Design, Synthesis and Biological Evaluation of 5-amino-3-aryl-1-(6'-chloropyridazin-3'-yl)pyrazoles and their Derivatives as Analgesic Agents

Authors
Swati[1], Ranjana Aggarwal[1,2], Pawan Kaushik[3], Ajay Kumar[3], Deepika Saini[3]

Affiliations
1 Department of Chemistry, Kurukshetra University, Kurukshetra, India
2 Department of Scientific and Industrial Research NISTADS, New Delhi, India
3 Department of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, India

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Correspondence
Ranjana Aggarwal
National Institute of Science Technology and Development Studies
110012 New Delhi
India
Tel.: (+91-11) 25846064, 25843227
director@nistads.res.in

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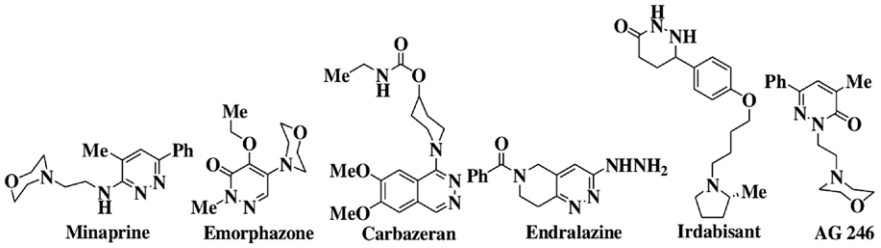
ABSTRACT

An efficient and environmental benign solvent-free synthesis of 5-amino-3-aryl-1-(6'-chloropyridazin-3'-yl)pyrazoles **(4a-e)** was accomplished by grinding 3-chloro-6-hydrazinopyridazine **(2)** and β-ketonitriles **(3a-e)** in the presence of *p*-toulenesulfonic acid as a catalyst. Subsequently, 6'-chloro group in **4a-e** was replaced with cyclic 2° amine derivatives *viz.* pyrrolidine **5a**, piperidine **5b** and morpholine **5c** to obtain **6a-e**, **7a-e**, **8a-e** respectively. The newly synthesized compounds were characterized by using IR, NMR (^{1}H and ^{13}C), mass spectral studies, elemental analyses. All the synthesized compounds were studied for their docking interaction with target protein 6COX and screened for their *in vivo* analgesic mode of action against swiss albino mice (animal model) using acetic-acid induced writhing test. Consequently, docking simulations data justifies the potential of synthesized series as an analgesic and very well correlated with *in vivo* study. Preliminary results revealed that most of the synthesized compounds exhibited moderate to good analgesic activity as compared to reference/standard drug (s) sodium diclofenac and candidates **4d** and **7c** protrude out as a promising lead for further investigation.

Introduction

Pain is an unpleasant sensory and emotional event that works as a piece of physiological advice to convey discomfort signals to the brain by sensory neurons and thereby alerting the body for potentially tissue-damaging situations which are triggered by aseptic stimuli [1, 2]. Body inflammation is a key element to pain induction which is an outcome of tissue damage developed by a sequence of pain, fever or microbial infection [3]. Several drugs are available in the market which nullifies and prevent the sensitization of nociceptors, e. g., dipyrone and indomethacin respectively, also, known as peripheral analgesic agents for their mode of action to regulate the inflammatory hyperalgesia [4]. Most of the NSAID exert their analgesic action by inhibition of the cyclooxygenase enzyme (COX), known for reducing the level of prostaglandins within the hypothalamic region, e. g. PGE2, which sensitizes nociceptors at nerve fibre terminals [5]. Moreover, 5-lipoxygenase (5-LOX) catalyses the conversion of arachidonic acid to leukotriene B4 which contributes to the hyperalgesia seen during inflammation [6, 7]. Neutrophils are the first responders of inflammatory cells which migrate to the site of inflammation following chemical signal, leukotriene, during the beginning phase of inflammation. For these reasons, compounds that achieve the dual inhibition of enzymes COX and 5-LOX become an important therapeutic strategy in the combat of the pain tolerable trauma.



▶ **Fig. 1** Marketed drugs containing pyridazine ring collaborated with cyclic secondary amines.

Out of the three isomeric forms of diazines: pyridazine, pyrimidine and pyrazine, pyridazine moiety does not show its abundant existence in any natural product probably due to difficulty in formation of N-N bond by living organisms. However, pyridazine nucleus has occupied an influential place in the therapeutic arena because of its bioavailability to the cell membrane, especially to CNS, due to its water soluble nature. Pyridazine scaffold participates as hydrogen bond receptor and has the highest capacity to complex with targets due to its highest dipole moment 3.9 D, as compared to pyrimidine 2.42 D and pyrazine 0.6 D [8].

Pyrrolidine, piperidine, morpholine are few cyclic secondary (2°) amines have received considerable attention in the pharmaceutical field due to their existence as an essential part in a wide range of drugs such as procyclidine, bepridil, droperidol, risperidone, melperone, meperidine, edronax, fenpropimorph, phendimetrazine etc. Also, these are present in many natural alkaloids: pyrrolidine in nicotine, hygrine, piperidine in solenopsins, piperine (black pepper), anabasine, lobeline, coniline and morpholine in polygonapholine, chelonine, acortatarins etc.

Evidence for the potent activity of pyridazine entity endowed with cyclic 2° amines in biological system has been highlighted by its remarkable presence in a number of pharmaceutical drugs such as minaprine (brantur), emorfazone (pentoil or nandron), carbazeran, endralazine, irdabisant and AG-246 etc (▶ **Fig. 1**). Considering our matter of interest, pyridazine moiety has emerged as an important target in pain therapy aiming at improved nonsteroidal analgesics which are effective pain killers without any gastric and renal ulcerations. Emorphazone is one of the best drug known which shows better gastric profile, marketed in Japan as an analgesic and anti-inflammatory drug [9, 10] and AG 246 is also known for its good analgesic action [11] etc.

The medicinal value of 5-aminopyrazoles is widespread and achieved a remarkable position in the biological system as antimicrobial, anticancer, antimalarial, antiprotozoal, anti-HIV agents and receptor antagonist [12–17] etc. This privileged scaffold has fascinated the attention of most of the researchers in the field of medicinally active candidates. The rapid growth of 5-aminopyrazole motif has been witnessed in a wide range of compounds. Fipronil, a member of 5-aminopyrazole family, is the most effective pesticide as it blocks the GABA-gated, glutamate-gate chloride channels and disrupts the central nervous system of insects. Interestingly, ethyl 5-amino-1-(2-(6-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yloxy)acetyl)-1*H*-pyrazole-4-carboxylate [18] and 5-amino-1-(2-fluorophenyl)-*N*-(5-(isoxazol-3-ylcarbamoyl)-2-methylphenyl-1*H*-pyrazole-4-carboxamide [19] are reported in the literature as an anti-inflammatory agent.

Aim: Designing of Target molecules

The above-mentioned applications envisaged us to combine these three active pharmacophores (pyridazine, cyclic 2° amines and 5-aminopyrazole) to generate a small library of novel compounds which are supposed to exhibit interesting biological properties in animal models. In literature, 5-aryl-1-(4-substitutedpyridazinyl) pyrazol-3-propanoic acids (**A**) have been reported to inhibit LTB_4 biosynthesis in human neutrophils with IC_{50} values of 12–14 μM [20, 21]. Keeping this in view and in continuation of our interest in 5-aminopyrazoles as precursors for the synthesis of heterocyclic rings and as a promising biological active entity [22–29], target molecules have been designed by replacing acidic chain of pyrazole ring with aryl ring, aryl ring at position-5 of pyrazole ring with amino group and widening the scope of substituents such as R group at phenyl ring present at position-3 of pyrazole ring, and substitution of Cl group by pyrrolidine, piperidine and morpholine of pyridazine ring (▶ **Fig. 2**).

Materials and Methods

Experimental protocol

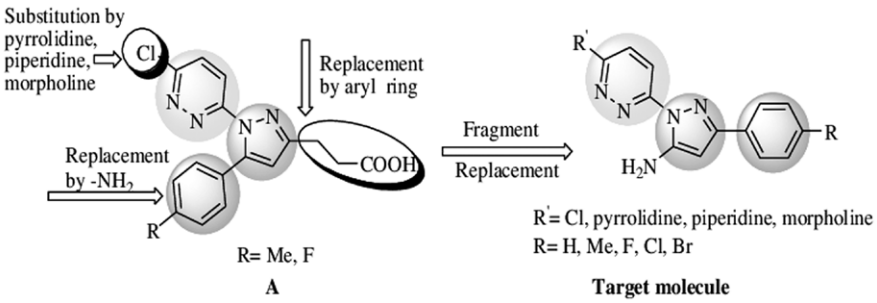
Melting points were determined in open capillaries on digital instrument Make. Infrared spectra were recorded on a spectrophotometer (IR M-500, Buck Scientific Inc, Norwalk, CT) in KBr pellets (ν_{max} in cm^{-1}). ^1H and ^{13}C NMR spectra for the analytical purpose were recorded in $CDCl_3$ on a Bruker instrument (Billerica, MA) at 400 and 100 MHz respectively. Tetramethylsilane was taken as an integral standard. Chemical shifts (δ) were measured in ppm. Coupling constants (J) are given in Hertz (Hz). Mass and elemental analyses were performed on Q-TOF micro mass spectrometer and CHNS-O elemental analyser at Sophisticated Analytical Instrumentation Facility, Panjab University, Chandigarh.

Step-wise solvent-mediated procedures

Synthesis of 1-(6'-Chloropyridazin-3'-yl)-3-phenyl-1*H*-pyrazole-5-amine **(4a)**

Ethanol medium

3-Chloro-6-hydrazinopyridazine (0.144g, 1mmol) was added to a solution β-ketonitrile **(3a)** (0.145g, 1mmol) in 15 ml ethanol followed by the addition of a catalytic amount of conc. HCl (4–5



▶ **Fig. 2** Designing of target molecules as analgesic agent w.r.t. model compound A.

drops) after 5 min. of refluxing the contents. Completion of the reaction was observed within 30 min. of refluxing. The reaction mixture was concentrated, cooled and left overnight. The solid separated was filtered, washed and crystallised with ethanol. The TLC (ethyl acetate/petroleum ether in 1:4) and ^{1}H NMR spectra showed the formation of a single product.

DMF medium

3-Chloro-6-hydrazinopyridazine (0.144g, 1mmol) and β-ketonitrile **(3a)** (0.145g, 1mmol) were refluxed in 6 ml DMF followed by the addition of a catalytic amount of Cs_2CO_3. Completion of the reaction was observed in 5–10 min. of refluxing and the excess of solvent was distilled off to about 1/3 of the volume *i.e* 2ml, cooled, water poured into it, sticky solid separated was filtered, washed with hot water repeatedly and crystallised with ethanol. The TLC (ethyl acetate/petroleum ether in 1:4) and ^{1}H NMR spectra showed the formation of a single product.

Similarly, compounds **4b-e** were synthesized using the procedure mentioned above.

Percentage yield reported in the experimental protocol is of the ethanolic medium.

1-(6'-Chloropyridazin-3'-yl)-3-phenyl-1H-pyrazole-5-amine (**4a**) [30], Cas No.: 122651–96–1

Yield 62.4 %; R_f = 0.66; M.Pt. 185 °C; IR (KBr, cm^{-1}): 3317 (symm.) and 3487 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.87 (s, 1H, 4-H); 5.99 (s, 2H, -NH_2); 7.39 (m, 3H, 3", 4", 5"-H); 7.58 (d, 1H, J = 9.4 Hz, 5'-H); 7.82 (m, 2H, 2", 6"-H); 8.34 (d, 1H, J = 9.4 Hz, 4'-H); Ms: *m/z* [M + 1]$^+$ 272/274 (3:1). Anal. Cal. for $C_{13}H_{10}ClN_5$: C, 57.47; H, 3.71; N, 25.78. Found: C, 57.00; H, 3.00; N, 25.58.

1-(6'-Chloropyridazin-3'-yl)-3-p-tolyl-1H-pyrazol-5-amine (**4b**), Cas No.: 1155511–89–9

Yield 66.9 %; R_f = 0.65; M.Pt. 173 °C; IR (KBr, cm^{-1}): 3325 (symm.) and 3433 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.39 (s, 3H, -CH_3); 5.86 (s, 1H, 4-H); 5.97 (s, 2H, -NH_2); 7.23 (d, 2H, J = 8.0 Hz, 3",5"-H); 7.59 (d, 1H, J = 9.3 Hz, 5'-H); 7.71 (d, 2H, J = 8.0 Hz, 2",6"-H); 8.35 (d, 1H, J = 9.3 Hz, 4'-H); MS: *m/z* [M + 1]$^+$ 286/288 (3:1). Anal. Cal. for $C_{14}H_{12}ClN_5$: C, 58.85; H, 4.23; N, 24.51. Found: C, 57.81; H, 3.76; N, 26.50.

1-(6'-Chloropyridazin-3'-yl)-3-(4"-fluorophenyl)-1H-pyrazol-5-amine (**4c**), Cas No.: 1154198–00–1

Yield 75.0 %; R_f = 0.66; M.Pt. 205.5 °C; IR (KBr, cm^{-1}): 3294 (symm.) and 3418 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.83 (s, 1H, 4-H); 6.01 (s, 2H, -NH_2); 7.15 (t, 2H, J = 8.7 Hz, 3", 5"-H); 7.60 (d, 1H, J = 9.3 Hz, 5'-H); 7.79 (m, 2H, 2", 6"-H); 8.33 (d, 1H, J = 9.3 Hz, 4'-H); MS: *m/z* [M + 1]$^+$ 290/292 (3:1). Anal. Cal. For $C_{13}H_9ClFN_5$: C, 53.90; H, 3.13; N, 24.17. Found: C, 53.00; H, 2.98; N, 23.98.

3-(4"-Chlorophenyl)-1-(6'-chloropyridazin-3'-yl)-1H-pyrazole-5-amine (**4d**), Cas No.: 1154568–20–3

Yield 88.1 %; R_f = 0.67; M.Pt. 203 °C; IR (KBr, cm^{-1}): 3294 (symm.) and 3418 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.92 (s, 1H, 4-H); 6.96 (s, 2H, -NH_2); 7.43 (d, 2H, J = 8.4 Hz, 3",5"-H); 7.84 (d, 2H, J = 8.6 Hz, 2", 6"-H); 7.97 (d, 1H, J = 9.4 Hz, 5'-H); 8.33 (d, 1H, J = 9.4 Hz, 4'-H); Ms: *m/z* [M]$^+$ 305/307/309 (9:6:1). Anal. Cal. For $C_{13}H_9Cl_2N_5$: C, 51.00; H, 2.96; N, 22.88. Found: C, 49.96; H, 2.90; N, 22.80.

1-(6'-Chloropyridazin-3'-yl)-3-(4"-bromophenyl)-1H-pyrazol-5-amine (**4e**), Cas No.: 1154319–76–2

Yield 74.6 %; R_f = 0.66; M.Pt 193.5 °C; IR (KBr, cm^{-1}): 3394 (symm.) and 3402 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.85 (s, 1H, 4-H); 6.01 (s, 2H, -NH_2); 7.54 (d, 2H, J = 8.5 Hz, 3", 5"-H); 7.61 (d, 1H, J = 9.3 Hz, 5'-H); 7.68 (d, 2H, J = 8.6 Hz, 2", 6"-H); 8.32 (d, 1H, J = 9.4 Hz, 4'-H); Ms: *m/z* [M]$^+$ 350/352/354 (3:4:1). Anal. Cal. For $C_{13}H_9ClBrN_5$: C, 44.53; H, 2.59; N, 19.98. Found: C, 44.40; H, 2.50; N, 19.80.

Step-wise solvent-mediated procedure

Synthesis of 3-phenyl-1-(6'-(pyrrolidin-1'"-yl)pyridazin-3'-yl)-1*H*-pyrazol-5-amine **(6a)**

Ethanol medium

1-(6'-Chloropyridazin-3'-yl)-3-phenyl-1*H*-pyrazole-5-amine **(4a)** (0.271g,1mmol) was treated with pyrrolidine (0.070g, 1mmol) in 15ml ethanol and refluxed for about 28hrs, then concentrated to about 1/5th of the volume, cooled, poured into water, solid separated, filtered, washed with water and crystallised from ethanol. The TLC (ethyl acetate/petroleum ether in 1:4) and ^{1}H NMR spectra showed the formation of a single product.

DMF medium

1-(6'-Chloropyridazin-3'-yl)-3-phenyl-1*H*-pyrazole-5-amine **(4a)** (0.271g, 1mmol) was treated with pyrrolidine (0.070g, 1mmol) in 5 ml DMF and refluxed for 30 min, then excess of solvent was distilled off to about ½ of the volume, cooled, poured into water, sticky solid separated, filtered, washed with water and crystallised from ethanol. The TLC (ethyl acetate/petroleum ether in 1:4) and ^{1}H NMR spectra showed the formation of a single product.

One-pot solvent-free procedure

Synthesis of 3-phenyl-1-(6'-(pyrrolidin-1'''-yl)pyridazin-3'-yl)-1*H*-pyrazol-5-amine **(6a)**

Equimolar amounts of 3-chloro-6-hydrazinopyridazine (0.144g, 1mmol) and 3-oxo-3-phenylpropanenitrile (0.145g, 1mmol) were ground with *p*-toulenesulfonic acid (PTSA) (0.344g, 2mmol) in mortar and pestle. The reaction was monitored on TLC (ethyl acetate/petroleum ether in 1:4) at regular intervals and indicated that reaction is complete in 5 min. Once the synthesis of **4a** accomplished, then *in situ* addition of pyrrolidine (0.070, 1mmol) was undertaken. The reaction mixture ground for another 15–20 min. and the spot corresponding **4a** disappeared and a new spot corresponding to **6a** appeared. Then ethanol is added to the reaction mixture, left overnight, solid separated, filtered through suction, washed with water and crystallised in alcohol. ^{1}H NMR spectra showed the formation of a single product.

Similarly, compounds **6b-e** and **7,8 a-e** with secondary amines piperidine **5b** and morpholine **5c** respectively, have been synthesized following the above procedure.

3-Phenyl-1-(6'-(pyrrolidin-1'''-yl)pyridazin-3'-yl)-1*H*-pyrazol-5-amine (**6a**)

Yield 88.8 %; R_f = 0.15; M.Pt. 187 °C; IR (KBr, cm^{-1}): 3317 (symm.) and 3425 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.08 (quintet, 4H, J = 3.1 Hz, 3''', 4'''-H); 3.57 (t, 4H, J = 6.6 Hz, 2''', 5'''-H); 5.86 (s, 1H, 4-H); 5.88 (s, 2H, -NH_2); 6.86 (d, 1H, J = 9.7 Hz, 5'-H); 7.32 (m, 1H, 4''-H); 7.40 (t, 2H, J = 7.2 Hz, 3'', 5''-H); 7.82 (d, 2H, J = 7.1 Hz,2'', 6''-H); 8.13 (d, 1H, J = 9.7 Hz, 4'-H); Ms: *m/z* [M + 1]$^+$ 307. Anal. Cal. For $C_{17}H_{18}N_6$: C, 66.65; H, 5.92; N, 27.43. Found: C, 66.50; H, 5.85; N, 27.30.

1-(6'-(Pyrrolidin-1'''-yl)pyridazin-3'-yl)-3-p-tolyl-1H-pyrazol-5-amine (**6b**)

Yield 89.3 %; R_f = 0.14; M.Pt. > 315 °C; IR (KBr, cm^{-1}): 3279 (symm.) and 3379 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.07 (quintet, 4H, 3''', 4'''-H); 2.37 (s, 3H, -CH_3); 3.56 (t, 4H, J = 6.6 Hz, J = 3.0 Hz, 2''', 5'''-H); 5.83 (s, 1H, 4-H); 5.87 (s, 2H, -NH_2); 6.85 (d, 1H, J = 9.7 Hz, 5'-H); 7.20 (d, 2H, J = 8.0 Hz, 3'', 5''-H); 7.71 (d, 2H, J = 8.1 Hz, 2'', 6''-H); 8.12 (d, 1H, J = 9.7 Hz, 4'-H); MS: *m/z* [M + 1]$^+$321. Anal. Cal. for $C_{18}H_{20}N_6$: C, 67.48; H, 6.29; N, 26.23. Found: C, 51.25; H, 4.53; N, 18.09.

3-(4''-Fluorophenyl)-1-(6'-(pyrrolidin-1'''-yl)pyridazin-3'-yl-1H-pyrazol-5-amine (**6c**)

Yield 89.8 %; R_f = 0.16; M.Pt. 218 °C; IR (KBr, cm^{-1}): 3310 (symm.) and 3425 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.08 (quintet, 4H, J = 3.1 Hz, 3''', 4'''-H); 3.56 (t, 4H, J = 6.5 Hz, 2''', 5'''-H); 5.81 (s, 1H, 4-H); 5.90 (s, 2H, -NH_2); 6.86 (d, 1H, J = 9.7 Hz, 5'-H); 7.08 (t, 2H, J = 6.7 Hz, 3'', 5''-H); 7.79 (m, 2H, 2'', 6''-H); 8.10 (d, 1H, J = 9.7 Hz, 4'-H); MS: *m/z* [M + 1]$^+$ 325. Anal. Cal. for $C_{17}H_{17}FN_6$: C, 62.95; H, 5.28; N, 25.91. Found: C, 62.80; H, 5.05; N, 25.80.

3-(4''-Chlorophenyl)-1-(6'-(pyrrolidin-1'''-yl)pyridazin-3'-yl-1H-pyrazol-5-amine (**6d**)

Yield 92.8 %; R_f = 0.15; M.Pt. 210 °C; IR (KBr, cm^{-1}): 3325 (symm.) and 3433 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.00 (s, 4H, 3''', 4'''-H); 3.49 (s, 4H, 1''', 2'''-H); 5.75 (s, 1H, 4-H); 5.83 (s, 2H, -NH_2); 6.79 (s, 1H, 5'-H); 7.24 (s, 2H, 3'', 5''-H); 7.59 (s, 2H, 2'', 6''-H); 8.01 (s,1H, 4'-H), MS: *m/z* [M + 1]$^+$341/343 (3:1). Anal. Cal. for $C_{17}H_{17}ClN_6$: C, 59.91; H, 5.03, N, 24.66. Found: C, 59.80; H, 4.95; N, 24.50.

3-(4''-Bromophenyl)-1-(6'-(pyrrolidin-1'''-yl)pyridazin-3'-yl-1H-pyrazol-5-amine (**6e**)

Yield 93.9 %; R_f = 0.17; M.Pt. > 315 °C; IR (KBr, cm^{-1}): 3325 (symm.) and 3433 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.08 (quintet, 4H, J = 3.8 Hz, 3''', 4'''-H); 3.56 (t, 4H, J = 6.4 Hz, 1''', 2'''-H); 5.82 (s, 1H, 4-H); 5.90 (s, 2H, -NH_2); 6.85 (d, 1H, J = 9.6 Hz, 5'-H); 7.51 (d, 2H, J = 8.4 Hz, 3'', 5''-H); 7.69 (d, 2H, J = 8.4 Hz, 2'', 6''-H); 8.08 (d,2H, J = 9.6 Hz, 4'-H), MS: *m/z* [M + 1]$^+$ 341/343. $C_{17}H_{17}BrN_6$: C, 53.00; H, 4.45; N, 21.81. Found: C, 52.95; H, 4.30; N, 21.75.

3-Phenyl-1-(6'-(piperidin-1'''-yl)pyridazin-3'-yl)-1H-pyrazol-5-amine (**7a**)

Yield 90.9 %; R_f = 0.44; M.Pt. > 315 °C; IR (KBr, cm^{-1}): 3325 (symm.) and 3425 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70 (s, 6H, 3''', 4'''-H); 3.62 (s, 4H, 2''', 6'''-H) 5.86 (s, 1H, 4-H); 5.89 (s, 2H, -NH_2); 7.14 (d, 1H, J = 9.8 Hz, 5'-H); 7.32 (m,1H, 4''-H); 7.40 (t, 2H, J = 7.2 Hz, 3'', 5''-H); 7.82 (d, 2H, J = 7.1 Hz, 2'', 6''-H); 8.12 (d, 1H, J = 9.8 Hz, 4'-H); MS: *m/z* [M + 1]$^+$ 321. Anal. Cal. for $C_{18}H_{20}N_6$: C, 67.48; H, 6.29; N, 26.23. Found: C, 67.30; H, 6.00; N, 26.10.

1-(6'-(Piperidin-1'''-yl)pyridazin-3'-yl)-3-p-tolyl-1H-pyrazol-5-amine (**7b**)

Yield 97.1 %; R_f = 0.45; M.Pt. 221 °C; IR (KBr, cm^{-1}): 3317 (symm.) and 3425 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70 (s, 6H, 3''', 4''', 5'''-H); 2.37 (s, 3H, -CH_3); 3.62 (s, 4H, 2''',6'''-H); 5.83 (s, 1H, 4-H); 5.87 (s, 2H, -NH_2); 7.14 (d, 1H, J = 9.9 Hz, 5'-H); 7.20 (d, 2H, J = 8.0 Hz, 3'', 5''-H); 7.70 (d, 2H, J = 8.1 Hz, 2'', 6''-Hz); 8.11 (d, 1H, J = 9.9 Hz, 4'-H); MS: *m/z* [M + 1]$^+$335. Anal. Cal. for $C_{19}H_{22}N_6$: C, 68.24; H, 6.63; N, 25.13. Found: C, 55.22; H, 4.40; N, 20.06.

3-(4''-Fluorophenyl)-1-(6'-(piperidin-1'''-yl)pyridazin-3'-yl-1H-pyrazol-5-amine (**7c**)

Yield 87.4 %; R_f = 0.43; M.Pt. > 315 °C; IR (KBr, cm^{-1}): 3256 (symm.) and 3364 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70 (s, 6H, 3''', 4''', 5'''-H); 3.62 (s, 4H, 2''', 6'''-H); 5.80 (s, 1H, 4-H); 5.91 (s, 2H, -NH_2); 7.08 (t, 2H, J = 8.5 Hz, 3'', 5''-H);7.15 (d, 1H, J = 9.9Hz, 5'-H); 7.79 (m, 2H, 2'', 6''-H); 8.09 (d, 1H, J = 9.5 Hz, 4'-H); MS: *m/z* [M + 1]$^+$ 339. Anal. Cal. for $C_{18}H_{19}FN_6$: C, 63.89; H, 5.66; N, 24.84. Found: C, 63.75; H, 5.50; N, 24.50.

3-(4''-Chlorophenyl)-1-(6'-(piperidin-1'''-yl)pyridazin-3'-yl-1H-pyrazol-5-amine (**7d**)

Yield 91.9 %; R_f = 0.44; M.Pt. 207 °C; IR (KBr, cm^{-1}): 3286 (symm.) and 3387 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70

(s, 6H, 3‴, 4‴, 5‴-H); 3.63 (s, 4H, 2‴, 6‴-H); 5.82 (s, 1H, 4-H); 5.89 (s, 2H, -NH_2); 7.13 (d, 1H, J = 9.8 Hz, 5'-H); 7.37 (d, 2H, J = 6.7 Hz, 3", 5"-H); 7.74 (d, 2H, J = 6.6 Hz, 2", 6"-H); 8.08 (d, 1H, J = 9.8 Hz, 4'-H); MS: *m/z* $[M+1]^+$ 355/357 (3:1). Anal. Cal. for $C_{18}H_{19}ClN_6$: C, 60.93; H, 5.40; N, 23.68. Found: C, 60.50; H, 5.30; N, 23.50.

3-(4"-Bromophenyl)-1-(6'-(piperidin-1‴-yl)pyridazin-3'-yl-1H-pyrazol-5-amine (**7e**)

Yield 86.5 %; R_f = 0.42; M.Pt. 224.5 °C; IR (KBr, cm^{-1}): 3402 (symm.) and 3294 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70 (s, 6H, 3‴, 4‴, 5‴-H); 3.63 (s, 4H, 2‴, 6‴-H); 5.82 (s, 1H, 4-H); 5.90 (s, 2H, -NH_2); 7.14 (d, 1H, J = 9.8Hz, 5'-H); 7.52 (d, 2H, J = 8.4 Hz, 3", 5"-H); 7.60 (d, 2H, J = 8.4 Hz, 2", 6"-H); 8.08 (d, 1H, J = 9.7 Hz, 4'-H); MS: *m/z* $[M+1]^+$ 400/402 (1:1). Anal. Cal. for $C_{18}H_{19}BrN_6$: C, 54.14; H, 4.80; N, 21.05. Found: C, 54.00; H, 4.58; N, 21.00.

1-(6'-Morpholinopyridazin-3'-yl)-3-phenyl-1H-pyrazol-5-amine (**8a**)

Yield 84.7 %; R_f = 0.19; M.Pt. 170.5 °C; IR (KBr, cm^{-1}): 3279 (symm.) and 3410 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.60 (t, 4H, J = 4.7 Hz, 3‴,5‴-H); 3.87 (t, 4H, J = 5.0 Hz,2‴,6‴-H); 5.87 (s, 1H, 4-H); 5.90 (s, 2H, -NH_2); 7.13 (d, 1H, J = 9.8 Hz, 5'-H); 7.33 (m, 1H, 4"-H); 7.40 (t, 2H, J = 7.7 Hz, 3", 5"-H); 7.82 (d, 2H, J = 7.2 Hz, 2", 6"-H); 8.19 (d, 1H, J = 9.8 Hz, 4'-H); MS: *m/z* $[M+1]^+$ 323. Anal. Cal. for $C_{17}H_{18}N_6O$: C, 63.34; H, 5.63; N, 26.07. Found: C, 63.20; H, 5.30; N, 25.95.

1-(6'-Morpholinopyridazin-3'-yl)-3-p-tolyl-1H-pyrazol-5-amine (**8b**)

Yield 79.5 %; R_f = 0.18; M.Pt. 214 °C; IR (KBr, cm^{-1}): 3295 (symm.) and 3401 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.39 (s, 3H, -CH_3); 3.60 (t, 4H, J = 4.7 Hz, 3‴,5‴-H); 3.87 (t, 4H, J = 5.1 Hz, 2‴, 6‴-H); 5.85 (s, 1H, 4-H); 5.88 (s, 2H, -NH_2); 7.13 (d, 1H, J = 9.8 Hz, 5'-H); 7.20 (d, 2H, J = 7.9 Hz, 3", 5"-H); 7.71 (d, 2H, J = 8.0 Hz, 2", 6"-H); 8.19 (d, 1H, J = 9.8 Hz, 4'-H); MS: *m/z* $[M+1]^+$ 337. Anal. Cal. for $C_{18}H_{20}N_6O$: C, 64.27; H, 5.99; N, 24.98. Found: C, 59.61; H, 4.71; N, 22.12.

3-(4"-Fluorophenyl)-1-(6'-morpholinopyridazin-3'-yl)-1H-pyrazol-5-amine (**8c**)

Yield 80.7 %; R_f = 0.17; M.Pt 220 °C; IR (KBr, cm^{-1}): 3402 (symm.) and 3294 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.60 (t, 4H, J = 4.7 Hz, 3‴,5‴-H); 3.87 (t, 4H, J = 5.1 Hz, 2‴,6‴-H); 5.81 (s,1H, 4-H); 5.92 (s, 2H, -NH_2); 7.08 (t, 2H, J = 6.7 Hz, 2", 6"-H); 7.14 (d, 1H, J = 9.8 Hz, 5'-H); 7.79 (m, 2H, 3", 5"-H); 8.17 (d, 1H, J = 9.8 Hz, 4'-H); MS: *m/z* $[M+1]^+$ 341. Anal. Cal. for $C_{17}H_{17}FN_6O$: C, 59.99; H, 5.03; N, 24.69. Found: C, 59.69; H, 4.98; N, 24.55.

3-(4"-Chlorophenyl)-1-(6'-morpholinopyridazin-3'-yl)-1H-pyrazol-5-amine (**8d**)

Yield 84.0 %; R_f = 0.19; M.Pt. >315 °C; IR (KBr, cm^{-1}): 3294 (symm.) and 3402 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.53 (t, 4H, J = 4.7 Hz, 3‴,5‴-H); 3.80 (t, 4H, J = 5.0 Hz, 2‴,6‴-H); 5.75 (s,1H, 4-H); 5.84 (s, 2H, -NH_2); 7.05 (d, 1H, J = 9.8 Hz, 5'-H); 7.30 (d, 2H, J = 8.5 Hz, 3", 5"-H); 7.67 (d, 2H, J = 8.4 Hz, 2", 6"-H); 8.08 (d, 1H, J = 9.8 Hz, 4'-H); MS: *m/z* $[M+1]^+$ 357/359 (3:1). Anal. Cal. for $C_{17}H_{17}ClN_6O$: C, 57.22; H, 4.80; N, 23.55. Found: C, 57.00; H, 4.58; N, 23.40.

1-(6'-morpholinopyridazin-3'-yl)-3-(4"-bromophenyl)-1H-pyrazol-5-amine (**8e**)

Yield 80.8 %; R_f = 0.18; M.Pt. >315 °C; IR (KBr, cm^{-1}): 3286 (symm.) and 3418 (asymm.) NH strech; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.61 (t, 4H, J = 4.7 Hz, 3‴,5‴-H); 3.87 (t, 4H, J = 5.0 Hz, 2‴,6‴-H); 5.83 (s,1H, 4-H); 5.91 (s, 2H, -NH_2); 7.13 (d, 1H, J = 9.8 Hz, 5'-H); 7.52 (d, 2H, J = 8.4 Hz, 3", 5"-H); 7.69 (d, 2H, J = 8.4 Hz, 2", 6"-H); 8.16 (d, 1H, J = 9.8 Hz, 4'-H); MS: *m/z* $[M+1]^+$ 402/404 (1:1). Anal. Cal. for $C_{17}H_{17}ClN_6O$: C, 50.89; H, 4.27; N, 20.94. Found: C, 50.45; H, 4.12; N, 20.85.

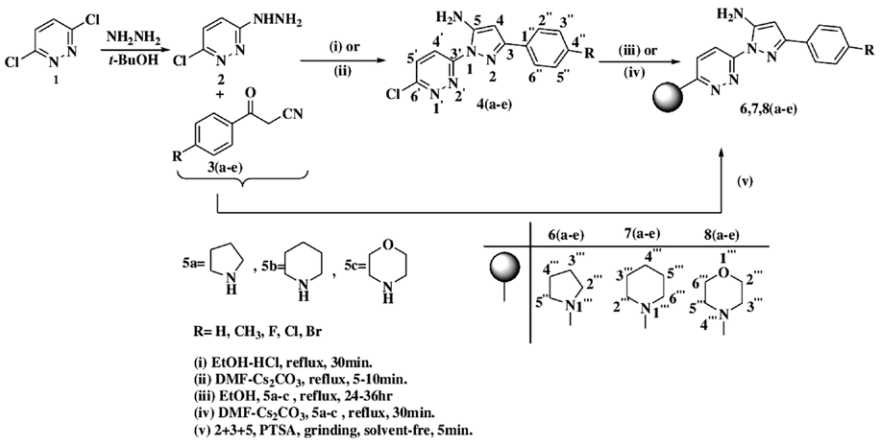
Molecular docking methodology

Analgesic effect of titled compounds can be understood by studying the interaction between the ligand and the protein receptor involved. A docking simulation study is the best way for understanding drug-receptor interaction that helps in drug discovery. Molecular docking has been performed using Algorithm genetic method of the AutoDock 4.0 [31]. Openbabel software tool was employed for converting all the mol, 2D structures of ligand molecules were converted into pdb, 3D format. To get an insight into the active residues having a vital role in an analgesic activity, the newly synthesized compounds were docked against target protein (PDB ID: 6COX) [32]. The protein, Cyclooxygenase-2 complexed with a selective inhibitor; SC-558, (PDB 6COX) was obtained from protein data bank (www.rcsb.org). The water of crystallization was removed while polar hydrogens and Gasteigere partial charges were added. All the parameters were set by default during docking analysis. The grid box was set to get the region of interest, active site, in the macromolecule under study. A grid box size of 40.0, 40.0, 40.0 Å was generated having x, y and z coordinates of 47.06, 25.441 and 36.961 respectively with a spacing of 0.375 Å for the crystal structure of COX-2. Docking results were analysed and chimera was used to get the pictorial presentation of drug-receptor interaction [33].

Pharmacology

Acetic-acid induced writhing test

The total number of writhings following intraperitoneal administration of acetic acid solution was (1 %, 10 mL/kg) was recorded over for 15 min, starting 5 min after acetic acid injection. The mice were treated with the test compound (50 mg/kg) or vehicle (tween 80 (5 %)) or standard drug (diclofenac sodium, 50 mg/kg), 30 min before administration of acetic acid and number of writhings and stretchings were recorded and permitted to express the percentage of protection. Calculation of percentage protection was undertaken by considering the following formula: % protection = (Mc-Mt/Mc) × 100; where Mt = Mean writhing of test group and Mc = Mean writhing of a control group and reported values were the average of six determinations SEM of n (6) animals per group. Results were expressed as mean + SEM differences between control and treatment group and were tested using one-way ANOVA followed by least significant differences. The $^*p<0.05$, $^{**}p<0.01$ values are considered as statistically significant or highly significant, respectively [34].



▶ **Fig. 3** Synthesis of 5-amino-3-aryl-1-(6'-chloro/heterocyclicpyridazin-3'-yl)pyrazoles.

Results

The synthesis of 5-amino-3-aryl-1-(6'-chloropyridazin-3'-yl)pyrazoles and their derivatives **4, 6–8 (a-e)** is outlined in ▶ **Fig 3**. The key precursor, 3-chloro-6-hydrazinopyridazine **2**, synthesized by treating 3,6-dichloropyridazine **1** with hydrazine hydrate following the literature procedure [35]. Initially, the reaction of 3-chloro-6-hydrazinopyridazine **2** with β-ketonitriles **3 (a-e)**, obtained by reaction of α-bromoacetophenones with potassium cyanide, was carried out under refluxing ethanol for 30 min. with 2–3 drops of concentrated HCl, which yielded single product 5-amino-3-aryl-1-(6'-chloropyridazin-3'-yl)pyrazoles **4 (a-e)** on ethyl acetate/petroleum ether (1:4) TLC system. Further, the synthesis of 5-amino-3-aryl-1-(6'-heterocyclicpyridazin-3'-yl)pyrazoles **(6–8)** was undertaken by amino-de-halogenation of **4 (a-e)** with cyclic secondary amines, *viz.* pyrrolidine **5a**, piperidine **5b**, morpholine **5c**, under refluxing ethanol for 24–36 hrs. Interestingly, another reaction condition was studied utilising DMF as a solvent with a catalytic amount of Cs_2CO_3 (high polarizability and size than Na_2CO_3 and K_2CO_3), the reaction time decreased drastically; **4 (a-e)** could be accomplished in 5–10 min. and **6–8 (a-e)** in 30 min., however, with low yields and tedious work-up. Hence, to overcome these drawbacks, an eco-friendly approach was applied resulting in minimum reaction time, high yield and easy work-up. Grinding of 3-chloro-6-hydrazinopyridazine **(2)** and β-ketonitrile **(3a-e)** in the presence of *p*-toulenesulfonic acid (PTSA) as a solid catalyst led to the formation of 5-aminopyrazoles **(4a-e)**, which were eventually converted to their 6'-pyrrolidine **(6a-e)**, 6'-piperidine **(7a-e)** and 6'-morpholine **(8a-e)** derivatives after *in situ* addition of cyclic 2° amines, pyrrolidine/piperidine/morpholine **5(a-c)** respectively. A comparative analysis of time for the completion of reaction under different reaction conditions is depicted in ▶ **Table 1**. The structure and purity of all the synthesized compounds were confirmed by TLC and corresponding spectral data (IR, MS, ^1H, ^{13}C) and elemental analysis.

The presence of amino group in compounds **4,6,7,8 (a-e)** was confirmed by IR spectra, which showed two sharp absorption bands in the range of 3252–3560 cm^{-1} (symm.) and 3356–3610 cm^{-1} (asymm.) due to NH_2 stretching.

▶ **Table 1** Comparative analysis of reactions under different reaction conditions.

Sr. No	Medium	Reaction time (4a–e)	Reaction time (6,7,8a–e)	Overall Yeild %
1	EtOH	30 min	24–36 hrs	55–81
2	DMR	5–10 min	30 min	35–40
3	Solvent-free	5 min (*in-situ*)	15–20 min	82–84

The ^1H NMR spectra also helped in recognising the NH_2 group in **4,6,7,8 (a-e)** as it displayed a characteristic singlet integrating for two protons at δ 5.84–6.96 ppm. Besides two doublets appearing for pyridazine protons 4'-H at δ 8.32–8.35 ppm and 5'-H at δ 7.58–7.97 ppm with coupling constant ^3J~ 9.3 Hz, a characteristic sharp singlet for 4-H pyrazole appeared at δ 5.83–5.92 ppm of compounds **4 (a-e)**. Nucleophilic substitution of chlorine by cyclic 2° amine resulted into an upfield shift in **6,7,8 (a-e)** and the signal appeared at δ 5.75–5.87, 6.79–7.15 ppm and 8.01–8.19 ppm for 4, 5' and 4'-H respectively. Besides, ^1H NMR of **6, 7** and **8** exhibited the characteristic splitting pattern of pyrrolidine, piperidine, morpholine in the aliphatic region.

Moreover, the structure of the compounds was supported by ^{13}C NMR spectroscopy and the signals appeared at δ 150, 85–87 and 151–152 ppm corresponding to C-3, C-4 and C-5 of the pyrazole ring, respectively. These values are following the literature [36]. The complete assignment of the signals in the ^{13}C NMR spectra of compounds **4,6,7,8 (a-e)** is given in ▶ **Tables 2** and ▶ **3**.

Discussion

Molecular docking study was performed to understand the interaction of potential sites of receptor protein with synthesized ligands. Aiming at the goal, 6COX was used to get an insight into the interaction pattern of ligands with COX-2 receptor. It was observed that N-atom present in synthesized series forms H-bond with various amino acid residues. Most common interacting residues were ASN375.A, ASN375.B and ASN537.B. Candidates **4d** and **7c** were docked into the active site of COX-2 and showed the same orien-

▶ **Table 2** C-13 NMR of compounds **4 (a-e)**.

Carbon	4a	4b	4c	4d	4e
C-3	149.9	149.8	150.0	150.8	150.0
C-4	87.5	87.4	87.3	85.2	87.3
C-5	152.7	152.6	152.8	152.0	152.9
C-3′	157.2	157.2	164.5	156.9	157.2
C-4′	130.6	130.5	121.6	131.3	130.6
C-5′	121.6	121.7	115.5	121.8	121.6
C-6′	154.2	154.3	153.3	152.2	153.1
C-1″	132.6	138.7	115.7	131.2	122.8
C-2″/6″	125.9	125.9	127.7	127.1	127.5
C-3″/5″	128.6	129.3	127.8	128.4	131.8
C-4″	128.8	129.7	130.6	133.2	131.5
CH_3		21.4			

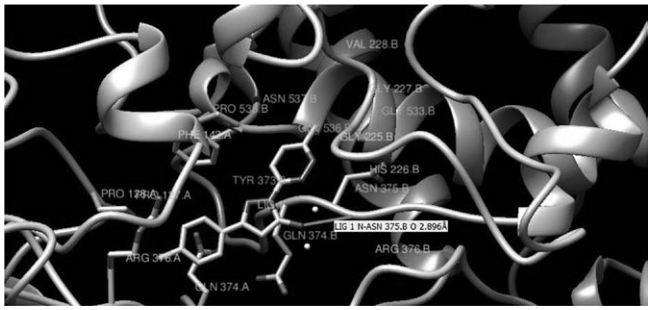
tation and binding mode to that of SC-588 (selective COX-2 inhibitor). In the binding mode, amino N of compound **4d** and pyridazine N of compound **7c** was potently bound to the active binding site of 6COX forming hydrogen bond with ASN375.B with dock score -8.61 and -8.23 kCal/mol as shown in ▶ **Figs. 4** and ▶ **5** respectively. Similarly, internal ligand, SC-558 was bound to the binding pocket of COX-2 with dock score -8.67 kCal/mol, also formed a hydrogen bond with ASN375.A as displayed in ▶ **Fig. 6**. The data of dock score and interactive amino acids of all the compounds of the series is provided in ▶ **Table 4**. Results from docking simulations justify the potential of synthesized series as an analgesic and very well correlated with *in vivo* study.

A screening protocol was used to select the structures from all the synthesized compounds possessing analgesic action and evaluation was undertaken by acetic-acid induced writhing test on swiss albino mice. The visceral pain induced by acetic-acid is due to the release of arachidonic acid *via* cyclooxygenase and prostaglandin (PG). A careful examination of the data in ▶ **Table 5** shows that all compounds, except **6b**, **8b**, **6d**, **8d** exhibited moderate-to-good analgesic action.

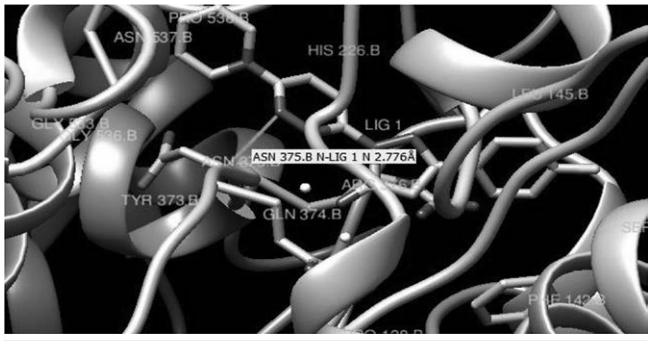
It is inferred from ▶ **Table 5** that analgesic potency estimated by classical acetic-acid induced constrictions/writhings for certain compounds **(8a, 4b, 4,7,8c, 4d)** is comparatively equal or highly related to reference/standard drug (s) sodium diclofenac. According to SAR study, considering fixed group present on 6'-position of pyridazine ring and variation is made around the R group present on *p*-position of phenyl framework (shown in ▶ **Fig. 7**). Antinociceptive action of 5-amino-3-aryl-1-(6'-chloropyridazin-3'-yl)pyrazoles (**4a-4e**) follows the sequence **4d > 4b > 4c > 4a > 4e**, which shows the enhancement of analgesic activity when Me, F, Cl group is present at *p*-position of phenyl group whereas Br group cause a decline. Candidates **4b** and **4d**, registered a noteworthy analgesic profile which is closest to standard reference drug (sodium diclofenac). Further, the sequence followed by 3-aryl-1-(6'-(pyrrolidin-1'''-yl)pyridazin-3'-yl)-1*H*-pyrazol-5-amines (**6a-6e**) for antinociceptive action is **6a > 6e > 6d > 6c > 6b**, which exhibit a decline in analgesic activity when phenyl group is substituted by Me, F, Cl, Br groups. Moreover, antinociceptive profile of 3-aryl-1-(6'-(piperidin-1'''-yl)pyridazin-3'-yl)-1*H*-pyrazol-5-amines (**7a-7e**) and 3-aryl-1-(6'-(morpholin-1'''-yl)pyridazin-3'-yl)-1*H*-pyrazol-5-amines

▲ **Table 3** C-13 NMR of compounds **6,7,8 (a-e)**.

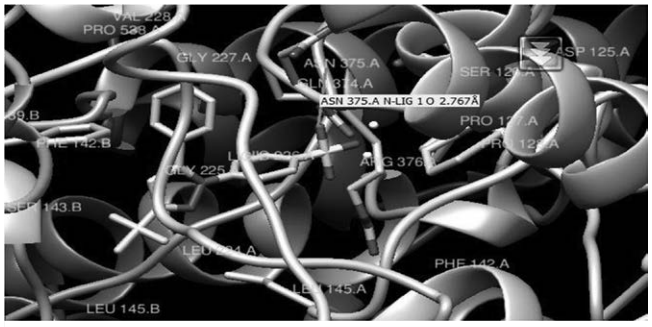
Carbon	6a	6b	6c	6d	6e	7a	7b	7c	7d	7e	8a	8b	8c	8d	8e
C-3	148.8	148.7	148.9	148.9	148.9	148.9	148.9	149.0	149.0	148.0	149.0	149.0	149.1	149.2	149.2
C-4	87.1	87.0	86.9	87.0	86.9	87.2	87.0	86.9	87.0	85.9	87.2	87.1	87.0	87.1	87.1
C-5	151.0	151.0	155.8	151.0	150.7	151.6	151.7	158.2	158.0	149.9	152.5	152.5	158.2	151.3	151.3
C-3′	155.8	155.8	164.0	155.8	155.8	158.2	158.3	164.0	158.3	157.2	158.2	158.1	164.1	158.2	158.2
C-4′	133.4	121.0	121.0	121.0	121.0	121.0	121.2	121.0	121.0	121.0	121.3	121.3	121.2	121.2	121.2
C-5′	115.8	115.8	115.8	115.8	115.8	116.8	116.8	116.8	116.7	115.7	116.5	116.5	116.6	116.5	116.5
C-6′	151.9	152.0	155.9	151.8	150.9	152.1	152.3	161.6	151.5	150.5	152.5	152.6	161.6	152.4	152.4
C-1″	128.5	137.8	129.6	131.9	121.9	133.3	138.0	129.5	131.9	121.0	133.2	138.1	129.4	131.7	122.2
C-2′/6′	121.1	125.6	115.4	127.0	127.3	125.8	125.7	115.4	127.0	126.3	125.8	125.7	115.5	127.0	127.3
C-3′/5″	128.0	129.2	127.4	128.7	131.6	128.5	129.3	127.4	128.7	130.6	128.5	129.2	127.5	128.7	131.6
C-4″	125.7	130.5	150.9	134.9	132.4	128.1	130.5	151.4	133.8	131.3	128.2	130.4	152.0	133.9	132.1
C-2‴/6‴	46.9	46.9	46.9	46.9	46.9										
C-2‴/5‴	25.5	25.5	25.5	25.5	25.5										
C-2‴/6‴						46.7	46.8	46.8	46.7	45.6	66.5	66.5	66.5	66.5	66.5
C-3‴/5‴						24.5	25.5	25.4	25.4	24.3	45.8	45.8	45.8	45.8	45.8
C-4‴						24.5	24.5	24.5	24.5	23.4					
CH_3		21.3					21.4					21.3			



▶ **Fig. 4** Docking pose of compound **4d** with COX-2 receptor (PDB ID: 6COX) showing hydrogen bond.



▶ **Fig. 5** Docking pose of compound **7c** with COX-2 receptor (PDB ID: 6COX) showing hydrogen bond.



▶ **Fig. 6** Docking pose of Internal ligand, SC-558 with COX-2 receptor (PDB ID: 6COX) showing hydrogen bond.

(**8a-8e**) follows the order: **7c > 7a > 7b > 7e > 7d** and **8c > 8a > 8e > 8d > 8b** respectively, exhibiting decrease in analgesic action by Me, Cl, Br groups present at *p*-position of phenyl framework and **8c** increment in analgesic action is comparable to reference drug.

▶ **Fig. 7** Graph representing % protection versus number of compounds and % protection obtained for compounds **4d**, **7c** comparable to reference/standard drug (s) sodium diclofenac.

Hence, analgesic study showed that candidates **(8a = 51.13 %, 4b = 57.59 %, 4c = 52.89 %, 7c = 59.08 %, 8c = 53.78 %, 4d = 65.01 %)** exhibited their best analgesic action by exerting their effect of action by inhibiting the enzymatic activity of COX and consequently reducing the level of prostaglandin (PGE_2) within the hy-

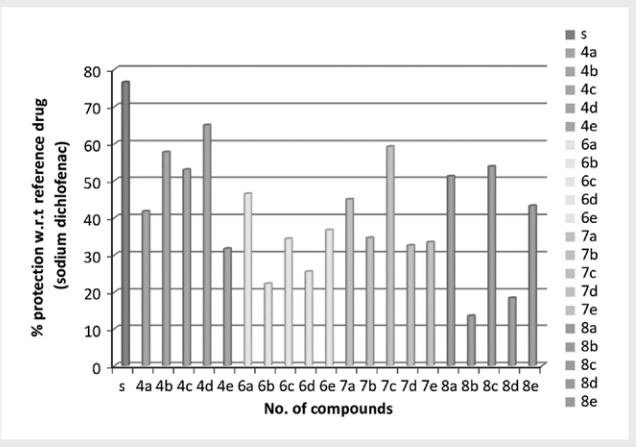
▶ **Table 4** Binding energy of synthesized compounds against COX-2 receptor (PDB ID: 6COX).

S.No	Compound	Docking score	Hydrogen bond	Amio acid
1	4a	−6.47		–
2	4b	−8.01	1	ASN375.B
3	4c	−7.26	1	ASN375.B
4	**4d**	**−8.61**	**1**	**ASN375.B**
5	4e	−6.76	1	ASN375.B
6	6a	−6.23	1	ASN375.B
7	6b	−6.11	1	ASN375.B
8	6c	−6.67	1	ASN375.A
9	6d	−6.23	1	ASN375.A
10	6e	−6.42	1	ASN375.B
11	7a	−6.27	1	ASN375.A
12	7b	−6.68	1	ASN375.A
13	**7c**	**−8.23**	**1**	**ASN375.B**
14	7d	−6.77	1	ASN375.B
15	7e	−6.99	1	ASN375.B
16	8a	−7.11	1	ASN375.B
17	8b	−5.36	–	–
18	8c	−7.94	2	ASN375.B
				ASN375.B
19	8d	−5.46	1	ASN375.B
20	8e	−6.69	1	ASN375.B
21	**SC-558**	**−8.67**	**1**	**ASN375.A**

▶ **Table 5** Analgesic action (percentage protection) of compounds using writhing test.

S.No	Groups	No of wriths	% Protection
1	**Control**	**5.56 ± 0.81**	**–**
2	**Standard(s)**	**13.1 ± 0.10 * ***	**76.5**
3	4a	33.00 ± 2.44 *	41.69
4	4b	24.00 ± 0.60 * *	57.59
5	4c	26.66 ± 0.57 * *	52.89
6	**4d**	**19.8 ± 0.40 * ***	**65.01**
7	4e	38.66 ± 0.17	31.69
8	6a	30.33 ± 0.91 *	46.41
9	6b	44.00 ± 0.7	22.26
10	6c	37.16 ± 0.90 *	34.34
11	6d	42.16 ± 1.4	25.51
12	6e	35.83 ± 1.5	36.69
13	**7a**	**31.16 ± 0.30 ***	**44.94**
14	7b	37.00 ± 0.8	34.62
15	**7c**	**23.16 ± 0.23 * ***	**59.08**
16	7d	38.16 ± 0.31	32.57
17	7e	37.66 ± 0.45	33.46
18	8a	27.66 ± 0.45 * *	51.13
19	8b	49.00 ± 0.5	13.42
20	8c	26.16 ± 0.15 * *	53.78
21	8d	46.16 ± 0.40	18.44
22	8e	32.16 ± 0.55 *	43.18

n = 6, All values are expressed as mean ± SEM of six swiss albino mice in each group. Statistically significant * * $p<0.01$, * $p<0.05$ compared to control.



▶ **Fig. 7** Graph representing % protection versus number of compounds and % protection obtained for compounds **4d, 7c** comparable to reference/standard drug (s) sodium diclofenac.

pothalamic region. Moreover, amination of 5-aminopyrazoles at 6'-position with pyrrolidine, piperidine, morpholine increases the analgesic mode of action in case of Ph, *p*-FPh, *p*-BrPh framework whereas reverse effects were obtained in case of *p*-MePh, *p*-ClPh.

Conclusion

A new series of biologically active compounds: 5-amino-3-aryl-1-(6'-chloro/heterocyclicpyridazin-3'-yl)pyrazoles **4,6,7,8 (a-e)** were synthesised and analgesic profile of synthesized compounds showed moderate-to-good results. Results from docking simulation suggested that compound **4d** and **7c** was the most potent among the series when docked against COX-2 receptor. The docking study investigated a binding mode for synthetic derivatives and provided insights for further inhibitor design. This study drives us to the path towards the development of pyridazine derivatives as lead molecules for further structural optimization as potential analgesic agents. Docking data was very well correlated to the results of *in vivo* study, also, candidates **4d** and**7c** exhibited comparable results regarding standard drug sodium diclofenac when tested with swiss albino mice using the acetic-acid induced writhing assay. Hence, compound **4d** and **7c** could be probed further as analgesic agents with application in the pharmaceutical industry, after testing its toxicity in humans.

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Conflict of Interest

The relationship among the authors (Dr. Ranjana Aggarwal, Swati (research scholar), Dr. Pawan Kaushik, Dr. Ajay Kumar, Dr. Deepika Saini) is purely a scientific collaboration.

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