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Research Article

SYNTHESIS, COMPUTER AIDED SCREENING AND PHARMACOLOGICAL EVALUATION OF 2/3-SUBSTITUTED-6(4-METHYLPHENYL)-4,5-DIHYDROPYRIDAZIN3(2H)-ONES, AND PYRIDAZINE SUBSTITUTED TRIAZINE

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ABSTRACT

The present research work involved synthesis of some new pyridazine derivatives and evaluation of their analgesic and anti-inflammatory activities in experimental animals to obtain safer non-steroidal anti-inflammatory agents (NSAIDs). Friedel-Crafts acylation reaction of succinic anhydride with toluene in the presence of anhydrous aluminum chloride gave 4-(4-methylphenyl)-4-oxo-butanoic acid (**1**). The aryl propionic acid **1** on reaction with phenyl hydrazine and hydrazine hydrate yielded the pyridazinone derivative **2** and **3**, respectively. Reaction of the compound **3** with phosphorus oxychloride ($POCl_3$) produced the corresponding chloropyridazine derivative **4**. A 4-hydroxymethyl derivative of dihydropyridazinone (**5**) was synthesized by condensing **3** with methanol and formaldehyde (HCHO). The compound **5** on further treatment with guanidine hydrochloride in ethanol gave the pyridazino-triazine (**6**). The synthesized compounds were investigated for their analgesic activity in mice and anti-inflammatory activity in Wistar albino rats. The molecular, pharmacokinetic and toxicity properties of the synthesized compounds were calculated by Molinspiration and Osiris property explorer software. The results of *in-vivo* anti-inflammatory studies revealed that the compound. **4** showed maximum inhibition in paw edema volume followed by compound **3** while the compound **4** exhibited excellent peripheral analgesic activity (**74%**) followed by the compound **5**. Compound s **4** and **5** also showed good central analgesic effect increased the reaction time to 90 minutes. All the title compounds except compound **5** are predicted to be safe by Osiris online software and are likely to have good oral bioavailability as they obey Lipinski's rule of five for drug likeness.

Key words: Pyridazinone, Hydrazine derivatives, Chloropyridazine, Triazin-2-imine

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications for the treatment and/or management of pain, fever, and inflammation. However, their long term use is associated with gastroenteropathy such as gastric irritation, ulceration, bleeding and renal toxicity that limit their therapeutic usefulness (Abouzid, *et al.,* 2012). Therefore, the synthesis of new compounds devoid of such side effects poses a challenging task for medicinal chemists. In recent years, synthesis of novel pyridazinone derivatives and investigation of their chemical and biological behavior have gained more importance due to their biological, medicinal, and agricultural reason. This privileged structure attracts the interest of medicinal chemists as a nucleus of potential therapeutic utility and exhibits several pharmacological activities such as analgesic (Asif, *et al.,* 2011), anti-inflammatory (Gokce, *et al.,* 2009), antidepressant (Coelho, *et al.,* 2003), antihypertensive (Demirayak, *et al.,* 2004), anticonvulsant (Rubat, *et al.,* 1990), cardiotonic (Amin, *et al.*, 2010), diuretic (Akahane, *et al.,*

1999), and anti-HIV (Livermone, *et al.,* 1993) activities. Certain pyridazinone derivatives containing the 2-phenyl-indolyl moiety have also shown anti-cancer activity (Murty, *et al.,* 2012). In addition, pyridazinones act as core nucleus in various drugs e.g. Sulmazole, Levosimendan, Amipizone, Indolidan, Imazodan, Pimobendan, Emorfazone, Zardaverine, Milrinone (Alam, *et al.,* 2014). The easy functionalization at various ring positions makes them an attractive synthetic building block for designing, synthesis and discovery of new drugs. The incorporation of this versatile biologically accepted pharmacophore in established medicinally active molecules results in wide range of pharmacological effects.

The currently used anti-inflammatory and analgesic compounds inhibit the synthesis of non selective or selective cyclooxygenases (COX1 & COX2) or prostaglandin, histamine and bradykinin (Sahina, *et al.,* 2004; Dogruer, *et al.,* 2003; Tazkoparan *et al.,* 2000). It has been suggested that prostaglandins and bradykinins play a major role in the analgesia or pain. So it may be predicted that the title compounds may act by inhibiting the synthesis of these chemical mediators involved in causing pain as well as inflammation (Raskin, 1999; McCarthy, 1998). The pyridazinones having characteristic pharmacological features, relative stability and ease of preparation contemplated us to synthesize some new derivatives of pyridazinones following molecular docking studies with a view to explore their potency as good analgesic and anti-inflammatory agents. Since it is impossible to synthesize all the possible compounds and to test all the available ones so molecular modeling makes this approach easier and limits to some fixed number of compounds (Regina, *et al.,* 2008). The compounds which were found to possess high Mol Dock Score and formed maximum number of H-Bond interaction with the receptors were synthesized in the laboratory and evaluated for their anti-inflammatory and analgesic activities.

MATERIAL AND METHODS

Chemistry

Chemicals and solvents were purchased from Sigma Aldrich and Merck for the synthesis of title compounds. All chemical reactions were monitored by thin layer chromatography (TLC) on glass plates coated with silica gel using solvent system Toluene: Ethyl Acetate: Formic acid (5:4:1). Melting points were determined on glass slides using Labindia MR-VIS visual melting range apparatus. Infrared spectra were recorded on Hitachi spectrophotometer and proton nuclear magnetic resonance was recorded on Bruker's 300Hz instrument with DMSO as solvent and TMS as an internal reference standard. Chemical shifts are reported in ä (parts per million values). Mass spectra were scanned on Brukers micrOTOF-QII, ESI Mass spectrophotometer.

*Synthesis of compounds (**2-6**)*

*4-(4-Methylphenyl)-4-oxobutanoic acid (**1**)*

It was synthesized as per the reported method (Asif *et al.*, 2011). Yield 45%, m.p. 104-105ºC, R_f 0.86, IR (KBr, cm^{-1}): $í_{max}$ 1582, 1682 (C=O), 3433 (OH). ^{1}H NMR (DMSO-d_6, ppm): *ä* 2.32 (s, 3H, CH_3), 2.68 (t, 2H, CH_2), 3.25 (t, 2H, CH_2), 7.03-7.06 (d, 1H, Ar), 7.66-7.68 (d, 1H, Ar), 7.86-7.88 (d, 1H, Ar), 8.09-8.11 (d, 1H, Ar), 11.95 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, ppm): *ä* 133.8 (C of C_1), 200.1 (C of C_2), 34.2 (CH_2 of C_3), 30.7 (CH_3 of C_4), 177.3 (C of C_5). EI-MS (*m/z*): 192 [M^+] $C_{11}H_{12}O_3$.

*6-(4-Methylphenyl)-2-phenylpyridazin-3(2H)-one (**2**)*

Required amount of phenyl hydrazine (2.14mmol) was added to a stirred and refluxing solution of compound **1** (2.13mmol) in aldehyde free ethanol (40mL). The reaction mixture was refluxed for 8h under continuous stirring. The reaction mixture was concentrated to half the volume and left overnight in refrigerator for crystallization. Yield 29.3%, m.p. 306ºC, R_f 0.51, IR (KBr, cm^{-1}): $í_{max}$ 1350, 1450, 1589 (Ar, str), 1674 (-C=N), 1713 (C=O), 3387 (Ar-H str). ^{1}H NMR (DMSO-d_6, ppm): *ä* 1.64-1.72 (t, 2H, CH_2), 1.99-2.38 (t, 2H, CH_2), 2.23 (s, 3H, CH_3), 7.11-7.14 (d, 1H, Ar), 7.31-7.34 (m, 3H, Ar), 7.42-7.48 (m, 3H, Ar), 7.88-7.93 (d, 1H, Ar), 7.96-8.05 (d, 1H, Ar). ^{13}C NMR (DMSO-d_6 ppm): *ä* 140.7 (C of C-1), 129.2 (CH of C-2, C-6), 129.1 (CH of C-3, C-5), 131 (C of C-4), 145.3 (C of C-7), 24.5 (CH_2

of C-8), 32.5(CH_2 of C-9), 173 (C of C-10). EI-MS (*m/z*): 264 [M^+] $C_{17}H_{16}N_2O$.

*6-(4-Methylphenyl)-4,5-dihydropyridazin-3(2H)-one (**3**)*

Required amount of hydrazine hydrate (2.14mmol) was added to a stirred and refluxing solution of compound **1** (2.13mmol) in aldehyde free ethanol (40mL). The reaction mixture was refluxed for 5-6h under continuous stirring. The reaction mixture was concentrated to half the volume and left overnight in refrigerator for crystallization. Yield 30.7 %, m.p. 224-226ºC, R_f 0.49, IR (KBr, cm^{-1}): $\acute{\imath}_{max}$ 1643 (-C=N), 1674 (C=O), 3364 (-N-H). ^{1}H NMR (DMSO-d_6, ppm): *ä* 1.63-1.71(t, 2H, CH_2), 1.89-2.33 (t, 2H, CH_2), 2.19 (s, 3H, CH_3), 7.10-7.14 (d, 1H, Ar), 7.34-7.37 (d, 1H, Ar), 7.42-7.46 (d, 1H, Ar), 7.77-7.91 (d, 1H, Ar), 9.20 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, ppm): *ä* 140.7 (C of C-1), 129.2 (C-H of C-2,C-6), 129.1 (C-H of C-3), 131 (C of C-4), 146.6 (C of C-7), 24.2 (CH_2 of C-8), 35.1 (CH_2 of C-9). EI-MS (*m/z*): 188 [M^+] $C_{11}H_{12}N_2O$.

*3-Chloro-6-(4-methylphenyl)-pyridazine (**4**)*

A mixture of compound **3** and $POCl_3$ (10 mL) was refluxed for 5h, cooled and treated with crushed ice. The solid so obtained was filtered off and crystallized from petroleum ether (b.p.80-100ºC). Yield 20%, m.p. 222-224ºC, R_f 0.78, IR (KBr, cm^{-1}): $\acute{\imath}_{max}$, 1342, 1445, 1579 (Ar, str), 1710 (C=O). ^{1}H NMR (DMSO-d_6, ppm): *ä* 1.59-1.70 (t, 2H, CH_2), 1.89-2.17 (t, 2H, CH_2), 2.20 (s, 3H, CH_3), 7.11-7.14 (d, 1H, Ar), 7.31-7.34 (d, 1H, Ar), 7.42-7.46 (d, 1H, Ar), 7.77-7.91 (d, 1H, Ar). ^{13}C NMR (DMSO-d_6, ppm): *ä* 129.2 (CH of C_1), 129.1 (CH of C_2), 140.7 (C of C_6), 131 (C of C_7), 24.3 (CH_3 of C_6). EI-MS (*m/z*): 206 [M^+] $C_{11}H_{11}ClN_2$.

*2-(Hydroxymethyl)-6-(4-methylphenyl)-4,5-dihydropyridazin-3(2H)-one (**5**)*

To a solution of compound **4** (0.001mol) in methanol (30mL), formaldehyde (37-41% aqueous solution) (2.5mL) was added and the mixture was refluxed for 9.5h. After completion of the reaction, methanol was distilled off and the residue was poured into crushed ice to separate out the product, (**5**). It was filtered and crystallized from methanol. Yield: 24%, m.p. 227-229ºC, R_f 0.81, IR (KBr, cm^{-1}): $\acute{\imath}_{max}$ 1677 (C=N), 1680 (C=O), 3411-3421 (b, OH). ^{1}H NMR (DMSO-d_6, ppm): *ä* 1.61-1.68 (t, 2H, CH_2), 2.02-2.28 (t, 2H, CH_2), 2.35 (s, 3H, CH_3), 4.02 (s, 1H, OH), 5.44 (s, 2H, CH_2), 7.14-7.18 (d, 1H, Ar), 7.28-7.33 (d, 1H, Ar), 7.55-7.63 (d, 1H, Ar), 7.72-7.84 (d, 1H, Ar). ^{13}C NMR (DMSO-d_6, ppm): *ä* 129.2 (CH_2 of C_1), 129.1 (CH_2 of C_2), 140.7 (CH of C_5), 146.6 (CH of C_6), 162.2 (C of C_9). EI-MS (*m/z*): 218 [M^+] $C_{12}H_{14}N_2O_2$.

*Preparation of compound 7-(4-methylphenyl)-3,4,8,9-tetrahydro-2H-pyridazino[1,6-á][1,3,5]triazin-2-imine (**6**)*

A mixture of compound **5** (0.001mol) and guanidine hydrochloride (0.001mol) was heated in an oil bath for 30 min, cooled and triturated with ethanol. The whole content was refluxed on a water bath for 8h. After completion of the reaction, ethanol was distilled off and the residue was poured into crushed ice to separate out the compound **6**. The separated solid was filtered and crystallized from ethanol. Yield: 30%, m.p. 185 ºC, R_f 0.69, IR (KBr, cm^{-1}): $\acute{\imath}_{max}$ 1549 (N-H), 1681 (C=N), 2923 (C-H). ^{1}H NMR ($CDCl_3$ ppm): *ä* 1.64-1.72 (t, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.53-2.79 (t, 2H, CH_2), 5.92 (s, 2H, CH_2), 7.16-7.22 (d, 1H, Ar), 7.29-7.35 (d, 1H, Ar), 7.53-7.61 (d, 1H, Ar), 7.80-7.92 (d, 1H, Ar), 8.50 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): *ä* 128.7(CH_2 of C_1), 129.3(CH_2 of C_2), 140.7(CH of C_6), 164(CH of C_7), 23.6(CH_2 of C_{11}), 20.1(CH_2 of C_{12}). EI-MS (*m/z*): 241 [M^+] $C_{13}H_{15}N_5$.

***In vivo* pharmacological activity**

Experimental animals

Male Swiss albino mice weighing 25-30g and Wistar albino rats weighing 180-250g were used for the evaluation of anti-inflammatory and analgesic activities. A prior approval for the study was obtained from Kurukshetra University animals ethics committee (Reg No. 562/GO/02/a/CPCSEA). The animals were fed *ad libitum* with standard laboratory rodent's chow and free access to drinking water. Animals were housed in polypropylene cages (5 per cage) with dust free rice husk as a bedding material under laboratory condition with controlled environment of temperature 25 ± 2°C and 12h Light/dark cycle as per CPCSEA

guidelines. The animals were given a week time to get acclimatized with laboratory conditions.

Determination of anti-inflammatory activity

The anti-inflammatory activity of synthesized compounds was determined using carrageenan induced rat paw edema as per the reported method (Winter, *et al.,* 1962). Rats were weighed, marked/numbered and divided in to seven groups having five animals in each. Group 1 was used for control (10mg/Kg), Group 2 for standard (Indomethacin 10mg/Kg) and Group 3-7 for test drugs (10mg/Kg). A mark was made on the left hind paw near tibio-tarsus junction so that every time the paw was dipped in the 0.5% NaCl solution column up to the fixed mark to ensure measurement of constant paw volume. Initial paw volume of all the animals was recorded. After 30 min. of administration of test and standard drug, carrageenan was injected intraperitoneally into the subplantar region of left hind paw of all animals. The paw volume was measured using a plethysmometer (model 7140, Ugo Basile, Italy) at 0h (before carrageenan injection), 1, 2, 3 and 4h after drug treatment (Vogel, 2002). The anti-inflammatory effect of synthesized compounds i.e. percent inhibition of inflammation (edema) was calculated by the following equation:

$$\% \text{ inhibition} = \frac{(Vc-Vt)}{Vc} \times 100$$

Where Vt is the paw volume in drug treated animals and Vc is the paw volume of control group of animals.

Determination of analgesic activity

The central and peripheral analgesic activity of the title compounds was studied by writhing tests and tail immersion method, respectively (Collier, *et al.,*, 1968; Seigmund, *et al.,* 1957).

Writhing test

Swiss albino mice of either sex weighing between 20-25g were used in the study. Acetic acid in a concentration of 1% v/v and 10mL/kg/ *i.p.* was administered to induce writhing. Seven groups having five animals in each were used for the study. Group 1 served as control (10mL/Kg), Group 2 received standard drug (Indomethacin 10mg/kg) and Group 3-7 were administered test compounds (10 mg/Kg). Group I was administered acetic acid solution alone and served as negative control. The test and standard groups of animals (groups 2-7) were administered the test compounds and the standard drug 30 min prior to acetic acid administration respectively. The mice were then observed for ten minutes and the numbers of writhing for each animal were recorded during that time period. For scoring purposes, writhing was indicated by stretching of the abdomen with simultaneously stretching of at least one hind limb.

Tail immersion method

Mice were held in position in a suitable restrainer with the tail protruding out. The tail up to 5 cm was dipped in a beaker of water at 55°C. All the dose of standard and test drugs (10mg/kg body weight) was prepared in 0.5% w/v Tween-80 which served as drug vehicle and administered orally. Indomethacin, (10mg /kg body weight i.e. 28g) was used as a reference drug for comparison purpose. The time taken by the mice to withdraw the tail clearly out of water was recorded as the reaction time. The reaction time was measured after 30, 60, 90, and 120min, respectively.

Calculation of pharmacokinetic parameters and toxicity potential

Molecular properties were calculated by using online Molinspiration software version 2011.06 (www.molinspiration.com) to evaluate the oral bioavailability and drug likeness of the synthesized compounds (Lipinski, *et al.*, 1997). The calculated properties were also compared with the reference drugs, indomethacin.

Pharmacokinetic parameters such as toxicity potential, solubility and overall drug-likeness of pyridazine compounds were predicted by using Osiris property explorer (www.organicchemistry.org/prog/peo/). The results of computer aided screening are valued and color coded green, yellow or red for properties such as effect on reproductive system, irritant effect, mutagenicity and tumorigenicity. High toxicity risks are shown in red color, while green color indicates a drug-conform behavior, and safety *in vivo*.

Statistical analysis

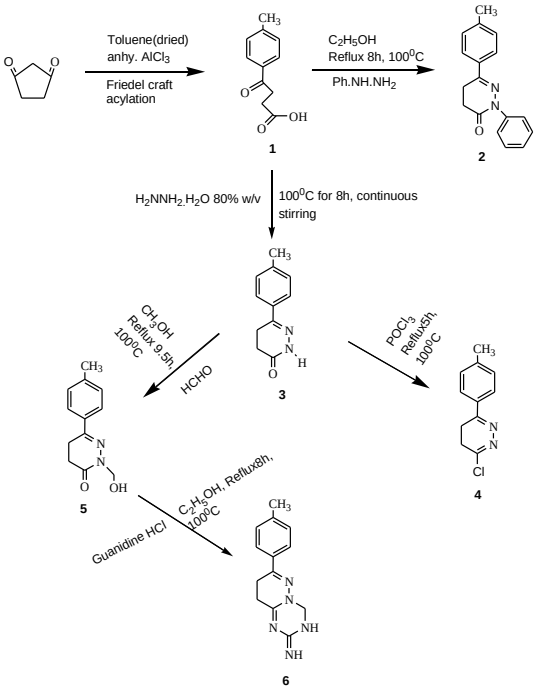
The statistical analysis was performed using GRAPHPAD INSTAT 3 software (Graph Pad Software Inc., San Diego, CA). Data obtained from animal experiments were expressed as arithmetic mean ±SEM. The comparison between various groups was performed by one-way analysis of variance (ANOVA) where $p<0.05$ was considered to be statistically significant (*$p<0.01$, **$p<0.05$ compared to control).

RESULT AND DISCUSSION

Chemistry

Pyridazine is an important heterocyclic scaffold for designing medicinal agents with varying biological actions. Pyridazinone, being an easy target for functionalization at various ring positions, makes it an interesting and attractive pharmacophore for the development of new and potent agents in anti-inflammatory therapy. A large number of substituted pyridazinone derivatives possessing analgesic activity along with other useful pharmacological properties have been reported (Gokce, *et al.,* 2009). Emorfazone (4-ethoxy-2-methyl-5-morpholino-3(2*H*)-pyridazinone) is a pyridazinone derivative which is currently used clinically in the management of pain and inflammation. Piaz *et al.,* in 1996 reported the synthesis and antinociceptive activity of 4-amino-2-methyl-6-phenyl-5-vinyl-3(*2H*)-pyridazinone which was found to be many fold potent than the emorfazone (Piaz, *et al.,* 1996). Prompted by these findings, a novel series of 2/3-substituted-6(4-methylphenyl)-4,5-dihydropyridazin3(*2H*)-ones and Pyridazine substituted Triazine have been synthesized. Synthetic routes were developed that allow efficient preparation of analogues with varied substitutions on the pyridazinone ring.

In the present study various compounds incorporating a 3(*2H*)-pyridazinone ring were synthesized as outlined in **Scheme I**. Friedel-Crafts acylation reaction of succinic anhydride with toluene in the presence of anhydrous aluminum chloride, gave 4-(4-methylphenyl)-4-oxo-butanoic acid (aryl propionic acid) **1**. The acid **1** with hydrazine derivatives produced the Pyridazinone derivative **2** and **3**. Reaction of the compound **3** with phosphorus oxychloride ($POCl_3$) gave the chloropyridazine derivative **4**. Reaction of the **3** with methanol (CH_3OH) and formaldehyde (HCHO) resulted in formation of 4-hydroxymethyl derivative of dihydropyridazinone **5**. The compound **5** on reaction with guanidine hydrochloride and ethanol yielded the compound **6**. Purity of compounds was checked by a single-spot TLC in solvent system toluene: ethyl acetate: formic acid (5:4:1). The spots on TLC were located under iodine vapors/UV light. Compounds were characterized on the basis of spectral data (IR, ^1H NMR, ^{13}C NMR and Mass spectrometry).



Scheme I: Synthesis of 2/3-substituted-6(4-methylphenyl)-4,5-dihydropyridazin3(*2H*)-ones and Pyridazine substituted Triazine

Spectral data of all the newly synthesized compounds were in full agreement with the proposed structures. In general, Infra Red spectra (IR) of title compounds showed presence of functional groups viz. C=N, C=O, N-H, O-H, at 1643-1677, 1674-1713, 3364, 3411-3421, cm^{-1}, respectively. The ^1H NMR spectra of the compounds exhibited the signal in the form of a singlet near *ä* 2.19-2.40 for tolyl CH_3 protons. The two methylene protons ($-CH_2$) in pyridazine ring appeared as triplet in the region *ä* 1.59-1.72 and *ä* 1.89- 2.79.

Table 1: Effect of compounds on carrageenan induced Paw edema volume in rats.

S. No	Treatment	Paw edema volume Mean±SEM (% inhibition)			
		30 min.	60 min	90 min.	120 min.
1	Control	0.4±0.02	0.7±0.02	0.7±0.0*	0.9±0.01*
2	Standard(a)	0.3±0.02(10%)	0.4±0.02*(36%)	0.5±0.0*(36%)	0.5±0.0 (46%)
3	2	0.5±0.01(17%)	0.4±0.05*(33%)	0.5±0.0*(36%)	0.5±0.02*(43%)
4	3	0.3±0.02(15%)	0.3±0.01*(44%)	0.4±0.08*(45%)	0. 5±0.03*(47%)
5	4	0.3±0.02(13%)	0.3±0.01*(44%)	0.4±0.08*(45%)	0. 5±0.03*(47%)
6	5	0.3±0.03(12%)	0.5±0.02*(30%)	0.5±0.01*(35%)	0. 7±0.01*(29%)
7	6	0.5±0.02(15%)	0.5±0.03*(24%)	0.5±0.0*(33%)	0. 6±0.02*(39%)

Values are expressed as mean ±SEM (N=5); a – Indomethacin (10 mg/kg, i.p.) used as a standard drug; *p<0.01 compared with vehicle control, **p<0.05 compared with vehide control (ANOVA).

An additional singlet of two protons at *ä* 5.92 in the spectrum of compound **6** was ascribed to –CH_2 group of triazine ring. Compound **3** and compound **6** showed a broad singlet at *ä* 8.5-9.2 due to NH protons. Aromatic protons were observed in the region ranging from *ä* 7.11-8.05. The ^{13}C NMR spectrum showed the signal with *ä* 140.7 at C-1, *ä* 129.2 at C-2, C-6, *ä* 129.1 at C-3, C-5, *ä* 131 at C-4, *ä* 145.3 at C-7, *ä* 24.5 at C-8, *ä* 32.5 at C-9, *ä* 173 at C-10. The molecular ion peak (M^+) for all the synthesized compounds was also obtained in mass spectra and was of good intensity.

Pharmacological evaluation

Anti-inflammatory activity

The synthesized compounds were screened for *in- vivo* anti-inflammatory activity by a commonly used experimental animal method of carrageenan induced rat paw edema (Winter, *et al.,* 1962). The edema induced by carrageenan involves release of various inflammatory mediators mainly prostaglandins, bradykinins and other related autocoids which cause migration of macrophages and polymorphonuclear neutrophils to the site of inflammation (Fernandes, *et al.,* 2007). The effect of tested compounds on % inhibition of edema was measured after 30, 60, 90 and 120 min and the results are presented in table I. A gradual increase in paw volume with time was observed in control group. Administration of standard and test compounds significantly reduced the paw volume after 120 min and thus showed anti-inflammatory activity. The % inhibition of rat paw volume due to the screened compounds at 30, 60, 90 and 120min time interval ranged 10-15%, 24-44%, 33-45% and 29-47%, respectively. Compounds, **3** and **4,** had smaller paw volume than the positive control and were found to be more potent. All the tested compounds except compound **5** exhibited the maximum reduction in paw volume at 120 min while **5** displayed the maximum protection at 90 min. It could be concluded that the anti-inflammatory activity of the synthesized compounds could be due to the inhibition of inflammatory mediators release and possibly due to the inhibition of cyclooxyenase synthesis similar to indomethacin. It was observed that substitution of 2-phenyl ring at *p*- position with –CH_2OH in compound 5, decreases the anti-inflammatory activity. However, the best activity is shown by compound *6-(4-methylphenyl)-4,5-dihydropyridazin-3(2*H*)-one* (**3**) having no substituent at 2nd position of pyridazine ring. Further, replacing an oxo group at 3rd position with a –chloro group does not lead to change in the activity.

Analgesic activity by acetic acid induced writhing method

The synthesized compounds were screened for analgesic activity by Acetic-acid induced writhing method. It is a sensitive screening method used to assess peripheral analgesic activity (Hunskaar and Hole, 1987). Standard drug indomethacin inhibited the acetic induced writhing by 76.3% while test compounds also showed good peripheral analgesic activity (59-74%) comparable to

Table II. Effect of compounds on Acetic-acid induced writhing in mice.

S. No.	Treatment	Dose (mg/kg)	Number of Writhes Mean ± SEM	% inhibition
1	Control	------	39.6 ± 2.1**	--------
2	Standard (a)	50	9.4 ± 0.5**	76.3%
3	2	50	16.2 ± 0.7*	59%
4	3	50	14.6 ± 1.7**	64.5%
5	4	50	10.0 ± 0.9**	74%
6	5	50	12.8 ± 1.0**	67.6%
7	6	50	14.6 ± 1.7*	63%

Values are expressed as mean ±SEM (N=5); a – Indomethacin (10 mg/kg, i.p.) used as standard drug in Acetic-acid induced writhing; *p<0.01 compared with vehicle control, **p<0.05 compared with vehicle control (ANOVA).

Table III. Effect of compounds on Tail immersion method.

S. No.	Treatment	Dose (mg/kg)	After Dose Reactiontime (insec.) Mean ± SEM			
			30 min.	60 min	90 min.	120 min.
1.	Control	-------	2.6±0.2*	2.6±0.2*	2.6±0.2*	2.4±0.2*
2.	Standard (a)	50	5.6±0.2*	7.8±0.5*	8.8±0.3*	8.2±0.3*
3.	2	50	4.6±0.4*	5.6±0.4*	7.0±0.3*	5.6±0.2*
4.	3	50	3.8±0.3	3.6±0.2	5.2±0.3*	4.4±0.2*
5.	4	50	5.4±0.2*	6.6±0.4*	8.4±0.4*	6.6±0.2*
6.	5	50	5.6±0.2	6.2±0.4**	7.2±0.5*	7.2±0.5*
7.	6	50	5.6±0.2*	5.6±0.2*	6.8±0.3*	6.8±0.3*

Values are expressed as mean ±SEM (N=); a – Indomethacin (10 mg/kg, i.p.) used as standard drug in Tail immersion method; *p<0.01 compared with vehide control, **p<0.05 compared with vehide control (ANOVA).

standard. In general the peripheral analgesic activity increased with time. Compound *3-chloro-6-(4-methylphenyl)-pyridazine* (**4**) was found to be the most potent analgesic agent with a % inhibition value of 74%. The results of analgesic activity are shown in table II.

Analgesic activity of compounds by tail immersion method

The effect of synthesized compounds on central analgesic activity was investigated by tail immersion method and the results are presented in table III. As it can be seen from table III, that after the administration of test compounds, reaction time (analgesic activity) gradually increased with time and the peak analgesic activity was observed at 90 min, after which analgesia starts to regress. Compound **4** showed comparable activity with respect to standard drug. Other compounds also showed moderate to good analgesic activity.

Pharmacokinetic parameters and toxicity potential

Lipinski's rule is commonly used in drug design to predict fate of drug *in vivo*. A compound that obeys follows Lipinski's rule of five is expected to be absorbed orally (Lipinski, *et al.,* 1997). To obey Lipinski's rule, a molecule should have a) molecular weight <500, b) log P<5, c) number of hydrogen bond donors (OH and NH groups) ≤5 and d) ≤10 hydrogen bond acceptors (notably N and O). Molecular properties including Log P, TPSA, number of hydrogen bond donor and acceptor, molecular weight etc were calculated with the help of Molinspiration online to evaluate the drug likeness of synthesized compounds as per the Lipinski's rule of five in search of the lead NSAID candidate(s). It can be seen from the results presented in the table 4 that all the synthesized pyridazine derivatives are likely to be absorbed orally as they did not show any

Table IV. Drug likeness score of synthesized compounds by molinspiration software.

Compound	miLog P[a]	TPSA[b]	*n* Atoms	n ON[c]	*n* OHNH[d]	*n* violation	*n* rotb[e]	MW[f]
2	3.7	32.7	20	3	0	0	2	264.3
3	1.8	41.5	14	3	1	0	1	188.2
4	3.2	24.7	14	2	0	0	1	206.7
5	1.4	52.9	16	4	1	0	2	218.3
6	1.9	63.8	18	5	2	0	1	241.3
Indomethacin	4.0	68.5	25	5	1	0	4	357.8

[a]Logarithm of partition coefficient between *n*-octanol and water (miLog P); [b]topological polar surface area (TPSA); [c]number of hydrogen bond acceptors (*n*-ON); [d]number of hydrogen bond donors (*n*-OHNH); [e]number of rotatable bonds (*n*-rotb);); [f]molecular weight (MW).

Table V. Drug-likeness/scores and toxicity calculations of pyridazine derivatives (2-6) based on Osiris property explorer.

Compound	Solubility	Drug-likeness	Drug score	Mutagenic	Tumorigenic	Irritant	Reproductive effect
2	-4.1	2.4	0.71	Green	Green	Green	Green
3	-3.3	1.9	0.83	Green	Green	Green	Green
4	-3.5	-0.7	0.54	Green	Green	Green	Green
5	-3.2	1.6	0.14	Yellow	Red	Red	Red
6	-3.2	2.8	0.87	Green	Green	Green	Green
Indomethacin	-5.4	9.4	0.56	Green	Green	Green	Green

violation of Lipinski's rule of five. Also the chemical descriptors to predict pharmaco-kinetic properties of compounds **2-6** are similar to the standard drug indomethacin.

Results of overall drug score and toxicity profile of title compounds **2-6** by Osiris property explorer are tabulated in table V. This online program predicts on the basis of functional group similarity of the investigated compound with the extensively *in-vitro* and *in-vivo* studied compounds present in its data base. Green color suggests low toxic tendency, yellow shows the mild toxicity and red color indicates high probability of toxicity. It is very obvious from the presented data that except compound **5**, all other compounds are predicted to be safe and expected to show low or no toxicity regarding mutagenicity, tumorigenicity, irritant effect and effect on reproductive system. The probability of developing the molecule in to a drug molecule was predicted on the basis of drug score which ranged from 0.14-0.87. However, the high drug score was obtained for compounds **3** and **6** (0.87 and 0.83) while **2** and **4** had the moderate drug score (0.71 and 0.54). The drug score of compound **4** (0.54) was at par with the drug score of indomethacin (0.56) but compounds **2**, **3** and **6** exhibited better score than the reference compound. It was interesting to note that compound **5** had the lowest drug score of 0.14 *vis a vis* it was predicted to be highly toxic with regards to tumorigenicity, irritant effect and reproductive system Solubility of pyridazine derivatives was also predicted to be within the permissible optimum range.

CONCLUSION

In the present study, some novel pyridazinone derivatives were designed, synthesized, characterized and evaluated for their *in-vivo* analgesic and anti-inflammatory activities. The results of the pharmacological screening indicated that pyridazine derivatives possess significant analgesic activity associated with NSAIDs properties. All title compounds exhibited anti-inflammatory activity (Table I) that lasted for 90min and the potency increased

with time. Among the synthesized pyridazinones, compound **4**, *3-chloro-6-(4-methylphenyl)-pyridazine* emerged as lead compound with good analgesic and anti-inflammatory activities at par with the reference drug. Most of the compounds exhibited analgesic effect by both peripheral and central mechanisms. The study confirmed the anti-inflammatory and analgesic potential of 2/3 substituted pyridazine derivatives. However, further detailed investigations are needed to establish the safety, efficacy and mechanism of this promising class of heterocyclic compounds.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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