C16	1.374(3)	C29	C28	1.382(3)
N8	C10	1.391(2)	C15	C20	1.384(3)	C25	C26	1.380(3)
N8	C7	1.374(2)	C16	C17	1.387(3)	C26	C27	1.370(3)
C10	C11	1.393(2)	C20	C19	1.390(3)	C28	C27	1.386(4)
C11	C12	1.397(2)	C18	C17	1.351(3)	C46	C45	1.388(3)
C11	C13	1.467(2)	C18	C19	1.372(4)	C42	C43	1.388(3)
C14	C24	1.448(3)	C34	C35	1.399(3)	C43	C44	1.375(4)
C14	C23	1.346(2)	C31	C30	1.475(2)	C45	C44	1.376(4)
C7	C8	1.368(3)	C31	C32	1.377(3)	C47	C48	1.486(3)
C7	C1	1.478(3)	N13	N12	1.415(2)	O5	C50	1.168(3)
C13	O1	1.231(2)	N13	C47	1.396(2)	C52	C50	1.428(4)
C8	C9	1.402(3)	N13	C49	1.480(2)	C51	C50	1.447(4)

Also, the π-π stacking of the pyrazole rings could be the contributing factor. In both molecules, the pyrazolo[1,5-*a*]pyrimidine rings are planar. The bond length and angles are within the normal ranges [38]. The bond distances for C35-C36, C33-C32, C9-C8 and C11-C12 are 1.388, 1.390, 1.402 and 1.397 Å, respectively, which are much shorter than that expected for C-C single bonds. Also the bond lengths of C34-C35, C31-C32, C10-C11 and C7-C8 are 1.399, 1.377, 1.393 and 1.368 Å, respectively, which are longer than C=C bond and close to that of C-C single bond. This indicates a considerable degree of delocalization of the aromatic 10-pi-electrons around the heterocyclic pyrazolopyrimidine ring system [38].

3.3. Biological activities

3.3.1. Anti-inflammatory assay

3.3.1.1. Percentage Inhibition of Paw Diameter

Untreated paw edema bearing mice served as control group. The mice (except the control) were then treated with the compounds (50 mg/kg body weight) *via* intraperitoneal (i.p) injection. The ability of the test compound to reduce the edema caused by Freund's Complete Adjuvant (FCA) was taken as a parameter. When induced with injection of FCA into the plantar side of left hind paw of the mice, all the groups of mice showed an increase in the paw diameter. In our study, we found that when these mice were treated with the test compounds (except the control which is untreated) some of the compounds showed a decrease in the paw diameter, which indirectly means that the edema has reduced. The ability of these test compounds to reduce the paw edema was calculated as percentage inhibition of paw diameter by using the formula ((a-b)/a)×100 [39] where 'a' and 'b' denote the mean increase in paw diameter of the control and drug treated mice respectively. The decrease/increase in the paw diameter was monitored at different intervals of zero, one, two, three and 24 h, respectively. Values are expressed as ±SEM.

Table 5. Selected bond angles for compound **6a** (°)

Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle
C13	N3	C14	124.55(16)	C4	C5	C6	121.1(2)	N4	C37	C35	114.44(17)
C9	N1	C10	115.84(16)	N9	N10	C22	113.35(17)	O3	C37	N4	123.36(18)
C12	N2	N8	103.34(14)	C23	N10	N9	105.25(14)	O3	C37	C35	122.16(18)
C33	N5	C34	115.53(16)	C23	N10	C22	118.16(16)	C29	C30	C31	118.25(19)
C36	N6	N7	103.22(15)	C24	N9	N10	110.77(15)	C29	C30	C25	118.40(18)
C37	N4	C38	126.10(17)	C24	N9	C15	127.45(16)	C25	C30	C31	123.33(17)
N6	N7	C34	111.76(14)	C15	N9	N10	120.53(15)	N4	C38	C39	120.26(17)
C31	N7	N6	125.94(15)	N9	C24	C14	104.21(16)	C47	C38	N4	130.70(18)
C31	N7	C34	122.24(15)	O2	C24	C14	130.40(18)	C47	C38	C39	109.03(16)
N2	N8	C10	112.15(14)	O2	C24	N9	125.28(18)	C31	C32	C33	120.61(18)
N2	N8	C7	125.92(15)	C14	C23	N10	109.90(17)	O4	C39	C38	129.87(18)
C7	N8	C10	121.92(15)	C14	C23	C21	130.48(18)	O4	C39	N12	124.77(18)
N1	C10	N8	122.37(15)	N10	C23	C21	119.60(16)	N12	C39	C38	105.33(16)
N1	C10	C11	131.74(15)	C16	C15	N9	118.75(18)	N5	C33	C32	124.65(18)
N8	C10	C11	105.88(15)	C16	C15	C20	120.4(2)	C46	C41	N12	120.91(19)
C10	C11	C12	104.28(15)	C20	C15	N9	120.9(2)	C42	C41	C46	120.6(2)
C10	C11	C13	128.06(16)	C15	C20	C19	119.0(2)	C42	C41	N12	118.5(2)
C12	C11	C13	127.66(16)	C17	C18	C19	119.6(2)	C28	C29	C30	120.8(2)
N3	C14	C24	121.04(16)	C18	C17	C16	121.5(3)	C26	C25	C30	120.1(2)
C23	C14	N3	129.44(18)	C18	C19	C20	120.5(2)	C27	C26	C25	121.3(2)
C23	C14	C24	109.41(16)	N5	C34	N7	122.14(16)	C29	C28	C27	120.3(2)
N2	C12	C11	114.34(16)	N5	C34	C35	131.75(17)	C41	C46	C45	119.8(2)
N8	C7	C1	120.75(16)	N7	C34	C35	106.11(15)	C41	C42	C43	119.3(2)
C8	C7	N8	115.04(16)	N7	C31	C30	121.13(16)	C26	C27	C28	119.2(2)
C8	C7	C1	124.21(17)	N7	C31	C32	114.81(16)	C44	C43	C42	120.1(3)
N3	C13	C11	114.37(16)	C32	C31	C30	124.05(17)	C44	C45	C46	119.5(3)
O1	C13	N3	123.06(17)	N12	N13	C49	113.85(15)	C43	C44	C45	120.7(2)
O1	C13	C11	122.56(16)	C47	N13	N12	105.77(14)	N13	N12	C41	119.13(15)
C7	C8	C9	121.05(17)	C47	N13	C49	118.22(15)	C39	N12	N13	109.40(14)
C2	C1	C7	117.81(18)	C34	C35	C37	130.17(17)	C39	N12	C41	124.40(16)
C6	C1	C7	123.48(17)	C36	C35	C34	103.82(16)	N13	C47	C48	119.71(16)
C6	C1	C2	118.67(19)	C36	C35	C37	125.82(17)	C38	C47	N13	109.46(16)
N1	C9	C8	123.77(17)	N6	C36	C35	115.08(18)	C38	C47	C48	130.75(18)
C3	C2	C1	120.5(2)	C4	C3	C2	120.4(2)	O5	C50	C52	122.5(3)
C5	C6	C1	119.9(2)	C5	C4	C3	119.5(2)	O5	C50	C51	119.9(4)

From the Table 6, it shows that compound **8b** led to a percentage inhibition in the paw diameter of the mice.

Table 6 reveals the percentage inhibition of paw edema of the different test groups. In this study, it was found that the standard drug ibuprofen could produce the highest percentage inhibition of up to 66.67 % at four hours but this inhibition was found to be absent at 24 hours. The highest percentage inhibition of the edema at 24 hours after intraperitoneal (i.p) drug administration was seen in case of test compound **8b** (25 %) and **13a** (16.57 %), respectively, while at four hours, this effect was seen to be highest in case of test compound **8b**, followed by test compounds **9** and **13a**, **13b**, **6a**, **6c**, **6e** and **10**, respectively.

3.3.1.2. Concentration of nitric oxide (NO)

This assay was used to assess the potentiality of the test compounds as anti-inflammatory agents. As nitric oxide is produced by inducible nitric oxide synthase (iNOS) in activated macrophages during inflammation, this compound therefore can serve as a good biological marker for inflammation. This means that the agents that can inhibit the over production of nitric oxide may have anti-inflammatory activities [36,40].

The concentration of nitric oxide was assessed in paw exudates and whole blood. Of the different compounds tested, compound **13b** was found to exert marked reduction in the concentration of NO in paw exudates (Figure 2) while in blood, compound **6a** showed some magnitude of reduction (Figure 3).

3.3.1.3. Differential WBC count in the blood

During inflammation, a variety of cells such as neutrophils, eosinophils and basophils mediate an effective immune response [41]. Differential leukocyte count in blood smear was performed for the test compounds (Table 7) to study its anti-inflammatory properties. When compared with the untreated blood sample count, the blood samples that showed a reduction in the percentage count of the neutrophils, eosinophils and basophils might have anti-inflammatory effect. Most of the compounds tested resulted in lower counts of both neutrophils and eosinophils as compared to control mice. The percentage count of neutrophils was found to be significantly reduced in case of **8b** and **13b** ($p < 0.0005$***), the effects of which are higher than that of ibuprofen ($p < 0.001$**) treated mice. The test compound **13a** showed comparable effects to ibuprofen in the reduction of neutrophils ($p < 0.001$**). In the reduction of eosinophils, test compounds **10** and **8a** ($p < 0.001$**) showed comparable activities to ibuprofen ($p < 0.001$**), while test compound **8b** showed lower effect ($p < 0.005$*). It is clearly understood that the compounds with the highest ability to reduce the marked indicators of inflammation, neutrophils and eosinophils are test compounds **8b** and **13b**.

3.3.2. Anti-cancer assay

3.3.2.1. MTT-based cytotoxic assay

The anti-cancer screening of the synthesized compounds were performed in cultured Chinese hamster ovary K1 (CHO K1) cell lines. The ability of the enzyme succinate tetrazolium reductase to metabolize 3-[4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide to purple formazan product after treatment with the test compounds in comparison to the untreated (control) cells was assessed. It was found that the test compounds **6a**, **6b**, **8a**, **8b**, **13a**, and **13b**, resulted in a decrease in metabolism of MTT by CHO K1 cells (Table 8). In particular, test compound **6a** produced the lowest metabolism of MTT by these cells (Figure 4), followed by test compounds **8b**, **13a**. This assay is commonly used to monitor cell viability and proliferation and hence can also be used for assessing cytotoxicity [36,42].

Table 6. Percentage inhibition of paw edema in two different test groups, one administered with test compounds and the other with ibuprofen.

Test groups	Mean paw diameter ± SEM (mm)					
	Before FCA injection	1 h	2 h	3 h	4 h	24 h
Control	2±0.00	3.5±0.00	3.7±0.17	3.3±0.17	3.5±0.29	3.2±0.57
Ibuprofen	2±0.00	4.2±0.17	3.8±0.17	3.6±0.28	3.0±0.28	3.8±0.17
6a	2±0.00	4.3±0.29	3.9±0.06	3.3±0.35	3.2±0.17	3.8±0.46
6b	2±0.00	4.0±0.00	4.0±0.00	3.7±0.23	3.8±0.17	4.3±0.46
6c	2±0.00	4.0±0.00	3.1±0.05	3.2±0.17	3.3±0.17	3.5±0.29
6d	2±0.00	4.0±0.00	3.7±0.34	3.5±0.29	3.5±0.35	3.9±0.57
6e	2±0.00	4.0±0.00	3.9±0.06	3.5±0.17	3.3±0.00	3.2±0.17
8a	2±0.00	3.8±0.17	3.9±0.06	3.6±0.17	3.5±0.17	3.2±0.17
8b	2±0.00	3.7±0.17	3.3±0.12	2.9±0.06	2.8±0.00	2.9±0.06
9	2±0.00	3.9±0.00	3.5±0.00	3.3±0.00	2.9±0.06	3.2±0.17
10	2±0.00	4.0±0.00	3.6±0.12	3.1±0.06	3.3±0.12	4.0±0.00
13a	2±0.00	3.7±0.17	3.5±0.17	3.3±0.23	2.9±0.06	3.0±0.12
13b	2±0.00	4.0±0.00	3.3±0.17	3.1±0.17	3.0±0.12	3.2±0.17

Test groups	Mean increase in paw diameter (mm)					
		1 h	2 h	3 h	4 h	24 h
Control		1.5	1.7	1.3	1.5	1.2
Ibuprofen		2.0	1.2	1.0	0.5	1.8
6a		2.3	1.9	1.3	1.2	1.8
6b		2.0	2.0	1.7	1.8	2.3
6c		2.0	1.1	1.2	1.3	1.5
6d		2.0	1.7	1.5	1.5	1.9
6e		2.0	1.9	1.5	1.3	1.2
8a		1.8	1.9	1.6	1.5	1.2
8b		1.7	1.3	0.9	0.8	0.9
9		1.9	1.5	1.3	0.9	1.2
10		2.0	1.6	1.1	1.3	2.0
13a		1.7	1.5	1.3	0.9	1.0
13b		2.0	1.3	1.1	1.0	1.2

Test groups	Percentage inhibition in paw diameter					
		1 h	2 h	3 h	4 h	24 h
Control						
Ibuprofen		-33.33	29.41	23.07	66.67	-50.00
6a		-53.33	-11.76	0.00	20.00	-50.00
6b		-33.33	-17.65	-30.77	-20.00	-91.67
6c		-33.33	35.29	7.69	13.33	-25.00
6d		-33.33	0.00	-15.38	0.00	-58.33
6e		-33.33	-11.76	-15.38	13.33	0.00
8a		-20.00	-11.76	-23.08	0.00	0.00
8b		-13.33	23.53	30.77	46.67	25.00
9		-26.67	11.76	0.00	40.00	0.00
10		-33.33	5.88	15.38	13.33	-66.67
13a		-13.33	11.76	0.00	40.00	16.57
13b		-33.33	25.53	15.38	33.33	0.00

Table 7. Differential WBC count in three different test groups: untreated paw edema bearing mice (control), ibuprofen treated paw edema bearing mice and test compounds treated paw edema bearing mice. The values are ±SEM (* denote significance against paw edema bearing control mice at $p < 0.0005$***, $p < 0.001$**, $p < 0.005$*).

Test groups	Lymphocytes	Neutrophils	Eosinophils	Basophils	Monocytes
Control	62.67	14.00	6.00	8.67	8.67
Ibuprofen	86.67	4.00	2.00	6.67	0.33
6a	68.00	16.00	6.00	7.30	2.70
6b	77.33	8.67	6.00	6.00	2.00
6c	69.33	19.33	7.33	3.33	0.67
6d	64.67	19.33	9.33	4.67	2.00
6e	78.67	10.00	8.00	2.67	0.67
8a	82.67	6.33	2.00	9.00	0.00
8b	93.00	1.67	3.00	3.00	0.00
9	76.00	10.00	8.67	4.67	0.67
10	71.33	27.33	0.67	0.67	0.00
13a	79.67	6.67	5.00	7.00	1.67
13b	88.00	3.00	4.00	3.00	2.00

Table 8. MTT assay in CHO K1 cell lines after exposure to 20 μL of 10 mg/mL test compounds (in 10 % DMSO). Absorbance was recorded at 570 nm. Cells exposed to 10 % DMSO served as control. Values are mean ± SD of three readings.

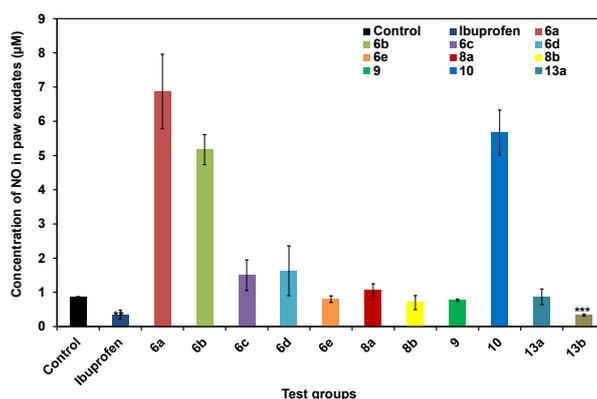| Test compounds	Absorbance at 570 nm	Standard deviation
Control	0.730	±0.3400
6a	0.020	±0.0145
6b	0.445	±0.0488
6c	0.726	±0.3530
6d	0.705	±0.1321
6e	0.687	±0.6026
8a	0.394	±0.2100
8b	0.197	±0.0600
9	0.654	±0.0770
10	0.646	±0.0682
13a	0.201	±0.0505
13b	0.370	±0.0912

Figure 2. Concentration of nitric oxide in paw exudates of different test groups (treated with test compounds and ibuprofen) in comparison to the control (untreated) paw edema bearing mice. The edema was induced by injection of FCA into the plantar side of left hind paw of the mice. The mice (except the control) were then treated with the compounds (50 mg/kg body weight) *via* i.p injection. After 24 h, the paws were excised, weighed, homogenized and centrifuged at 12,000 rpm in 10 per cent ice-cold normal saline. The supernatant was collected and used for NO assay. Absorbance was recorded at 520 nm and the concentration of NO was calculated using the standard nitrite curve. Values are expressed as mean ±SEM (*denotes significance against paw edema bearing control mice at $p < 0.0005$***, $p < 0.001$**, $p < $*0.005).
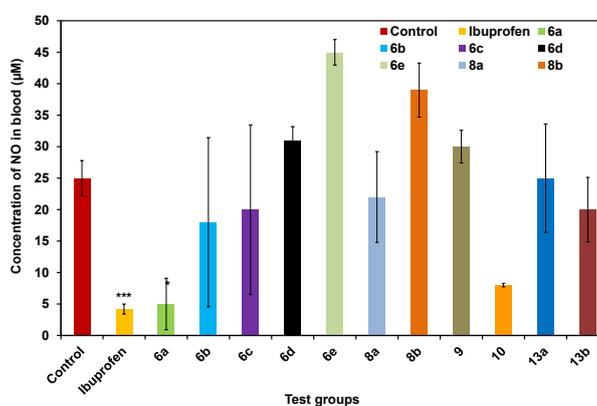
Figure 3. Concentration of nitric oxide (NO) in blood groups of treated and untreated paw edema bearing mice. Untreated groups served as control. The edema was induced by injection of FCA into the plantar side of the left hind paw of the mice. These mice (except the control) were then treated with the compounds (50 mg/kg body weight) *via* i.p injection. After 24 h, blood was collected by retro-orbital bleeding and used for nitric oxide assay. Absorbance was recorded at 520 nm and the concentration of nitric oxide was quantified using the standard nitrite curve. Values are expressed as mean ±SEM (*denotes significance against paw edema bearing control mice at $p < 0.0005$***, $p < 0.001$**, $p < $*0.005).
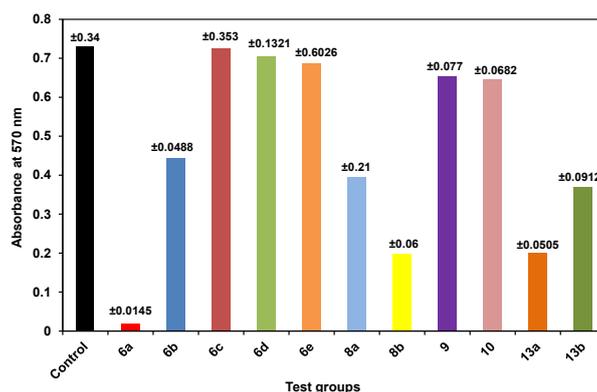

Figure 4. The cytotoxic effects of the test compounds on CHO K1 cell lines were compared against the control using the MTT based assay. CHO K1 cells were grown in microtiter plates and treated with 20 μL of 10 mg/mL test compounds (in 10 % DMSO). Their ability to metabolise MTT was measured by taking the absorbance at 570 nm. Cells exposed to 10 % DMSO were used as control.

This means that the lower the production of purple formazan in cells treated with the test compounds, the lower is the number of viable cells, the higher is their cytotoxic effects and hence the greater is their potential as anticancer compounds. Hence, these compounds **6a**, **6b**, **8a**, **8b**, **13a**, **13b** may be concluded to have cytotoxic effect on CHO K1 cell lines.

4. Conclusion

We were able to successfully combine the bio-labile rings together in a molecular framework. The synthetic protocol features a new route that utilizes ultrasound irradiation for the diversification of pyrazolopyrimidine derivatives. The

starting materials used were developed by methods reported from our group from the commercially available active proton compounds suggesting that further modification of this component may be a viable strategy for future development of bioactive compound libraries. From the results obtained, the test compound **13b** can be selected as a good candidate for anti-inflammatory effects. This test compound showed an ability to decrease the paw edema up to 33.33 % at four hours and also show a potential to decrease the concentration of nitric oxide to a level that is at par with the known drug ibuprofen.

On the basis of the anti-cancer assay, test compound **6a** was found to show the maximum cytotoxic effect, followed by compounds **8b**, **13a**, **13b**, **8a** and **6b**, respectively. The percentage inhibition of these test compounds was found to be compounds **6a** (97.10 %), **8b** (72.96 %), **13a** (72.46 %), **13b** (49.24 %) and **8a** (45.75 %).

Further investigation on these compounds are therefore crucial in order to elucidate the exact mechanism of action on target cells and hence to therefore add them in the list of potent anti-inflammatory and anti-cancer drugs.

Acknowledgements

Supporting information Ⓢ

CCDC-1401935 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

Disclosure statement ⒹⓈ

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References

[1]. Elkhawaga, A. M.; El-Dean, A. M. K.; Radwan, S. M.; Ahmed, M. M. *Bull. Korean Chem. Soc.* **2009**, *30*, 561-566.
[2]. Dreassi, E.; Zizzari, A. T.; Mori, M.; Fillipi, I.; Belfiore, A.; Naldini, A.; Carraro, F.; Santucci, A.; Shenone, S.; Botta, M. *Eur. J. Med. Chem.* **2010**, *45*, 5958-5964.
[3]. Earl, R. A.; Pugmire, R. J.; Revanker, G. R.; Townsend, L. B. *J. Org. Chem.* **1975**, *40*, 1822-1828.
[4]. Curran, K. J.; Verheijen, J. C.; Kaplan, J.; Richard, D. J.; Toral-Barza, L.; Hollander, I.; Lucas, J.; Ayral-Kaloustian, S.; Yu, K.; Zask, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1440-1444.
[5]. Hassan, A. S.; Hafez, T. S.; Osman, S. A. *Sci. Pharm.* **2015**, *83,* 27-39.
[6]. Ferrari, S. M.; Motta, C. L.; Sartini, S.; Baldini, E.; Materazzi, G.; Politti, U.; Ruffilli, I.; Ulisse, S.; Miccoli, P.; Antonelli, A.; Fallahi, P. *Mini. Rev. Med. Chem.* **2016**, *16*, 86-93.
[7]. Abdou, I. M.; Saleh, A. M.; Zohdi, H. F. *Molecules* **2004**, *9*, 109-116.
[8]. El-Feky, S. A.; Abd El-Samii, Z. K. *Pharmazie* **1996**, *51*, 540-543.
[9]. Davies, L. P.; Brown, D. J.; Chow, S. C.; Johnston, G. A. *Neurosci. Lett.* **1983**, *41*, 189-193.
[10]. Ghorab, M. M.; Ismail, Z. H.; Abdel-Gawad, S. M.; Aziem, A. A. *Heteroatom. Chem.* **2004**, *15*, 57-62.
[11]. Mohamed, H. A.; Abdel-Wahab, B. F.; Abdel-Latif, E. *J. Chem. Pharm. Res.* **2015**, *7*, 1218-1222.
[12]. Stefano, A.; Anna, A.; Maurizio, B.; Alessandra, T.; Francisco, O.; Francesco, O.; Silvia, S.; Chiara, B.; Matilde, Y. *Chem. Med. Chem.* **2010**, *5*, 1242-1251.
[13]. Fraley, M. E.; Hambaugh, S. R.; Rubino, R. S.; Hungate, R. W. *US Patent* 2007, 7, 262, 199.
[14]. Senga, K.; Novinson, T.; Wilson, H. R. *J. Med. Chem.* **1981**, *24*, 610-613.
[15]. Schenone, S.; Brullo, C.; Bruno, O.; Bondavalli, F.; Mosti, L.; Maga, G.; Crespan, E.; Carraro, F.; Manetti, F.; Tintori, C.; Botta, M, *Eur. J. Med. Chem.* **2008**, *43*, 2665-2676.
[16]. George, C. F. P. *Lancet* **2001**, *358*, 1623-1626.
[17]. Lutsevich, A. N.; Bender, K. I.; Reshetko, O. V. *Eksp. Klin. Farmakol.* **1995**, *58*, 51-55.
[18]. Turan-Zitouni, G.; Sivaci, M.; Kiliç, F. S.; Erol, K. *Eur. J. Med. Chem.* **2001**, *36*, 685-689.
[19]. Acheson, R. M. Introduction to Chemistry of Heterocyclic Compounds, 4th edition, John Wiley & Sons, 2009, p. 354.
[20]. Bondock, S.; Rabie, R.; Etman, H. A.; Fadda, A. A. *Eur. J. Med. Chem.* **2008**, *43*, 2122-2129.
[21]. Metwally, M. A.; Gouda, M. A.; Harmal, A. N.; Khalil, A. M. *J. Med. Chem.* **2012**, *56*, 254-262.
[22]. Yoo, J.; Thai, K.; Kim, D.; Lee, J. Y.; Park, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4271-4274.
[23]. Devi, A. S.; Kaping, S.; Vishwakarma, J. N. *Mol. Divers.* **2015**, *19*, 759-771.
[24]. Kaping, S.; Kalita, U.; Sunn, M.; Singha, L. I.; Vishwakarma, J. N. *Monatsh. Chem.* **2016**, *147*, 1257-1276.
[25]. Kaping, S.; Boiss, I.; Singha, L. I.; Helissey, P.; Vishwakarma, J. N. *Mol. Divers.* **2016**, *20*, 379-390.
[26]. Buriol, L.; Munchen, T. S.; Frizzo, C. P.; Marzari M. R. B.; Zanatta N.; Bonacorso, H. G.; Martins, M. A. P. *Ultrason. Sonochem.* **2013**, *20*, 1139-1143.
[27]. Bharath, Y.; Rao, M. V. B.; Pal, M. *Lett. Drug Design Discover.* **2017**, *14*, 1206-1214.
[28]. Dandia, A.; Gupta, S. L.; Parewa, V. *RSC Adv.* **2014**, *4*, 6908-6915.
[29]. Farrugia, L. J.; *J. Appl. Cryst.* **2012**, *45*, 849-854.
[30]. Chanda, K.; Dutta, M. C.; Karim, E.; Vishwakarma, J. N. *J. Indian Chem. Soc.* **2004**, *81*, 791-793.
[31]. Dutta, M. C.; Chanda, K.; Karim, E.; Vishwakarma, J. N. *Ind. J. Chem.* **2004**, *36*, 2471-2474.
[32]. Fadda, A. A.; Bondock, S.; Rabie. R.; Etman, H. A. *Turk. J. Chem.* **2008**, *32*, 259-286.
[33]. Gopalsamy, A.; Ciszewski, G.; Shi, M.; Berger, D.; Hu, Y.; Lee, F.; Feldberg, L.; Frommer, E.; Kim, S.; Collins, K.; Wojciechowicz, D.; Mallon, R. *Biorg. Med. Chem. Lett.* **2009**, *19*, 6890-6892.
[34]. Lai, S. C.; Peng, W. H.; Huang, S. C. Ho, Y. L.; Huang, J. H.; Lai, Z.; Chang, Y. *Am. J. Chin. Med.* **2009**, *37*, 573-588.
[35]. Houwen, B. *Lab. Hematol.* **2000**, *6*, 1-7.
[36]. Freshney, R. I.; Culture of animal cells: A Manual of Basic Technique and Specialized Application, 6th edition; John Wiley and Sons: New Jersey, 2010, p. 373-375.
[37]. Sheldrick, G. M. SHELXL97. Program for the Refinement of Crystal Structures, University of Gottingen, Germany, 1997.
[38]. Portilla, J.; Quiroga, J.; Cobo, J.; Low, J. N.; Glidewell, C. *Acta. Cryst. C* **2006**, *62*, 186-189.

[39]. Ahmad, F.; Khan, R.; Rasheed, S. *J. Islamic Acad. Sci.* **1992**, *5*, 111-114.

[40]. Kang, O. H.; Chae, H. S.; Oh, Y. C.; Choi, J. G.; Lee, Y. S.; Jang, H. J.; Kim, J. H.; Kim, Y. C.; Sohn, D. H.; Park, H.; Kwon, D. Y. *Am. J. Chin. Med.* **2008**, *36*, 913-928.

[41]. Khan, F. H. The Elements of Immunology, 1st edition, Pearson Education India, 2009.

[42]. Alam, A.; Imliwati, L.; Rapthap, C.; Singh, V. *Ind. J. Exp. Biol.* **2001**, *39*, 201-208.