Accepted Manuscript

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PII: S0223-5234(15)30085-4

DOI: 10.1016/j.ejmech.2015.06.011

Reference: EJMECH 7938

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 23 December 2014

Revised Date: 2 June 2015

Accepted Date: 3 June 2015

Please cite this article as: R. Aggarwal, G. Singh, P. Kaushik, D. Kaushik, D. Paliwal, A. Kumar, Molecular docking design and one-pot expeditious synthesis of novel 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines as anti-inflammatory agents, *European Journal of Medicinal Chemistry* (2015), doi: 10.1016/j.ejmech.2015.06.011.

Graphical Abstract

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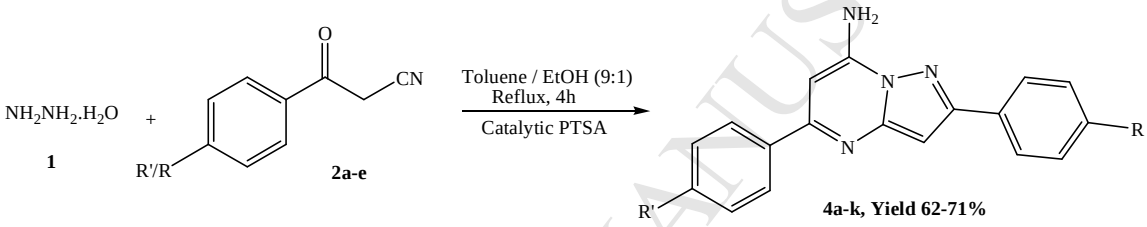

4	a	b	c	d	e	f	g	h	i	j	k
R	H	H	H	CH_3	CH_3	Cl	CH_3	Cl	Br	CH_3	Br
R'	CH_3	Cl	Br	H	F	CH_3	Cl	Br	Cl	Br	CH_3

Molecular docking design and one-pot expeditious synthesis of novel 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines as anti-inflammatory agents

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Abstract

A series of novel 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines were designed as COX-2 inhibitors by molecular docking studies and their synthesis was accomplished *via* an expeditious one-pot reaction. Structures of the compounds were established by NMR (^{1}H-^{13}C), IR spectroscopy and high resolution mass spectrometry. All the eleven compounds have been screened for their *in vivo* anti-inflammatory activity on rats by carrageenan-induced rat paw edema assay. Correlation studies of calculated moldock score and observed percentage inhibition have also been carried out which concluded that the synthesized 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines act as potent anti-inflammatory agents up to the 4th hour of study.

Keywords 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines, hydrazine hydrate, 3-aryl-3-oxopropanonitriles, *p*-toluenesulphonic acid, anti-inflammatory agents, molecular docking.

1. Introduction

Inflammation, an important natural and beneficial response imposed by the body defence mechanism in order to cease infections, involves certain inflammatory reactions like pain, swelling and mental discomfort. Thus relieving and reducing the severity of symptoms stand as an important goal with the use of therapeutic agents. Use of non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), corticosteroids and immunosuppressive agents is generally accepted stepwise chemotherapeutic approach to treat the inflammatory disorder.

Pyrazole [1] and fused pyrazole [2] with six-membered ring nucleus are main skeletal feature among different types of non-steroidal anti-inflammatory drugs. Among these, pyrazolo[1,5-*a*]pyrimidine system, due to its purine analogy, represents an important class and is found to display anti-inflammatory activity by acting as selective cyclooxygenase-2 (COX-2) inhibitor [3]. Almansa *et al* have synthesized a series of pyrazolo[1,5-*a*]pyrimidines (**I**) which exhibit *in vitro* inhibitory activitiy of COX-1 and COX-2 in a human whole blood (HWB) assay along with *in vivo* anti-inflammatory action studies in rat carrageenan-induced paw edema assay [4]. Structure activity relationship (SAR) studies have proposed that the compounds having substitution at the 5^{th}, 6^{th} and 7^{th} position of pyrazolo[1,5-*a*]pyrimidine nucleus display anti-inflammatory activity (**Figure-1**). The compound, 3-(4-fluorophenyl)-6,7-dimethyl-2-(4-(methylsulphonyl)phenyl)pyrazolo[1,5-*a*]pyrimidine (**II**), was found to be the most promising among the series of compounds listed as **I** where the position 6 and 7 was occupied by methyl groups.

Along with the bio-active profile, synthesis of pyrazolo[1,5-*a*]pyrimidine by developing simpler routes from readily available reagents and compounds stands as a challenging task in organic synthesis. Multi-component condensation reactions (MCRs) and one-pot condensation reactions provide a fair pathway to overcome this obstacle where the ease of simplicity in procedures, high atom economy, high yields and avoidance of intermediate isolation are observed [5]. Moreover, multi-component condensation reactions may also be a useful synthetic strategy to bring out the regioselectivity in the reaction [6].

Thus keeping in mind the anti-inflammatory ability of pyrazolo[1,5-*a*]pyrimidine nucleus, it was envisaged in the present study to design some new pyrazolo[1,5-*a*]pyrimidines by applying few more structural modifications to the compound **I** (**Figure-1**) considering the SAR studies of Almansa *et al* [4]. Pyrazolo[1,5-*a*]pyrimidines thus designed were synthesized by one pot efficient and regioselective multicomponent reaction and were evaluated for their anti-inflammatory activity.

2. Molecular Docking Designing

In an effort to develop some novel pyrazolo[1,5-*a*]pyrimidines, the compounds **4a-k** (**Figure-2**) were taken into the consideration where methyl group at 7th position of the pyrazolo[1,5-*a*]pyrimidine is replaced by amino group having steric profile comparable to methyl group (**Compound-II** in **Figure-1**) but having additional advantage of ability to bind with H-bonds in active site of responsible enzyme. Position-2 of the nucleus is occupied with the aryl residue similar to compounds (**I**) but with a difference of substitution at the *para* position. Variously *para* substituted aryl residues were shifted from position-3 to 5 of the pyrazolo[1,5-*a*]pyrimidine nucleus so as to bring out structural deviation towards planarity in the whole structure for better fitting compared to compound **II** (**Figure-1**).

The core domain of COX-2 with a standard ligand SC-558 was chosen for the interactive studies of designed compounds **4a-k**. In an effort to understand the binding between pyrazolo[1,5-*a*]pyrimidines (**4a-k**) and COX-2, these compounds were docked to the core domain of COX-2. Molegro Virtual Docker allows the flexible docking of ligands into its site of action. It has the ability to use all the rotatable bonds of the ligands to give a number of conformations from which the best mode could be achieved. All compounds were embedded in the hydrophobic pocket formed by the amino acids. The results of docking studies with respect to COX-2 inhibitor are summarised in **Table 1**.

Standard compound indomethacin showed three hydrogen bond interactions with OH and C=O of carboxylic acid group, -O- of methoxy group, respectively. Compound **II** also showed three interaction *via* hydrogen bonds with N of pyrimidine part and oxygen of SO_2 region. Nitrogen of the sulphonamide group of internal ligand SC-558 of PDB (Protein Data Bank) showed two hydrogen bond interactions with amino acids Ser353 & His90 with hydrogen bond length 2.76Aº & 2.96Aº, respectively. Oxygen of sulphonamide group of SC-558 showed interaction with Arg513 having distance 3.27Aº. Among these three, indomethacin and compound **II** underwent a common hydrogen bond interaction with amino acid residue Tyr355. Likewise, in most of the compounds (**4**), N of NH_2 group showed interaction with amino acid Tyr355 (2.55Aº -2.84Aº) and His90 (3.42Aº-3.52Aº) except compound **4j** and **4e**, which showed interaction with Phe518 (2.54Aº-2.56Aº) and Gly519 (3.50Aº-3.57Aº). N of pyrazole ring showed strong interactions with amino acids Tyr355 and Arg120 in compound **4d**,. The secondary structure of PDB COX-2, compound **SC-558** and **4k** embedded in PDB were shown in Figure **3** and **4**, respectively.

Molecular docking results indicated that the designed compounds **4** may be potential COX-2 inhibitor, therefore a reterosynthetic strategy was taken in account so as to achieve this scaffold from readily available starting materials such as hydrazine hydrate (**1**) and 3-aryl-3-oxopropanonitriles (**2a-e**) (**Figure-5**).

3. Result and Discussion

In our earlier attempts, the reaction of hydrazine hydrate (**1**), 3-oxo-3-phenylpropanonitriles (**2a**) and 3-oxo-3-*p*-tolylpropanonitrile (**2b**) was carried out in refluxing ethanol with or without the presence of *p*-toluenesulphonic acid (PTSA). However, it led to the formation of mixture of 5-aminopyrazoles corresponding to two 3-aryl-3-oxopropanonitriles. Performing reaction at elevated temperature by refluxing in toluene without PTSA again afforded 5-aminopyrazoles mixture. When the reaction of hydrazine hydrate (**1**) with successive addition of **2a** followed by **2b** was performed with catalytic PTSA in refluxing toluene, formation of the desired product **4a** was observed but with poor yield. Finally, on turning the reaction solvent from toluene to a mixture of toluene and ethanol (9:1), the reaction led to desired product 2-phenyl-5-*p*-tolylpyrazolo[1,5-*a*]pyrimidin-7-amine **4a** in 65% yield within 4 hours (**Scheme-1**).

The structure elucidation of the product formed (**4a**) was carried out by TLC, ^{1}H NMR, ^{13}C NMR, IR spectroscopy and high resolution mass spectrometry.

IR spectrum of **4a** showed two characteristic absorption stretches at 3325 and 3350 cm^{-1} corresponding to the symmetric and asymmetric N-H stretches of 7-amino group. ^{1}H NMR spectrum of **4a** showed two singlets of one proton intensity each at δ 6.59 ppm and 6.90 ppm of protons 6-H and 3-H, respectively. A broad singlet exchangeable with D_2O of two protons intensity at δ 7.72 ppm was observed for amino group at position-7 of pyrazolo[1,5-*a*]pyrimidine nucleus. A sharp singlet appeared at δ 2.38 ppm for the methyl group of *p*-tolyl residue at position-5 besides the protons of aryl groups. In ^{13}C NMR, two signals at δ 84.7 ppm and δ 91.5 ppm were observed corresponding to 6-C and 3-C. Signal for 7-C appeared at δ 148.45 of pyrazolo[1,5-*a*]pyrimidine nucleus thus confirming the formation of 2-phenyl-5-*p*-tolylpyrazolo[1,5-*a*]pyrimidin-7-amine isomer (**4a**). The spectral data is in conformity with our recent report where in regioselective synthesis of 7-aminopyrazolo[1,5-*a*]pyrimidines have been achieved by the reaction of 3(5)-amino5(3)-hydrazinopyrazole with 3-oxo-3-phenylpropanonitrile (**2a**) and the position of substituents on pyrazolopyrimidine ring has been established by the combined use of NMR (HMBC & HMQC) and DFT calculations [7]. High resolution mass spectrum of compound **4a** showed a mass peak at m/z value of 301.1363 $[M+1]^{+}$.

With the optimized conditions in hand, further reaction of hydrazine hydrate (**1**) with two different 3-aryl-3-oxopropanonitriles (**2a-e**) in refluxing toluene/ethanol (9:1) in presence of catalytic *p*-toluenesulphonic acid (**Scheme-2**) resulted in a series of 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines (**4b-k**) in 60-70% yield.

4. Biological Activity

All the synthesized compounds (**4a-k**) have been tested for their anti-inflammatory using Winter et al. carrageenan-induced rat paw edema assay protocol approved by the Institutional Animal Ethics Committee (IAEC) [8]. 50 mg/kg body weight dose level of each test compound was given orally to the test animals 30 min prior to induction of inflammation by carrageenan injection. Indomethacin, reference anti-inflammatory drug was given as a dose of 10 mg/kg, orally. The results of anti-inflammatory activity of the evaluated compounds and the reference non-steroidal anti-inflammatory drug (indomethacin) are listed in **Table 2** and **Table 3**.

Examination of activity results from **Table 1** and **Table 2** showed that all the tested compounds **4a-k** were having moderate to comparable activity as comparable to the standard drug indomethacin. Activity tables shows that the synthesized compounds **4c**, **4f**, **4g**, **4h**, **4j** and **4k** start acting in first hour and also remain active up to the 4^{th} hour of study. Except compound **4d**, all the remaining tested compounds show their potency as anti-inflammatory agent in the 4^{th} hour which can be observed in **Figure-5** where hour wise % inhibition is plotted of tested compounds and the standard drug.

On observing the dock results in **Table-1** and **Figure 6**, this can be ascertained that interaction of compounds **4a-k** with amino acid Tyr355 in COX-2 active pocket is an important interaction as observed by the compound **II** and standard drug as well. Whereas, the excessive Tyr355 interaction in case of compound **4d** might have caused the deformation in the active site as it stands as the least active compound in activity comparison graph (**Figure-6**). Interaction with amino acid His90 common in case of compounds **4c**, **4g**, **4k** and **SC-558** also stands an important interaction for activity. Noteworthy interaction of amino group nitrogen with amino acid residue Tyr355 in all compounds **4a-k** makes this an important SAR around pyrazolo[1,5-*a*]pyrimidine nucleus (**Table-1**) as this is the only bonding that compounds **4a**, **b**, **f**, **h** and **i** show in their docking results but still these compounds are prominently active.

Without specific tests, it is quite difficult to hypothesize the mechanism of action of active compounds. Probable inhibition of the cyclooxygenase enzymes like other non-steroidal anti-inflammatory agents might be the mode of action in case of active compounds.

After comparing the moldock score with data obtained from biological evaluation, an interesting correlation was obtained between the two. We studied the correlation between moldock score and percentage inhibition of these compounds as given in **Figure-7**. As the anti-inflammatory effect of our compounds was evaluated further after fix interval of times the r^2 value between moldock score and percentage inhibition also increased proportionally. But after 3hr the correlation value slightly decrease and then increases. This interesting relation confirms that our compounds are potential anti-inflammatory agents which were confirmed from the biological evaluation as well as molecular docking studies.

5. Conclusion

In conclusion, we have described an efficient, simple, expeditious one-pot protocol for the preparation of novel 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines (**4a-k**) designed by molecular docking. Compounds have been screened for their anti-inflammatory activity and their activity results have been correlated with dock score. Good agreement in anti-inflammatory potential of screened compounds (**4a-k**) and molecular docking studies make these a new scaffold in the development of potent anti-inflammatory therapeutics.

6. Experimental

6.1 Materials and methods

Melting point were determined in open capillaries with an electrical apparatus and are uncorrected, The IR spectra of the compounds were recorded on ABB MB3000 FT-IR laboratory analyser combined with Horizon MB^{TM} FTIR software (ν_{max} in cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker instrument at 300 and 75 MHz, respectively. Chemical shifts are expressed in δ-scale downfield from TMS as an internal standard. High resolution mass spectra (HRMS) were measured in EI mode on a Kratos MS-50 spectrometer. Hydrazine hydrate (**1**) was commercially available and 3-aryl-3-oxopropanonitriles (**2a-e**) were prepared according to literature procedure [9].

6.2 General procedure for synthesis 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines (4a-k):

To the 1 mol eq. of 3-aryl-3-oxopropanonitrile (**2**) was added 1 mol eq. of hydrazine hydrate (**1**) in Toluene/EtOH (9:1) and the reaction mixture was refluxed for 30 min then another 1

mol eq. of different 3-aryl-3-oxopropanonitrile (**2**) and catalytic amount of PTSA (0.01 mol eq.) was added. Again reaction mixture was refluxed up to 4 hr. On completion of the reaction, excess solvent was distilled off. The solid so obtained was filtered and washed with cold ethanol. Solid was neutralised with aq. sodium bicarbonate solution and again filtered. Compound thus obtained was air dried and recrystallized from ethanol.

Analytical and spectral data of synthesized compounds **4a-k**:

6.2.1. 2-Phenyl-5-*p*-tolylpyrazolo[1,5-*a*]pyrimidin-7-amine (4a):

Yield 65%; Mp: 218-220°C; IR (cm^{-1}): 3325, 3350 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 2.38 (s, 3H, 4''-CH_3), 6.59 (s, 1H, 6-H), 6.90 (s, 1H, 3-H), 7.31-7.50 (m, 5H, Ar-H), 7.72 (bs, 2H, 7-NH_2), 7.93-8.09 (m, 4H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 21.30, 84.70, 91.51, 126.61, 127.10, 129.15. 129.74, 133.49, 135.82, 139.77, 148.45, 150.77, 154.90, 154.97, 156.23; HRMS (EI) m/z for $C_{19}H_{16}N_4$ calculated: 300.1375, found 301.1363 $[M+1]^+$.

6.2.2. **2-Phenyl-5-*p*-chlorophenylpyrazolo[1,5-*a*]pyrimidin-7-amine (4b)**:

Yield 62%; Mp: 178-180°C; IR (cm^{-1}): 3317, 3410 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 6.60 (s, 1H, 6-H), 6.93 (s, 1H, 3-H), 7.42-7.58 (m, 5H, Ar-H), 7.72 (bs, 2H, 7-NH_2), 8.05-8.08 (m, 4H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 84.84, 92.07, 126.63, 128.96, 129.17, 129.29, 133.36, 134.88, 137.40, 148.57, 150.47, 154.94, 155.09; HRMS (EI) m/z for $C_{18}H_{13}ClN_4$ calculated: 320.0829, found: 321.0815 $[M+1]^+$, 323.0816 $[M+1+2]^+$(3:1).

6.2.3. 2-Phenyl-5-*p*-bromophenylpyrazolo[1,5-*a*]pyrimidin-7-amine (4c):

Yield 69%; Mp: 198-200°C; IR (cm^{-1}): 3171, 3286 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 6.60 (s, 1H, 6-H), 6.93 (s, 1H, 3-H), 7.42-7.72 (m, 5H, Ar-H), 7.81 (bs, 2H, 7-NH_2), 7.98-8.08 (m, 4H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 84.74, 92.10, 122.49, 123.63, 126.64, 128.59, 129.22, 132.12, 133.40, 137.83, 148.58, 150.68, 154.95, 155.08; HRMS (EI) m/z for $C_{18}H_{13}BrN_4$ calculated: 364.0324, found: 365.0314 $[M+1]^+$, 366.0316 $[M+1+2]^+$(1:1).

6.2.4. 2-*p*-Tolyl-5-phenylpyrazolo[1,5-*a*]pyrimidin-7-amine (4d):

Yield 63%; Mp: 236-238°C; IR (cm^{-1}): 3325, 3350 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 2.37 (s, 3H, 4'-CH_3), 6.60 (s, 1H, 6-H), 6.87 (s, 1H, 3-H), 7.29-7.32 (d, 2H, Ar-H), 7.49-7.52 (m, 3H, Ar-H), 7.72 (bs, 2H, 7-NH_2), 7.95-7.98 (d, 2H, Ar-H), 8.03-8.05 (d, 2H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 21.38, 84.81, 91.69, 126.55, 127.15, 129.14, 129.72, 130.03, 130.75, 138.71, 148.47, 150.58, 155.02, 156.11; HRMS (EI) m/z for $C_{19}H_{16}N_4$: calculated 300.1375, found: 301.1373 $[M+1]^+$.

6.2.5. 2-*p*-Tolyl-5-*p*-fluorophenylpyrazolo[1,5-*a*]pyrimidin-7-amine (4e):

Yield 69%; Mp: 202-204°C; IR (cm^{-1}): 3250, 3290 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 2.37 (s, 3H, 4'-CH_3), 6.57 (s, 1H, 6-H), 6.87 (s, 1H, 3-H), 7.32-7.41 (m, 4H, Ar-H), 7.75 (bs, 2H, 7-NH_2), 7.95-7.98 (m, 2H, Ar-H), 8.08-8.11 (m, 2H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 21.37, 84.79, 91.07, 126.52, 127.16, 129.18, 129.69, 130.10, 130.71, 138.75, 148.45, 150.52, 155.08, 156.16; HRMS (EI) m/z for $C_{19}H_{15}FN_4$ calculated: 318.1281, found: 319.1278 $[M+1]^+$.

6.2.6. 2-*p*-Chlorophenyl-5-*p*-tolylpyrazolo[1,5-*a*]pyrimidin-7-amine (4f):

Yield 70%; Mp: 264-266°C; IR (cm^{-1}): 3190, 3260 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 2.38 (s, 3H, 4"-CH_3), 6.60 (s, 1H, 6-H), 6.93 (s, 1H, 3-H), 7.31-7.38 (d, 2H, Ar-H), 7.55-7.58 (d, 2H, Ar-H), 7.73 (bs, 2H, 7-NH_2), 7.93-7.95 (d, 2H, Ar-H); 8.08-8.10 (d, 2H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 21.31, 84.80, 92.07, 126.63, 128.96, 129.17, 133.36, 134.88, 137.40, 148.57, 154.94, 155.09; HRMS (EI) m/z for $C_{19}H_{15}ClN_4$ calculated: 334.0985, found: 335.0978 $[M+1]^+$, 337.0976 $[M+1+2]^+$(3:1).

6.2.7. 2-*p*-Tolyl-5-*p*-chlorophenylpyrazolo[1,5-*a*]pyrimidin-7-amine (4g):

Yield 65%; Mp: 240-242°C; IR (cm^{-1}): 3240, 3279 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 2.37 (s, 3H, 4-CH_3), 6.59 (s, 1H, 6-H), 6.88 (s, 1H, 3-H), 7.29-7.32 (d, 2H, Ar-H), 7.56-7.58 (d, 2H, Ar-H), 7.77 (bs, 2H, 7-NH_2), 7.95-7.98 (d, 2H, Ar-H); 8.05-8.08 (d, 2H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 21.38, 84.67, 91.80, 126.56, 128.92, 129.17, 129.72, 130.68, 134.80, 137.53, 138.68, 148.53, 150.68, 154.77, 155.13; HRMS (EI) m/z for $C_{19}H_{15}ClN_4$ calculated : 334.0985, found: 335.1066 $[M+1]^+$, 337.1047 $[M+1+2]^+$(3:1).

6.2.8. 2-*p*-Chlorophenyl-5-*p*-bromophenylpyrazolo[1,5-*a*]pyrimidin-7-amine (4h):

Yield 69%; Mp: 252-254°C; IR (cm^{-1}): 3240, 3286 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 6.61 (s, 1H, 6-H), 6.96 (s, 1H, 3-H), 7.56-7.59 (d, 2H, Ar-H), 7.70-7.72 (d, 2H, Ar-H), 7.84 (bs, 2H, 7-NH_2), 7.98-8.01 (d, 2H, Ar-H); 8.08-8.11 (d, 2H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 84.97, 92.25, 123.70, 128.31, 128.51, 128.59, 129.22, 132.10, 133.97, 137.73, 148.57, 150.72, 153.88, 155.15; HRMS (EI) m/z for $C_{18}H_{12}BrClN_4$ calculated: 397.9934, found: 398.9929 $[M+1]^+$, 400.9928 $[M+1+2]^+$, 402.9927 $[M+1+4]^+$, (3:4:1).

6.2.9. 2-*p*-Bromophenyl-5-*p*-chlorophenylpyrazolo[1,5-*a*]pyrimidin-7-amine (4i):

Yield 62%; Mp: 262-264°C; IR (cm^{-1}): 3210, 3250 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 6.61 (s, 1H, 6-H), 6.96 (s, 1H, 3-H), 7.55-7.58 (d, 2H, Ar-H), 7.69-7.72 (d, 2H, Ar-H), 7.83 (bs, 2H, 7-NH_2), 8.01-8.07 (m, 4H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 84.91, 92.30, 123.66, 128.31, 129.22, 132.12, 137.79, 148.50, 150.76, 153.84; HRMS (EI) m/z for $C_{18}H_{12}BrClN_4$ calculated: 397.9934, found: 398.9987 $[M+1]^+$, 400.0022 $[M+1+2]^+$, 402.9937 $[M+1+4]^+$ (3:4:1).

6.2.10. 2-*p*-Tolyl-5-*p*-bromophenylpyrazolo[1,5-*a*]pyrimidin-7-amine (4j):

Yield 67%; Mp: 258-260°C; IR (cm^{-1}): 3294, 3441 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 2.37 (s, 3H, 4'-CH_3), 6.58 (s, 1H, 6-H), 6.88 (s, 1H, 3-H), 7.29-7.32 (d, 2H, Ar-H), 7.69-7.72 (d, 2H, Ar-H); 7.78 (bs, 2H, 7-NH_2), 7.95-8.00 (m, 4H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 21.38, 84.63, 91.82, 123.58, 126.56, 129.19, 129.72, 130.67, 132.10, 137.89, 138.68, 148.53, 150.68, 154.84, 155.14; HRMS (EI) m/z for $C_{19}H_{15}BrN_4$ calculated: 378.0480, found: 379.0547 $[M+1]^+$, 381.0526 $[M+1+2]^+$(1:1).

6.2.11. 2-*p*-Bromophenyl-5-*p*-tolylpyrazolo[1,5-*a*]pyrimidin-7-amine (4k):

Yield 71%; Mp: 238-240°C; IR (cm^{-1}): 3279, 3402 (-NH_2); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 2.38 (s, 3H, 4"-CH_3), 6.60 (s, 1H, 6-H), 6.93 (s, 1H, 3-H), 7.31-7.33 (d, 2H, Ar-H), 7.69-7.72 (d, 2H, Ar-H); 7.59 (bs, 2H, 7-NH_2), 7.93-7.95 (d, 2H, Ar-H), 8.01-8.04 (d, 2H, Ar-H); ^{13}C-NMR δ (DMSO-d_6): 21.32, 84.82, 92.01, 122.39, 127.17, 128.56, 129.75, 131.90, 132.12, 132.84, 135.84, 139.76, 150.88, 153.68, 156.31; HRMS (EI) m/z for $C_{19}H_{15}BrN_4$ calculated: 378.0480, found: 379.0471 $[M+1]^+$, 381.0469 $[M+1+2]^+$(1:1).

7. Pharmacological assay

7.1 *In vivo* anti-inflammatory assay

Carrageenan-induced rat paw edema assay-

Male wistar albino rats weighing 200-250 g were used for the study. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. Animals were deprived of the food 12 hr before and during experimental hours. Three groups of five rats each were divided as control, standard drug and synthesized compounds treated. Control group of five rats was given tween 80 (95%) only. Standard group received indomethacin at a dose of 10 mg/kg body weight orally. Test group of animals was treated with test compounds at a dose of 50 mg/kg body weight. A mark was made on the left hind paw just beyond the tibiotarsal articulation for constant paw volume measurement. 0.1 ml freshly prepared suspension of carrageenan (1% in 0.9% saline) was injected under the planter region of the left hind paw of each rat. Test compounds and standard drug were administered orally to the animals, respectively 30 min before the carrageenan injection. The paw volume measurements of each rat were carried out at 0 hr (before carrageenan injection) and after 1 hr, 2 hr, 3 hr and 4 hr of carrageenan treatment with the help of a Plethysmometer (model 7140, Ugo Basile, Italy. The anti-inflammatory effect was calculated by the following equation:

Anti-inflammatory activity (%) = $(V_c-V_t/V_c) \times 100$

Where V_t and V_c are the volume of edema in test compound/standard drug and control group, respectively.

8. Molecular docking material and method

The 3D structures of the inhibitors were constructed using standard geometric parameters of the molecular modelling software package Marvin Sketch [10]. All the structures were minimized and optimized with the Merck Molecular Force Field (MMFF) method taking the root mean square gradient (RMS) of 0.01 kcal/molA° and the iteration limit to 10,000. All the structures were ionized at neutral pH 7. Conformers for each structure were generated using Monte Carlo by applying MMMF force field method and least energy conformer was selected for further study [11]. Molecular docking and scoring protocols as implemented in Molegro Virtual Docker [12] were used to investigate the possible binding conformations of the ligands within the COX-2 binding pocket. The X-ray crystallographic data for COX-2 determined at 2.05Aº (PDB ID 1COX-2) [13] used in the docking simulations were retrieved from the Protein Data Bank (PDB) and the A-chain residues of 1COX-2 were remained to dock. Before docking the protein is prepared by using the protein preparation wizard, removing the water molecule and cofactors from the proteins, optimizing hydrogen bonding and deleting the ligand present in crystal structure. Solvent molecules were deleted and bond order for crystal ligand and protein were adjusted and minimized up to 0.30 A° RMS distance.

Acknowledgement

We are thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India for providing financial assistance (JRF) to Gulshan Singh and the Mass Spectrometry Facility, University of California, San Francisco, USA for running the mass spectra.

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Table 1: Docking results of pyrazolo[1,5-*a*]pyrimidines (4a-k) by Molegro Virtual Docker

Compound No.	Mole Dock Score	No of H-Bonds	Atoms of Ligand/Compounds	Amino acid	Distance (A°)
4a	-125.811	1	N of NH_2	Tyr355	2.62
4b	-115.933	1	N of NH_2	Tyr355	2.60
4c	-124.808	2	N of NH_2	Tyr355	2.63
			N of NH_2(weak)	His90	3.52
4d	-102.883	4	N of NH_2	Tyr355	2.59
			N_1 of Pyrazole Ring	Tyr355	2.69
			N_{7a} of Pyrazole Ring	Tyr355	2.48
			N_{7a} of Pyrazole Ring	Arg120	3.41
4e	-119.854	2	N of NH_2	Phe518	2.56
			N of NH_2(very weak)	Gly519	3.57
4f	-133.205	1	N of NH_2	Tyr355	2.84
4g	-129.829	2	N of NH_2	Tyr355	2.61
			N of NH_2(weak)	His90	3.50
4h	-131.838	1	N of NH_2	Tyr355	2.81
4i	-131.662	1	N of NH_2	Tyr355	2.78
4j	-118.342	2	N of NH_2	Phe518	2.54
			N of NH_2(very weak)	Gly519	3.50
4k	-132.439	2	N of NH_2	Tyr355	2.67
			N of NH_2	His90	3.42
SC-558	-156.097	3	O of SO_2NH_2	Arg513	3.27
			N of SO_2NH_2	Ser353	2.76
			N of SO_2NH_2	His90	2.96
Indomethacin	-138.644	3	OH of COOH	Leu352	3.12
			C=O of COOH	Tyr355	2.88
			O of OCH_3	Arg120	2.79
Compound II	-135.815	3	N_4 of Pyrimidine	Tyr355	2.75
			O of SO_2CH_3	Tyr285	2.51
			O of SO_2CH_3	Ser530	2.30

Table 2: Anti-inflammatory activity of test compounds (carrageenan-induced paw edema test in rats)

Sr. No.	Compounds	Mean value of edema volume			
		1hr	2hr	3hr	4hr
1	4a	0.37±0.09	0.50±0.08	0.56±0.13	0.38±0.08**
2	4b	0.41±0.012	0.69±0.04	0.59±0.05	0.41±0.10**
3	4c	0.18±0.04	0.29±0.06**	0.36±0.05*	0.28±0.04**
4	4d	0.38±0.07	0.66±0.03	0.54±0.06	0.55±0.05*
5	4e	0.37±0.15	0.28±0.10**	0.60±0.18	0.30±0.11**
6	4f	0.22±0.08	0.20±0.08**	0.25±0.11**	0.16±0.05**
7	4g	0.21±0.06	0.26±0.03**	0.29±0.04**	0.27±0.05**
8	4h	0.21±0.07	0.32±0.11**	0.24±0.07**	0.20±0.04**
9	4i	0.45±0.07	0.22±0.06**	0.36±0.08*	0.29±0.07**
10	4j	0.15±0.05*	0.35±0.07**	0.36±0.07*	0.28±0.05**
11	4k	0.17±0.05*	0.19±0.11**	0.22±0.10**	0.30±0.09**
12	Indomethacin	0.14±0.05*	0.16±0.06**	0.14±0.02**	0.13±0.05**
13	Control (DMSO)	0.52±0.17	0.80±0.05	0.82±0.13	0.84±0.03

All values are expressed as mean ±SEM of five rats in each group. Statistically significant **$p<0.01$,*$p<0.05$ compared to control.

Table 3: Anti-inflammatory activity of test compounds showing percent inhibition (carrageenan-induced paw edema test in rats)

Sr. No.	Compounds	Percent inhibition			
		1hr	2hr	3hr	4hr
1	**4a**	28.84	37.50	31.70	54.76
2	**4b**	21.15	13.75	28.04	51.16
3	**4c**	65.38	63.75	56.09	66.66
4	**4d**	26.92	17.50	34.14	34.52
5	**4e**	28.84	65.00	26.82	64.28
6	**4f**	57.69	75.00	69.51	80.95
7	**4g**	59.61	67.50	64.63	67.85
8	**4h**	59.61	60.00	70.73	76.19
9	**4i**	13.46	72.50	56.09	65.47
10	**4j**	71.15	56.25	56.09	66.66
11	**4k**	67.30	76.25	73.17	64.28
12	**Indomethacin**	73.07	80.00	82.92	84.52

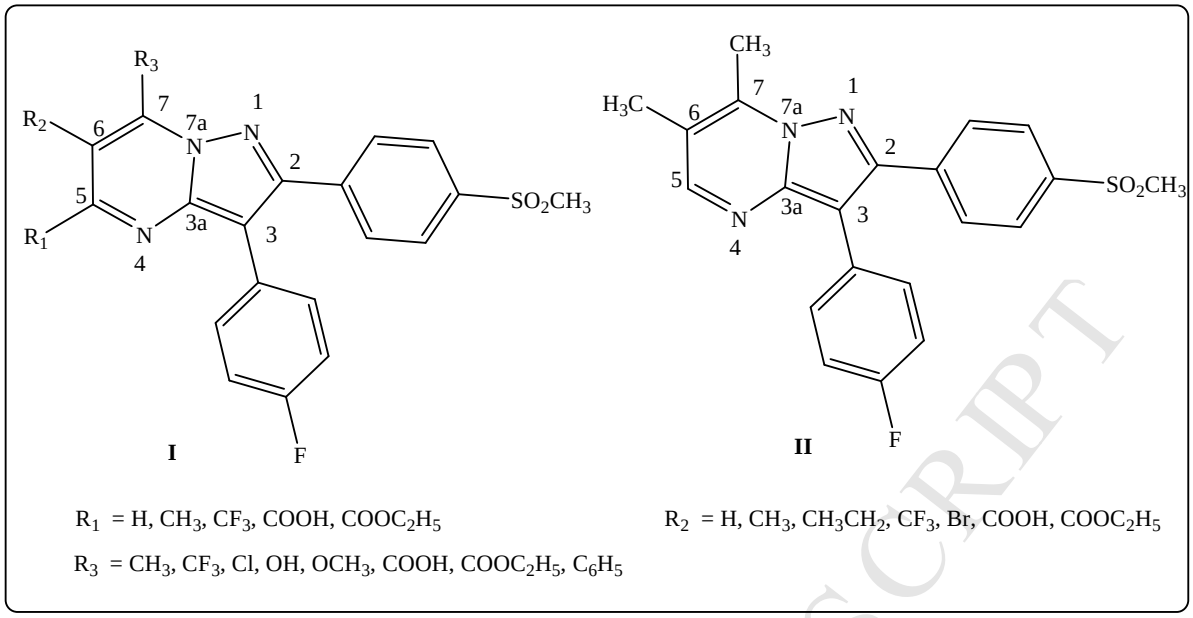
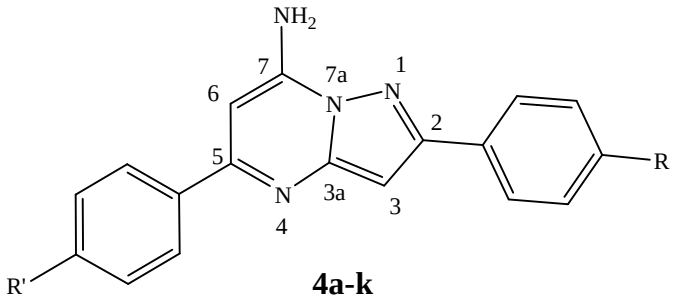


Figure 1: Structure of pyrazolo[1,5-*a*]pyrimidines (**I** and **II**) showing variation at 5^{th}, 6^{th} and 7^{th} position of nucleus



4a-k

4	a	b	c	d	e	f	g	h	i	j	k
R	H	H	H	CH_3	CH_3	Cl	CH_3	Cl	Br	CH_3	Br
R'	CH_3	Cl	Br	H	F	CH_3	Cl	Br	Cl	Br	CH_3

Figure 2: Pyrazolo[1,5-*a*]pyrimidines (**4a-k**)

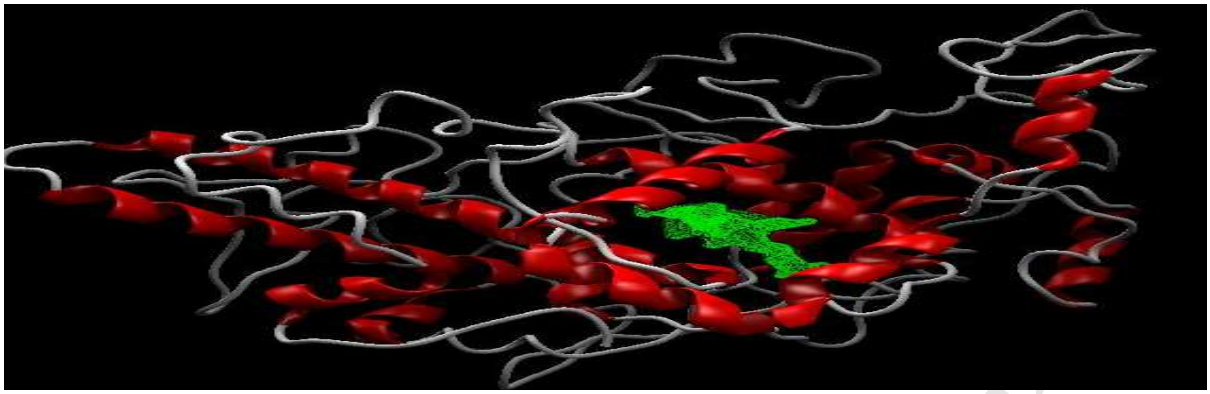


Figure 3: Secondary Structure of COX-2 with active binding pocket

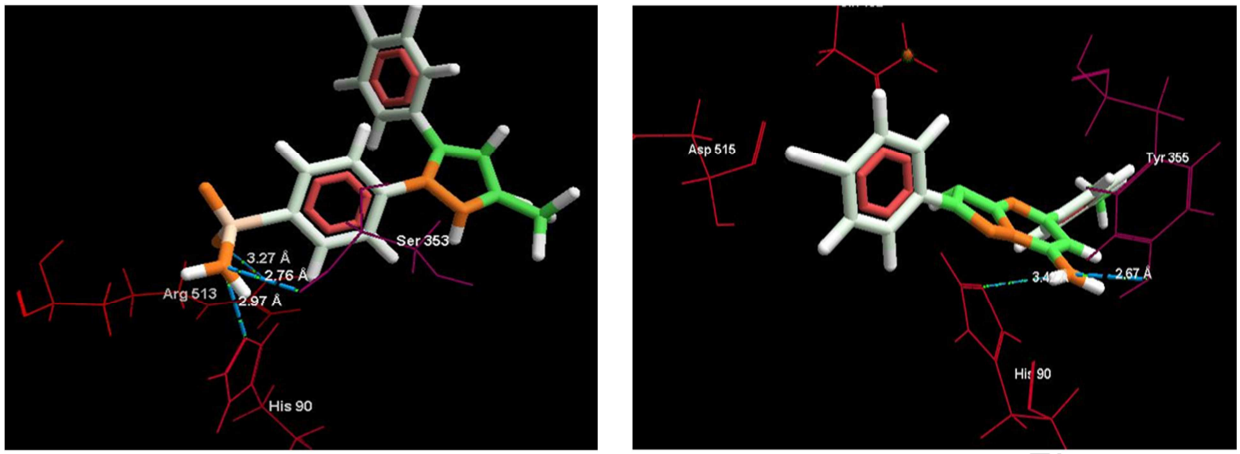


Figure 4: Binding mode of compound **SC-558** and **4k** into COX-2 pocket

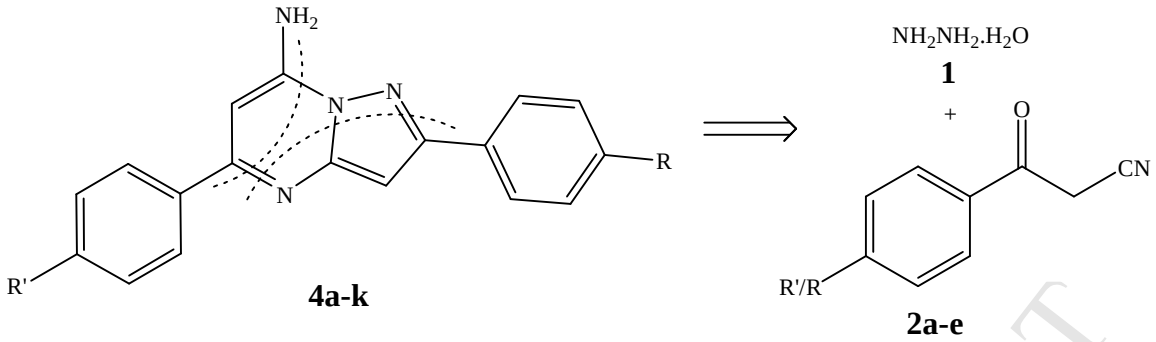


Figure 5: Disconnection of 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amine

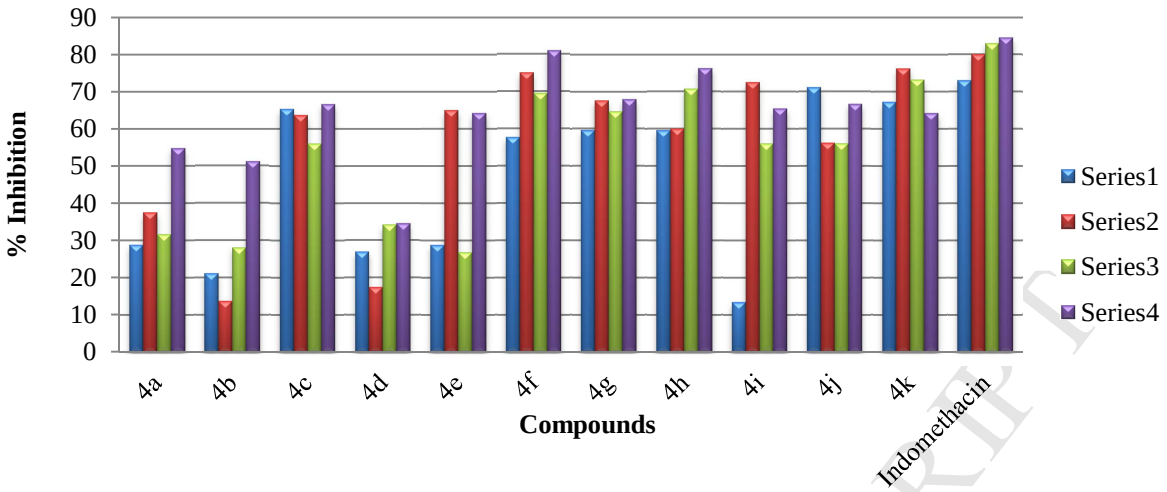
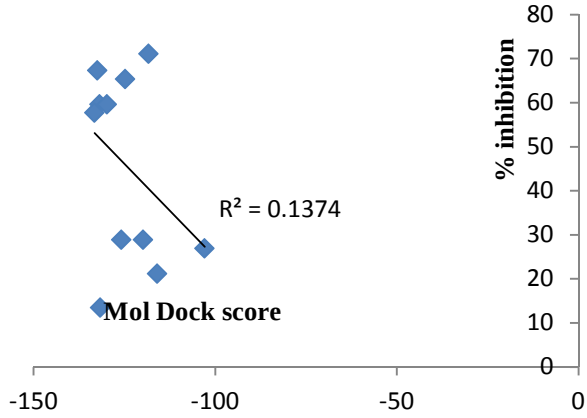
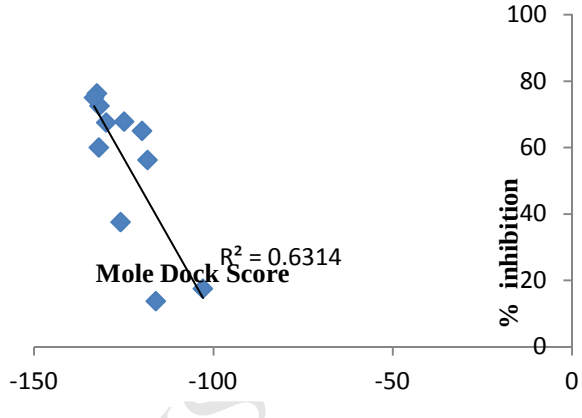


Figure 6: Comparison graph between tested compounds and standard drug

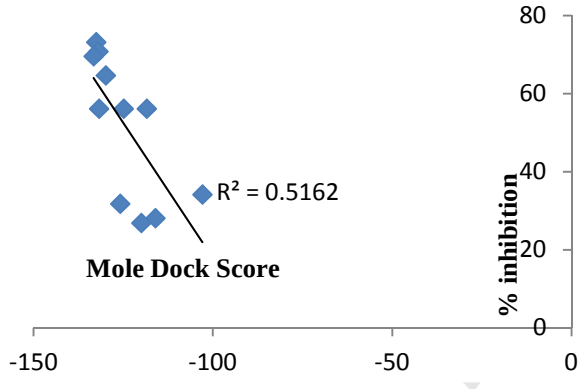
Correlation Value	Time (hr)			
	1	2	3	4
	0.137	0.631	0.516	0.706



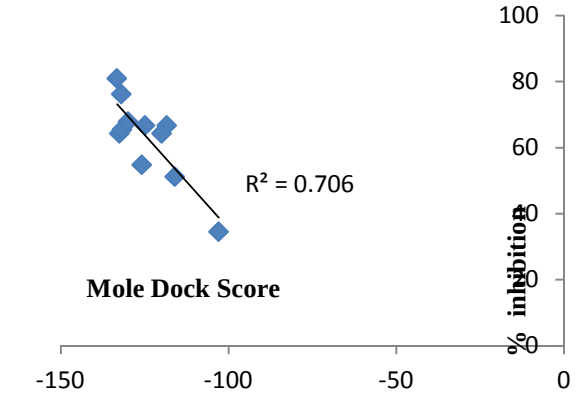
Mole dock score v/s % inhibition after 1 hr



Mole dock score v/s % inhibition after 2 hr

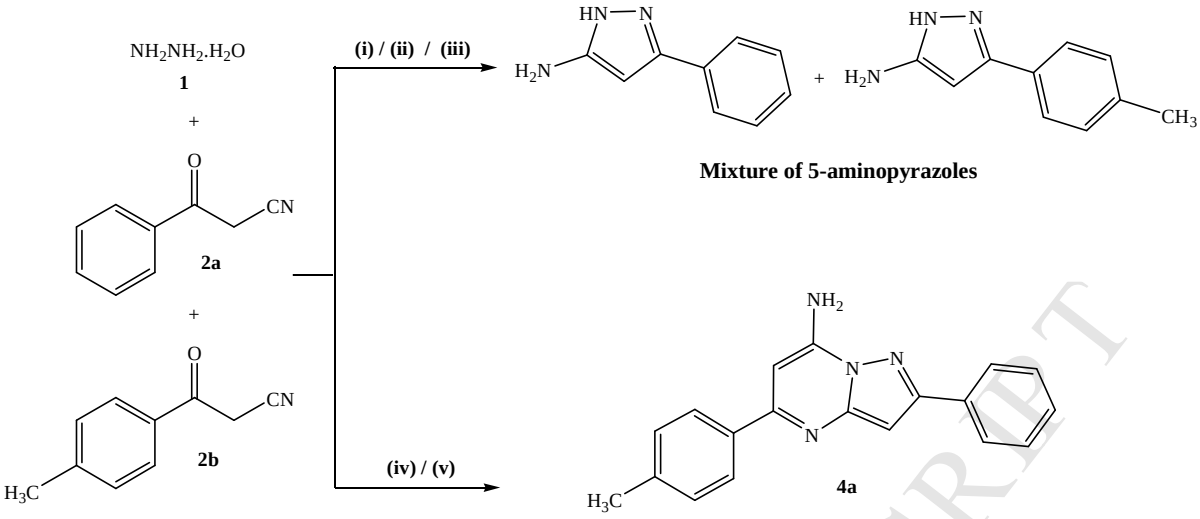


Mole dock score v/s % inhibition after 3 hr



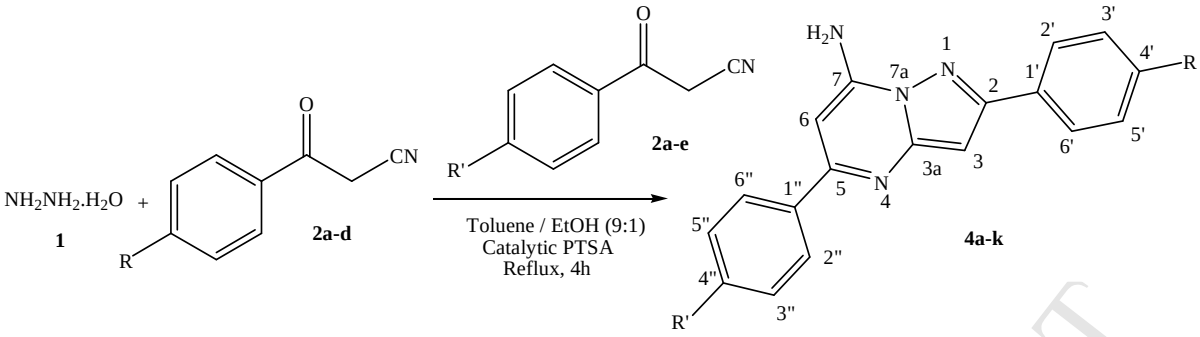
Mole dock score v/s % inhibition after 4 hr

Figure 7: Correlation value at different time interval



Reaction Conditions: (i) Ethanol, Reflux; **(ii)** Ethanol, PTSA, Reflux;**(iii)** Toluene, Reflux; **(iv)**Toluene, PTSA, Reflux, Yield 30%; **(v)** Toluene / EtOH (9:1), Catalytic PTSA, Reflux, 4h,Yield 65 %.

Scheme 1: Reaction profile of hydrazine hydrate (**1**) and 3-aryl-3-oxopropanonitriles (**2**) in different conditions



2	a	b	c	d	e
R, R'	H	CH_3	Cl	Br	F

4	a	b	c	d	e	f	g	h	i	j	k
R	H	H	H	CH_3	CH_3	Cl	CH_3	Cl	Br	CH_3	Br
R'	CH_3	Cl	Br	H	F	CH_3	Cl	Br	Cl	Br	CH_3

Scheme 2: General Scheme for synthesizing 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines (**4a-k**)

Research highlights

- Molecular docking design and synthesis of some novel 2,5-diarylpyrazolo[1,5-*a*]pyrimidin-7-amines.
- Efficient, simple, expeditious one pot protocol for the preparation.
- *In vivo* anti-inflammatory activity on rats by carrageenan-induced rat paw edema assay.
- Correlation of activity results with dock score.
- Potency as anti-inflammatory agents up to the 4^{th} hour of study