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ORIGINAL PAPER

Design, synthesis, structure elucidation, and biological activities of 3-(substituted amino)-1-(pyridin-4-yl)propenones and 5-isonicotinoyl-1,2,3,4-tetrahydropyrimidine–adamantane hybrids

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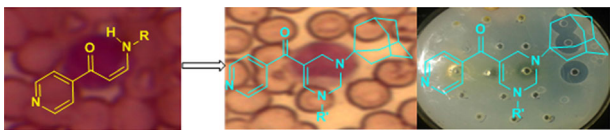
Abstract Novel molecular hybrids of 5-isonicotinoyl-1,2,3,4-tetrahydropyrimidine–adamantane have been synthesized in excellent yields by the reaction of enaminones, 1-adamantanamine, and formaldehyde. Enaminones (*Z*)-3-alkyl/aralkyl/aryl-1-(pyridin-4-yl)prop-2-en-1-ones have been synthesised by the reaction of 4-acetylpyridine with *N*,*N*-dimethylformamide dimethyl acetal and its bioassay test showed good anti-inflammatory property, but no zone of growth inhibition against *Bacillus subtilis* (MTCC Code-121), *Staphylococcus aureus* (MTCC Code-9886), *Escherichia coli* (MTCC Code-1302), and *Salmonella enterica* (MTCC Code-3232) bacterial strains. The structures of the synthesized compounds have been established with the help of spectral and analytical data. X-ray analysis of a representative candidate of the series 5-isonicotinoyl-1,2,3,4-tetrahydropyrimidine–adamantane was done for the final confirmation of the structure.
Graphical abstract



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Introduction

Nitrogen-containing heterocyclic compounds have a diverse range of biological and pharmacological properties [1–4]. The pyridine nucleus is an important heteroaromatic class of compounds with a wide range of activities and is present in many products such as drugs, vitamins, food, flavorings, plants, dyes, rubber products, adhesives, insecticides, and herbicides. Analogs of isonicotinic acid and isonicotinoyl group are well documented in the literature [5–7]. Literature survey reveals that compounds containing isonicotinoyl groups have been synthesized and were tested for their chelating [8–10] and biological properties [11–14]. Isonicotinic acid hydrazide (isoniazid) is reported to be a well-acknowledged drug and is one of the primary drugs used in combination with ethanbutol, rifampin, streptomycin, and pyrazinamide to treat tuberculosis [15, 16]. A large number of compounds containing the isoniazid moiety have been synthesized and tested, and further studies are going on due to increasing resistance of bacterial strains to certain type of antibiotics [17]. In the light of the importance of isonicotinoyl derivatives, our group has also reported [18] the synthesis and antibacterial activities of novel 5-isonicotinoyl-1,2,3,4-tetrahydropyrimidines and bis-(5-isonicotinoyl-1,2,3,4-tetrahydropyrimidines), envisaging that the presence of the isonicotinoyl group in position 5 of the tetrahydropyrimidine ring could have an important impact on the biological activities of these molecules. Most of the compounds were found to possess antibacterial activity on four Gram-positive bacteria used in the study. Enaminones are useful precursors for the synthesis of

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tetrahydropyrimidines [18] which are known to possess important biological properties such as antitumor [19], antimicrobial [19], anticonvulsant [20], anti-inflammatory [21], analgesic [21], and ulcerogenic properties [22]. Patel and coworkers have recently reported [23] the synthesis of substituted (*E*)-3-(benzo[d]thiazol-2-ylamino)phenylprop-2-en-1-one which had antidiabetic activity.

Moreover, the adamantane nucleus was found to be a very important pharmacophore in many therapeutic agents [24–30]. The incorporation of an adamantyl moiety into a pharmacologically active molecule resulted in many cases in improving the therapeutic profile of the parent drug [31]. Since the discovery of amantadine in 1966 as the first antiviral therapy for systemic use [32], several hundreds or even thousands of adamantane derivatives were synthesized and proved to be effective against several pathogenic microorganisms and beneficial in improving various physiological disorders. In addition, adamantane and 1,2,3,4-tetrahydropyrimidines derivatives have recently been reported to possess promising antimicrobial and anti-inflammatory activities [33, 34]. Based on these findings, we have recently reported the synthesis and biological activities of enaminones containing the adamantane moiety [35].

In the design of new drug prototypes, the concept of molecular hybridization is a useful tool and is based on the combination of pharmacophoric moieties of different bioactive substances to produce a new hybrid compound with improved affinity and efficacy. This strategy has resulted in compounds with modified selectivity profile, different and/or dual mode of action and reduced undesired side effects [36, 37]. Hybrid molecules or conjugates that combine two heterocyclic structural units of different nature generally lend themselves well to rational drug design and often possess improved biological activities [38, 39]. A number of biologically potent molecular hybrids have been described in the literature such as steroid antibiotics [40], steroid nucleosides [41], triterpenoid peptides [42], and DNA cleaving-agent amino acids [43]. Literature survey revealed that hybrids of 1,2,3,4-tetrahydropyrimidines with in-built isonicotinoyl group at C-5 and adamantane are unknown in the literature and hence their biological activities remain unexplored.

Prompted by the absence of 1,2,3,4-tetrahydropyrimidine–adamantane hybrids containing the isonicotinoyl group at C-5 in the literature and in continuation with our studies on adamantane derivatives [35, 44], we present herein a facile one-pot synthesis of hitherto unreported [3-(adamantan-1-yl)-1-alkyl/aralkyl/aryl-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanones to evaluate their biological properties. The synthesis of these molecular hybrids was achieved via (*Z*)-3-alkyl/aralkyl/aryl-1-(pyridin-4-yl)prop-2-en-1-ones, derived from commercially available 4-acetylpyridine by following our previously reported procedure [18].

Results and discussion

Synthesis of enaminones

To synthesize the proposed 1,2,3,4-tetrahydropyrimidine–adamantane hybrids bearing isonicotinoyl group in position 5 of the ring, we required enaminones of the type **3** derived from 4-acetylpyridine. Compound **3** was prepared from **2** following our previously reported procedure [18], and **2**, in turn, was synthesized by the reaction of **1** with dimethylformamide dimethyl acetal (DMF-DMA) under MWI [18].

Thus, when compound **2** was stirred with an equimolar amount of aniline in acetic acid at room temperature, workup of the reaction mixture resulted in the formation of the desired enaminone **3a** in 82% yield, which was characterized as 3-anilino-1-(pyridin-4-yl)prop-2-en-1-one (Table 1), on the basis of physical and spectral data and also by comparing with those already reported [18]. Similarly, the reaction of **2** with other primary aromatic amines

Table 1 Synthesis of (*Z*)-3-alkyl/aralkyl/aryl-1-(pyridin-4-yl)prop-2-en-1-ones **3a–3j**

Entry	Compound	R/R[1]	Reaction time/h	Yield/%	M.p. (lit. m.p.)/°C
1	**3a**	C_6H_5	45	82	148–149 (149–150 [18])
2	**3b**	4-MeC_6H_4	64	81	131–133
3	**3c**	4-MeOC_6H_4	45	83	115–116
4	**3d**	4-ClC_6H_4	44	95	122–123
5	**3e**	4-BrC_6H_4	64	96	140–141
6	**3f**	3-HOC_6H_4	5	94	217–218
7	**3g**	4-$NO_2C_6H_4$	65	85	>280
8	**3h**	CH_3	63	81	Gum [18]
9	**3i**	CH_3CH_2	68	91	66–67
10	**3j**	$C_6H_5CH_2CH_2$	50	92	Gum

Table 2 Synthesis of [3-(adamantan-1-yl)-1-alkyl/aralkyl/aryl-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanones **5a–5j**

Entry	Compound	R′	Reaction time/h	Yield/ %	M.p./°C
1	**5a**	C_6H_5	4	98	118–120
2	**5b**	4-$CH_3OC_6H_4$	5	98	112–113
3	**5c**	4-ClC_6H_4	3	96	165–166
4	**5d**	4-BrC_6H_4	3	96	170
5	**5e**	4-$CH_3C_6H_4$	6	97	150–151
6	**5f**	4-$NO_2C_6H_4$	10	91	205–207
7	**5g**	CH_3	6	91	136–137
8	**5h**	CH_3CH_2	6	96	112–114
9	**5i**	$PhCH_2CH_2$	2	93	187–188
10	**5j**	3-HOC_6H_4	7	94	116–118

gave the desired enaminones **3b–3g** in 81–96% overall yields.

However, the reaction of **2** with alkyl/aralkylamines under similar reaction conditions failed to give 3-(alkylamino)- or 3-(aralkylamino)enaminones and ended up in the formation of a complex mixture from which isolation of the desired product was not possible. Interestingly, when the reaction of **2** with alkyl or aralkyl amines was carried out in refluxing ethanol, the desired enaminones **3h–3j** were obtained in 81–95% overall yields. It is important to note that **2** failed to react with primary aromatic amines in refluxing ethanol, as these amines are weaker nucleophiles compared to alkyl and aralkylamines.

The structures of the enaminones were well established with the help of spectral and analytical data and also by comparison with those already reported [18]. Thus, the IR spectra of **3b** showed peaks at 1535, 1645, and 3285 cm^{-1} due to carbonyl and NH groups. In the ^1H NMR spectra, the α-vinylic proton appeared as a doublet at 5.96 ppm ($J = 7.4$–7.8 Hz), while the β-vinylic proton gave a double doublet at 7.55 ppm ($J = 7.6$, 12.8 Hz) due to its coupling with α-vinylic as well as NH protons. The NH proton resonated at 10.5–12.0 ppm, indicating its hydrogen-bonded state with carbonyl oxygen. The low coupling constant of the vinylic protons ($J = \sim 6$ Hz) and the appearance of the NH signal (10.55–12.34 ppm) at low fields confirm the *Z*-configuration of the enaminone. Synthesis of the desired tetrahydropyrimidine–adamantane hybrids was subsequently undertaken.

Synthesis of 1,2,3,4-tetrahydropyrimidine–adamantane hybrids

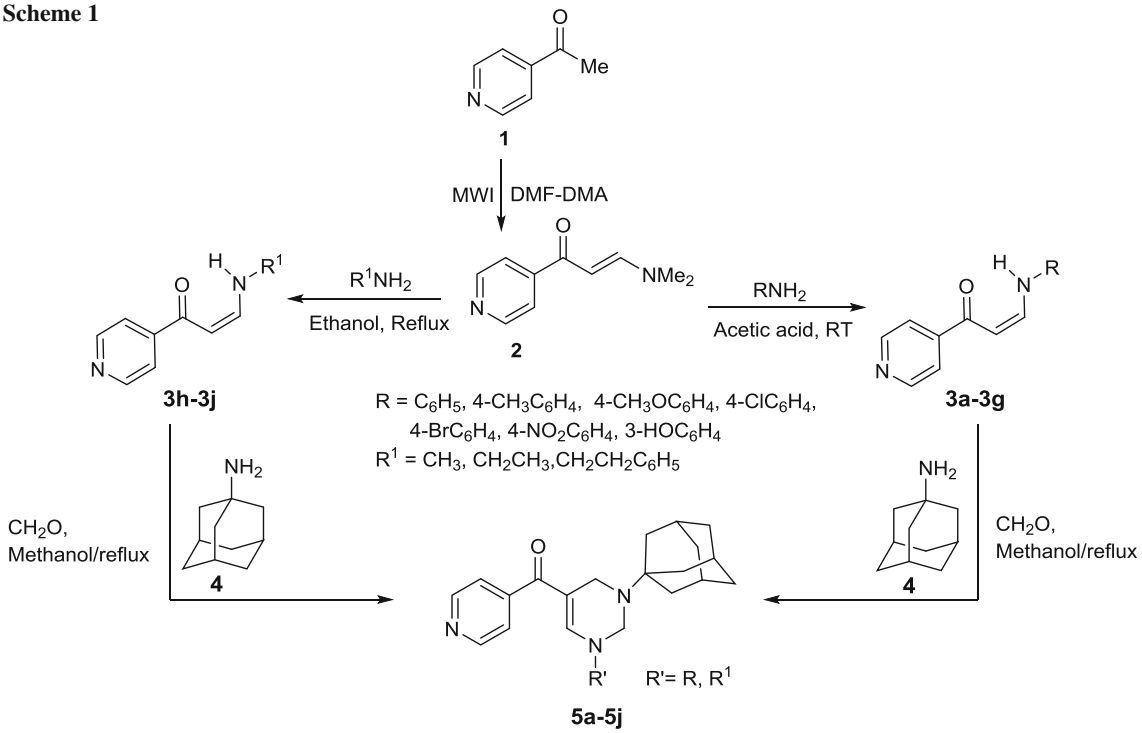
When a mixture of enaminone **3a**, 1-adamantanamine (**4**), and formaldehyde (1:1:2) in 4 cm^3 methanol was heated at reflux for 4 h, workup of the reaction mixture gave **5a** in 98% yields (Table 2), the structure of which was proposed to be [3-(adamantan-1-yl)-1-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone on the basis of spectral and analytical data. The reaction conditions could easily be extrapolated for the synthesis of **5b–5j** in 91–98% overall yields in 2–10 h (Scheme 1).

The structures of the products were established without any ambiguity. The infrared spectra of **5a–5j** showed strong peaks in the region of 1571–1638 cm^{-1} due to extensively delocalized double bonds and carbonyl groups. The C–H stretching due to adamantane gave characteristic bands close to 2906 and 2850 cm^{-1}. In the ^1H NMR spectra of **5a–5j**, the signals due to aromatic protons appear between 6.63 and 8.68 ppm. The C_6–H proton of the tetrahydropyrimidine ring resonated as a singlet between 6.52 and 6.96 ppm in **5a–5j** and 7.31–7.51 ppm in **5d–5f**, whereas it remained obscured by the aromatic protons in **5a–5c**. The CH_2 protons at C-2 of the tetrahydropyrimidine ring appeared between 4.06 and 4.70 ppm, while those at C-4 gave their signals in the range of 3.70–3.92 ppm. The three sets of protons of the adamantyl group resonated as three distinct multiplets in the ranges 1.64–1.73, 1.84–1.92, and 2.14–2.18 ppm. In the ^{13}C NMR spectra of the tetrahydropyrimidines, the most striking signal was due to carbonyl carbon (close to 190 ppm) and those due to adamantyl group carbon atoms appearing in the ranges of 29.4–29.6, 36.0–36.1, 43.1–43.4, and 53.2–53.8 ppm. The proposed cyclic structures for the tetrahydropyrimidines are further supported by the absence of NH ($\sim$12.00 ppm) and vinylic C–H ($\sim$6.00 ppm) proton signals of the starting enaminones in the spectra of **5a–5j**. The mass spectra of the products were also in conformity with the proposed structures.

Crystal structure of 5d

Furthermore, the proposed structure of [3-(adamantan-1-yl)-1-(4-bromophenyl)-1,2,3,4-tetrahydropyrimidin-5-yl]

Scheme 1



(pyridin-4-yl)methanone (**5d**) was unambiguously confirmed by single crystal X-ray crystallography (Fig. 1) with CCDC no. 996549. The crystals of **5d** suitable for X-ray analysis were obtained by crystallization from ethyl acetate. The crystal belongs to triclinic, space group P1 with $a = 16.3481\ (5)$ Å, $b = 12.3215\ (4)$ Å, $c = 11.0874\ (3)$ Å, $\beta = 93.619\ (2)°$, $V = 2228.92\ (12)$ Å^3, and $Z = 4$. The molecular graphics was performed using ORTEP-3 and displacement ellipsoids were drawn at 30% probability level.

Biological evaluation

The biological activities of the synthesized compounds **3a**–**3j** and **5a**–**5j** were assessed by carrying out anti-inflammatory and antibacterial tests.

Anti-inflammatory activity

For our study, mice bearing FCA-induced paw edema were treated with an intra-peritoneal injection of test compounds at a dose of 50 mg/kg body weight and were used as test subjects after 1 h. Mice bearing paw edema without subsequent treatment with the compounds and mice treated with ibuprofen (an NSAID) served as controls and positive controls, respectively.

(a) Inhibition of FCA-induced paw edema

The ability of the compounds **3a**–**3j** and **5a**–**5j** to reduce FCA-induced paw edema in mice was used as a physical parameter to test the anti-inflammatory property. All the compounds showed a reduction of paw edema (Tables 3, 4).

(b) Nitric oxide level in the paw exudates

The assay of NO in exudates of FCA-induced paw in mice was used as a biochemical indicator of inflammation. The enzyme NO synthase (NOS) synthesizes NO from the terminal guanidine nitrogen atom of L-arginine. The inducible form of NOS generates NO in endothelial cells, smooth muscle cells and macrophages, upon stimulation with lipopolysaccharide (LPS), the endotoxin component of FBS [45]. In this study, the lowering of the NO level in the paw exudate by the test compounds in inflammation-bearing mice indicates their anti-inflammatory activities. Measurements of NO in paw exudates of mice showed the highest reduction in mice treated with **3i**, followed by **3e**, **3f**, and **3j**, but **3a**, **3b**, **3c**, and **3d** showed no reduction of NO levels (Fig. 2). On the other hand, measurements of NO in paw exudates of mice showed no reduction when treated with compounds **5a**–**5j** (Fig. 3).

(c) Level of nitric oxide concentration in whole blood

The NO level in the blood of mice bearing paw edema was also used as an indicator of inflammation. LPS, a component of FCA, is a potent stimulator of NO synthesis by

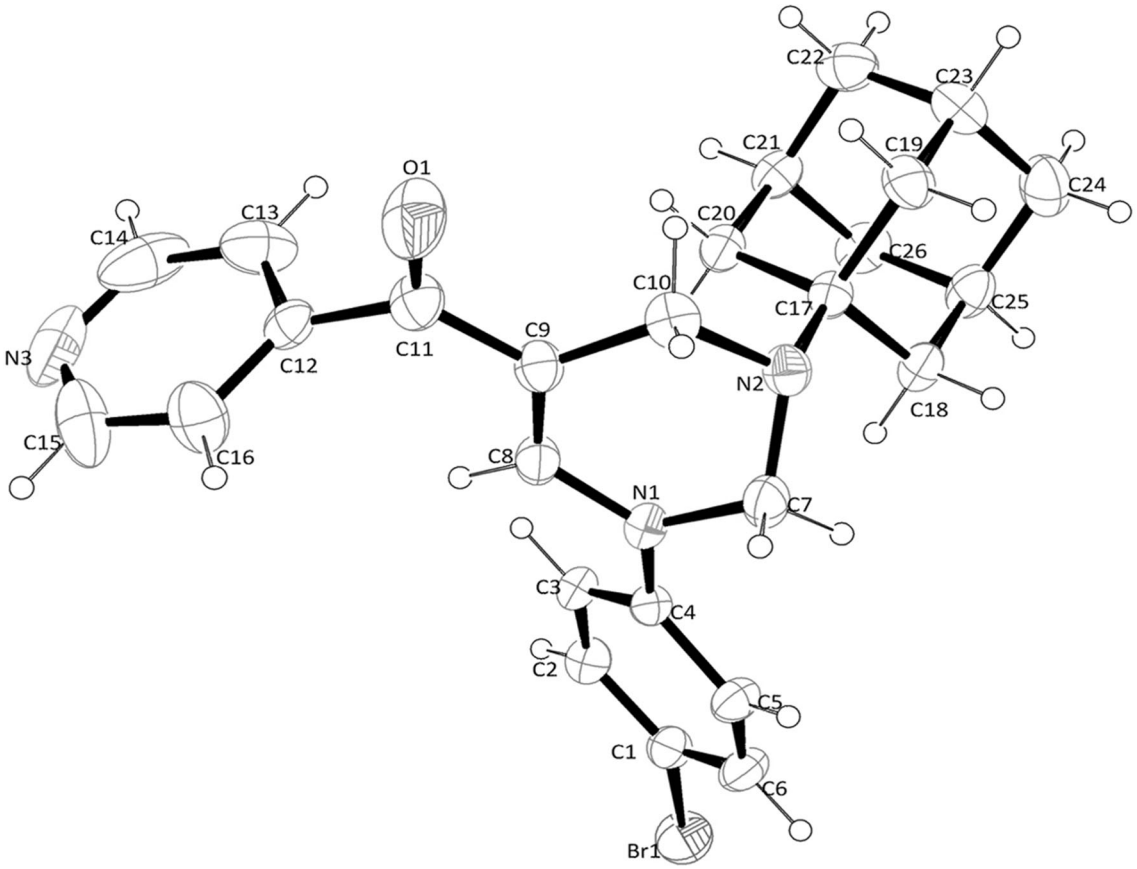


Fig. 1 Ortep diagram of **5d** with atom numbering scheme. Displacement ellipsoids are drawn at 30% probability level

several cells including endothelial cells and leads to vasodilation and hypotension [46–48]. Lowering of NO levels in the blood of inflammation-bearing mice treated with the test compounds suggests the anti-inflammatory effect of the compounds. The nitric oxide concentration in blood in response to the test compounds and ibuprofen is given in Figs. 4 and 5. Enaminones **3a**, **3b**, **3c**, and **3h** showed reduction in NO levels as compared to the control, but not as much as shown by ibuprofen and the molecular hybrids **5a**, **5f**, and **5i** that showed the highest reduction of NO levels in blood, which is also comparable with that of ibuprofen.

(d) Differential leukocyte count in blood

To determine any alteration in the number of different leukocytes in response to the test compounds and ibuprofen in mice bearing paw edema, a differential leukocyte count in peripheral blood was performed. The results were reported as a percentage of total leukocytes (Figs. 6, 7). The compounds brought about a reduction in the basophil and eosinophil percentages. A high percentage of neutrophils (neutrophilia) are indicative of inflammation or bacterial infection [49]. The results obtained in this test clearly indicated the anti-inflammatory effects of the test compounds.

Paw thickness was taken as a physical parameter for this investigation. From the reduction in paw thickness, it is clear that **3i**, **3e**, and **3d** have the highest ability to reduce paw edema followed by **3a**, **3g**, and **3f** (Table 3). Similarly, compounds **5b** and **5c** exhibited the highest reduction after 24 h, followed by compounds **5j**, **5f**, **5a**, **5i**, **5h**, and **5g** in decreasing order (Table 4). The other inflammatory parameters used for the investigation were NO level in paw exudates and whole blood. Measurements of NO in paw exudates of mice exhibited the highest reduction in mice treated with **3i**, followed by **3e**, **3f**, and **3j**, but **3a**, **3b**, **3c**, and **3d** showed no reduction of NO levels (Fig. 2). On the other hand, measurements of NO in paw exudates of mice displayed no reduction in mice treated with compounds **5a**–**5j** (Fig. 3). This was also observed in mice treated with ibuprofen, which showed little reduction comparable with the untreated control (Fig. 3).

Table 3 Paw thickness at different time intervals of the different test compounds **3a–3j** (50 mg/kg), ibuprofen (30 mg/kg) and an untreated control in mice carrying FCA-induced paw edema in comparison to untreated control

Treatment group	Time/h	Paw edema	Increase in paw thickness from 0 h	Percentage increase/decrease in paw thickness
Control	0	3.08 ± 0.07	0.00	0.00
	1	3.27 ± 0.28	0.19	6.17
	3	3.42 ± 0.29	0.34	11.04
	24	3.69 ± 0.27	0.61	19.81
Ibuprofen	0	3.97 ± 0.06	0.00	0.00
	1	3.97 ± 0.06	0.00	0.00
	3	3.13 ± 0.12	−0.84	−21.16
	24	3.80 ± 0.17	−0.17	−4.28
3a	0	4.00 ± 0.00	0.00	0.00
	1	3.93 ± 0.12	−0.07	−1.75
	3	3.47 ± 0.29	−0.53	−13.25
	24	3.93 ± 0.12	−0.07	−1.75
3b	0	4.00 ± 0.00	0.00	0.00
	1	4.00 ± 0.00	0.00	0.00
	3	3.60 ± 0.17	−0.40	−10.00
	24	3.77 ± 0.25	−0.23	−5.75
3c	0	4.00 ± 0.00	0.00	0.00
	1	3.83 ± 0.06	−0.17	−4.25
	3	3.50 ± 0.00	−0.50	−12.50
	24	3.83 ± 0.21	−0.17	−4.25
3d	0	4.00 ± 0.00	0.00	0.00
	1	3.97 ± 0.06	−0.03	−0.75
	3	3.23 ± 0.25	−0.77	−19.25
	24	3.70 ± 0.17	−0.30	−7.50
3e	0	4.00 ± 0.00	0.00	0.00
	1	3.83 ± 0.06	−0.17	−4.25
	3	3.57 ± 0.12	−0.43	−10.75
	24	3.70 ± 0.17	−0.30	−7.50
3f	0	4.00 ± 0.00	0.00	0.00
	1	4.00 ± 0.00	0.00	0.00
	3	3.70 ± 0.17	−0.30	−7.50
	24	3.90 ± 0.10	−0.10	−2.50
3g	0	4.00 ± 0.00	0.00	0.00
	1	3.83 ± 0.29	−0.17	−4.25
	3	3.20 ± 0.17	−0.80	−20.00
	24	3.90 ± 0.10	−0.10	−2.50
3h	0	4.00 ± 0.00	0.00	0.00
	1	4.00 ± 0.00	0.00	0.00
	3	3.50 ± 0.30	−0.50	−12.50
	24	3.80 ± 0.00	−0.20	5.00
3i	0	4.00 ± 0.00	0.00	0.00
	1	3.87 ± 0.12	−0.13	−3.25
	3	3.30 ± 0.00	−0.70	−17.50
	24	3.50 ± 0.00	−0.50	−12.50
3j	0	4.00 ± 0.00	0.00	0.00
	1	3.93 ± 0.12	−0.07	−1.75
	3	3.43 ± 0.32	−0.57	−14.25
	24	3.87 ± 0.12	−0.13	−3.25

The levels of NO in blood showed the highest reduction in case of mice treated with **3a**, **3b**, **3c**, and **3h,** which is comparable with ibuprofen. Compounds **3e**, **3i**, and **3j** caused lower reduction, while **3d** and **3g** showed no reduction (Fig. 4). The levels of NO in blood showed the highest reduction in the case of mice treated with **5a**, **5f**, and **5i,** which is comparable with ibuprofen and is followed by **5j**, **5b**, **5d**, **5e**, and **5g**, although **5c** and **5h** showed no reduction in NO levels (Fig. 5) Basophil and eosinophil counts were lower in mice treated with **3g**, **3b**, **3j**, and **3f** followed by **3d** and **3i** (Fig. 6), and these counts were lower in mice treated with **5c**, **5f**, and **5i** (Fig. 7).

The above results led to the conclusion that among enaminones **3a–3j**, **3i** and **3e** have the highest potential as anti-inflammatory agents which were followed by **3d**; in the case of **5a–5j**, compounds **5f** and **5i** were found to be most potent. Considering parameters like paw thickness, NO level in blood and differential WBCs count, compounds **3i**, **3e**, **3d**, **5f**, and **5i** exhibited anti-inflammatory activities in most of the above tested parameters and in some of the parameters these compounds showed activities comparable with the selected drug.

Antibacterial test

The results of the antibacterial activity were recorded as zone of inhibition in millimeters for all the compounds. The compound **3a–3j** did not show any zone of growth inhibition against the four bacterial strains. The compounds **5a–5j** did show some significant activity. Compound **5a** exhibited a clear zone of inhibition against *Staphylococcus aureus* and **5d** also showed a significant zone (14 mm) against *Salmonella enterica*. However, compound **5h** displayed different activity against the strains producing maximum zones of inhibition against *Salmonella* (22 mm), followed by *Escherichia coli* (16 mm) and *Staphylococcus* (14 mm) (Table 5). *Bacillus subtilis* was found to be resistant to both **3a–3j** and **5a–5j**. Inhibition zones of different test organisms for the standard antibiotic ampicillin were significantly different. Ampicillin strongly inhibited the growth of *Staphylococcus*, *Salmonella*, and *Bacillus*, but exhibited a lower zone of inhibition against *E. coli*.

Table 4 Paw thickness at different time intervals of the different test compounds **5a**–**5j** (50 mg/kg), ibuprofen (30 mg/kg) and an untreated control in mice carrying FCA-induced paw edema in comparison to untreated control

Treatment groups	Time/h	Paw edema	Increase in paw thickness from 0 h	Percentage increase/decrease in paw thickness
Control	0	3.08 ± 0.07	0.00	0.00
	1	3.27 ± 0.28	0.21	6.82
	3	3.42 ± 0.29	0.34	11.04
	24	3.69 ± 0.27	0.61	19.81
Ibuprofen	0	3.97 ± 0.06	0.00	0.00
	1	3.97 ± 0.06	0.00	0.00
	3	3.13 ± 0.12	−0.84	−21.16
	24	3.80 ± 0.17	−0.17	−4.28
5a	0	4.00 ± 0.00	0.00	0.00
	1	4.00 ± 0.00	0.00	0.00
	3	3.80 ± 0.00	−0.20	−5.00
	24	3.77 ± 0.25	−0.23	−5.75
5b	0	4.00 ± 0.00	0.00	0.00
	1	4.00 ± 0.00	0.00	0.00
	3	3.60 ± 0.17	−0.40	−10.00
	24	3.60 ± 0.17	−0.40	−10.00
5c	0	4.00 ± 0.00	0.00	0.00
	1	3.97 ± 0.06	−0.03	−0.75
	3	3.33 ± 0.42	−0.67	−16.75
	24	3.60 ± 0.17	−0.40	−10.00
5d	0	4.00 ± 0.00	0.00	0.00
	1	4.00 ± 0.00	0.00	0.00
	3	3.60 ± 0.10	−0.40	−10.00
	24	3.93 ± 0.17	−0.07	1.75
5e	0	4.00 ± 0.12	0.00	0.00
	1	4.00 ± 0.00	0.00	0.00
	3	3.00 ± 0.00	−1.00	−2.50
	24	4.00 ± 0.00	0.00	0.00
5f	0	4.00 ± 0.00	0.00	0.00
	1	3.90 ± 0.00	−0.10	−2.50
	3	3.13 ± 0.12	−0.87	−21.75
	24	3.70 ± 0.17	−0.30	−7.50
5g	0	4.00 ± 0.00	0.00	0.00
	1	3.87 ± 0.06	−0.13	−3.25
	3	3.00 ± 0.00	−1.00	−25.00
	24	3.87 ± 0.12	−0.13	−3.25
5h	0	3.97 ± 0.06	−0.03	−0.75
	1	3.80 ± 0.00	−0.20	−5.00
	3	3.30 ± 0.00	−0.70	−17.50
	24	3.83 ± 0.29	−0.17	−4.50
5i	0	3.90 ± 0.10	−0.10	−2.50
	1	3.60 ± 0.17	−0.40	−2.50
	3	3.50 ± 0.00	−0.50	−12.50
	24	3.80 ± 0.00	−0.20	−5.00
5j	0	4.00 ± 0.00	0.00	0.00
	1	4.00 ± 0.00	0.00	0.00
	3	3.43 ± 0.12	−0.57	−14.25
	24	3.63 ± 0.23	−0.37	−9.25

The test compounds **3a**–**3j** did not possess any antimicrobial activity. However, compounds **5a**, **5d**, and **5h** showed clear antibacterial activity. Compound **5h** had a potent antibacterial activity against the highly infectious *Salmonella* and *E. coli* (Gram-negative bacteria) and against *Staphylococcus* (Gram-positive bacteria). These test compounds might have several invasive targets that could lead to the inhibition of the bacteria. However, *B. subtilis* was found to be resistant, as it did not show any zone of inhibition against the test compounds. Thus, compounds **5a**, **5d**, and **5h** may have therapeutic value as an antibacterial agent against pathogenic bacteria.

Experimental

Melting points were recorded by the open capillary method. The IR spectra were recorded on a Perkin-Elmer 983 spectrometer (Perkin-Elmer). The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-ECS 400 taking Me_4Si as the internal standard in $CDCl_3$. In the NMR spectral data, the abbreviations d, dd, s, m, and t, stand for doublet, double doublet, singlet, multiplet, and triplet, respectively. The X-ray diffraction data were collected at 296 K with Mo Kα radiation ($\lambda = 0.71073$ Å) using a Bruker Nonius SMART APEX II CCD diffractometer equipped with a graphite monochromator. The structures were solved by direct methods (SHELXS97) and refined by full-matrix least squares based on F^2. All calculations were carried out using WinGX system version 1.80.05. All the non-H atoms were refined in the anisotropic approximation: the H atoms were located at calculated positions. The electron spray mass spectra were recorded on a Waters ZQ-4000 mass spectrometer. Elemental analysis was performed on a Vario-EL III instrument. Microwave irradiation was carried out in a CEM Discover Benchmate microwave digester at 850 W for 5 min in an open reaction vessel. Formylated product **2** was synthesized by our previously reported procedure [18] and experimental data for compounds **3a** and **3h** have been previously described in the literature [18].

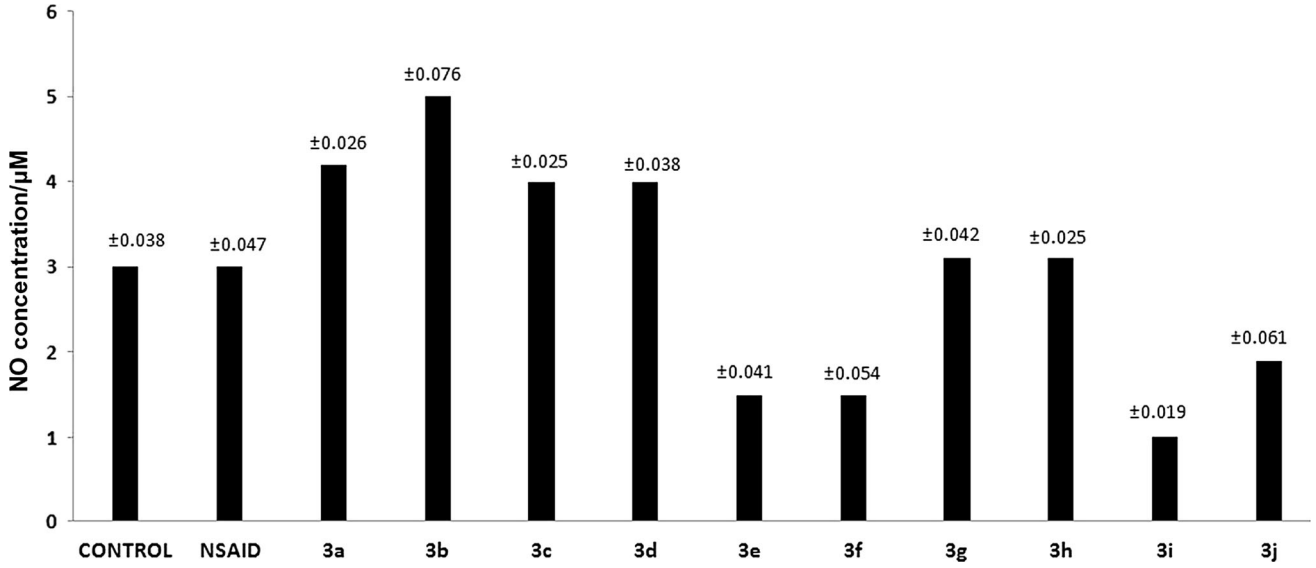


Fig. 2 Concentration of nitric oxide (in μM) in paw exudates of different treatment groups. Mice from different groups (3 mice each) were killed after 24 h from FCA injection, and the hind paw was excised and homogenized in 1 cm^3 normal saline. The NO level was measured using Griess reaction with standard nitrite reference curve. Each group represents the mean ± SEM ($n = 3$). *$P < 0.05$ statistical significance compared to the control (unpaired Student's t test)

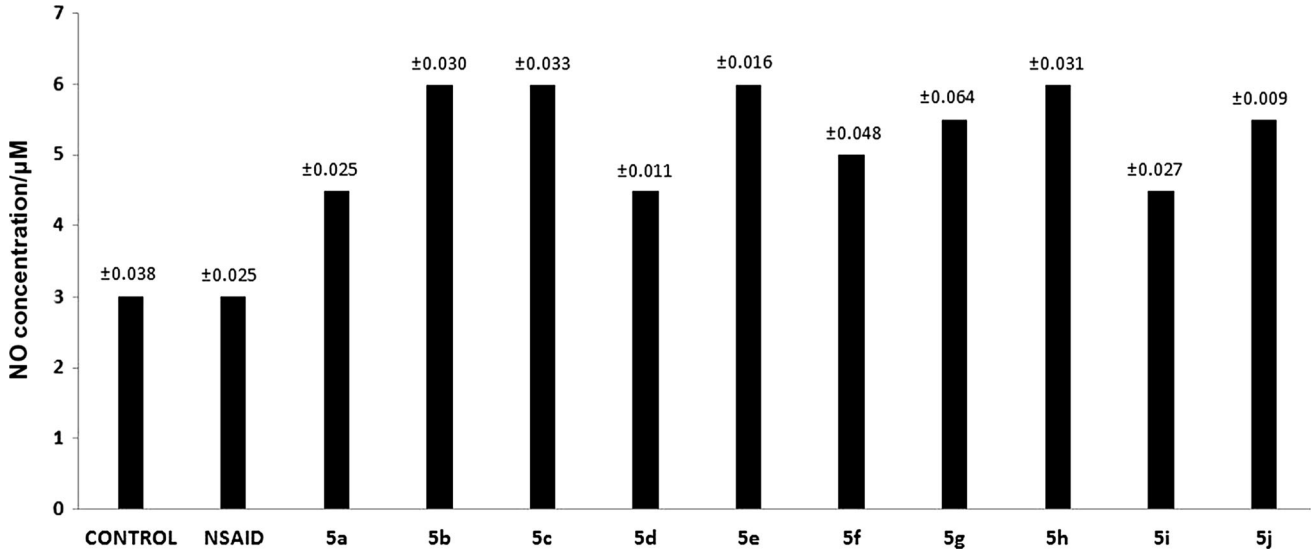


Fig. 3 Concentration of nitric oxide (in μM) in paw exudates of different treatment groups. Mice from different groups (3 mice each) were killed after 24 h from FCA injection, and the hind paw was excised and homogenized in 1 cm^3 normal saline. NO level was measured using Griess reaction with standard nitrite reference curve. Each group represents the mean ± SEM ($n = 3$). *$P < 0.05$ statistical significance compared to control (unpaired Student's t test)

*General procedure for the synthesis of (Z)-1-(pyridin-4-yl)-3-(p-arylamino)prop-2-en-1-ones **3a–3g***

To a solution of **2** (1 mmol) in 2 cm^3 acetic acid, aromatic amine (1 mmol) was added and the resulting mixture was stirred at room temperature for 44–65 h (takes 5 h in case of **3f**) when a solid product precipitated. After completion of the reaction (monitored by thin-layer chromatography), the mixture was poured over chilled water and the precipitated product was collected by filtration, washed repeatedly by water to ensure complete removal of acid, and dried to give practically pure product **3a–3g** in 81–96% overall yields. Further purification was achieved by column chromatography (silica gel, 20% EtOAc–hexane).

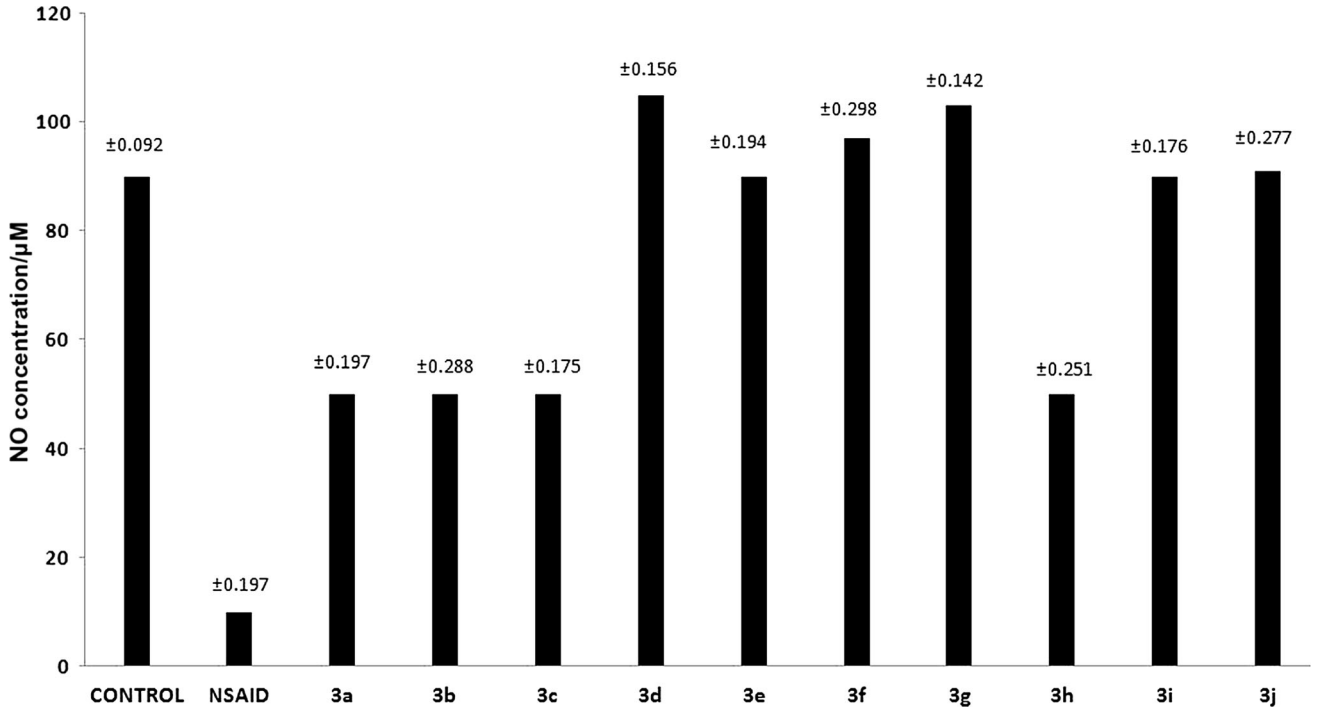


Fig. 4 Concentration of nitric oxide (in μM) in whole blood of different treatment groups. Paw edema was induced by injecting FCA and 1 h later mice were given intra-peritoneal injections of test compounds (50 mg/kg bw) and for ibuprofen 30 mg/kg bw. Blood was collected by retro-orbital bleeding from mice of different groups (3 mice each) and the whole blood was used to measure the level of NO. Each group represents the mean ± SEM ($n = 3$). *$P < 0.05$ statistical significance compared to the control (unpaired Student's t test)

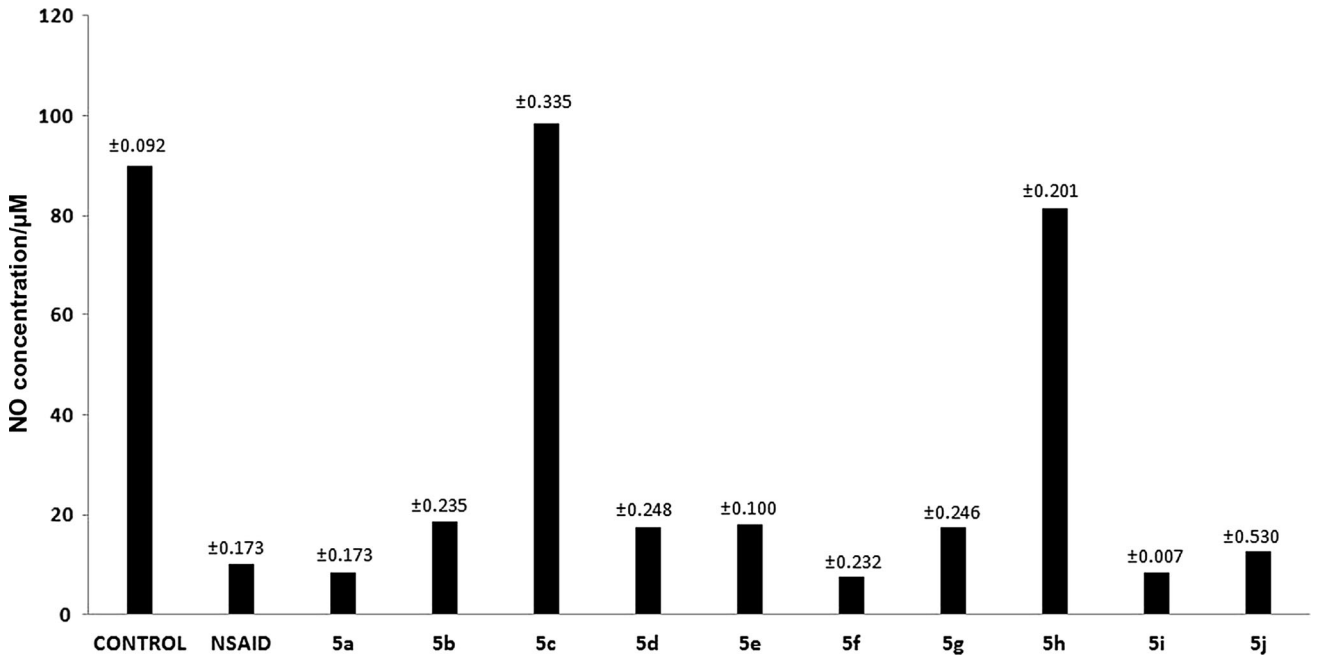


Fig. 5 Concentration of nitric oxide (in μM) in whole blood of different treatment groups. Paw edema was induced by injecting FCA and 1 h later mice were given intra-peritoneal injections of test compounds (50 mg/kg bw) and ibuprofen 30 mg/kg bw. Blood was collected by retro-orbital bleeding from mice of different groups (3 mice each) and the whole blood was used to measure the level of NO. Each group represents the mean ± SEM ($n = 3$). *$P < 0.05$ statistical significance compared to the control (unpaired Student's t test)

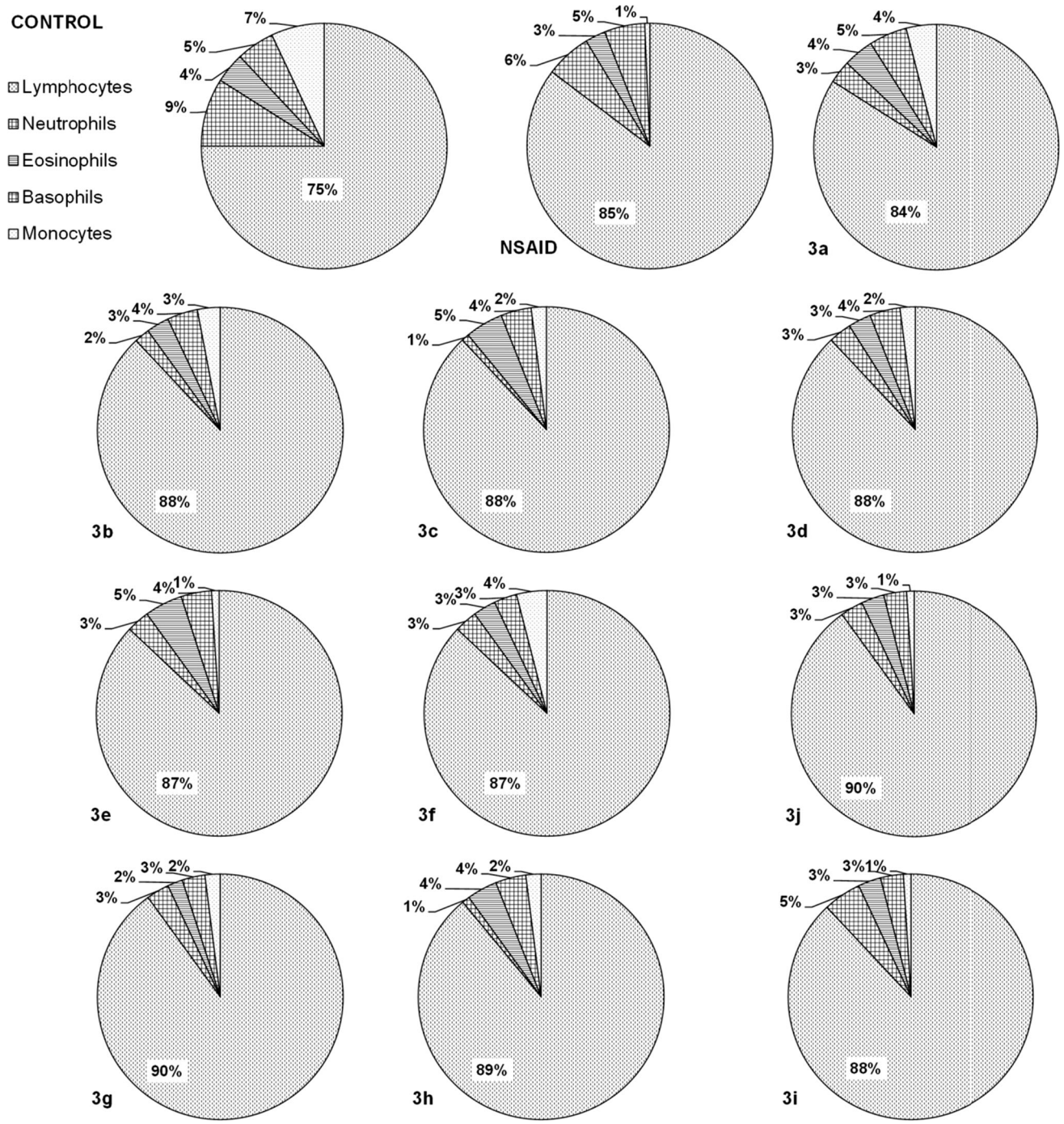


Fig. 6 Percentage counts of different types of leukocytes in mice treated with test compounds and ibuprofen in comparison to untreated control. Mice were killed 24 h after FCA injection. Blood smear was prepared and the slides were stained with Wright's stain and cells were counted under a microscope

(Z)-1-(Pyridin-4-yl)-3-(p-tolylamino)prop-2-en-1-one (**3b**, $C_{15}H_{14}N_2O$)

Yellow solid; yield: 193 mg (81%); m.p.: 131–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.33$ (s, 3H, CH_3), 5.96 (d, 1H, $J = 7.8$ Hz, H_α), 7.03 (d, 2H, $J = 8$ Hz, ArH), 7.17 (d, 2H, $J = 8$ Hz, ArH), 7.57 (dd, 1H, CH, $J = 7.8$, 12.4 Hz, H_β), 7.71 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.74 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 12.25 (d, 1H, $J = 12.4$ Hz, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 20.8$ (CH_3), 92.8 (CH-α), 116.7 (2, CH-phenyl), 120.7 (2, CH-phenyl), 130.3 (2, CH-4-pyridyl), 134.3 (Cq), 137.2 (Cq), 145.7 (Cq), 146.8 (CH-β), 150.5 (2, CH-4-pyridyl), 188.4 (CO) ppm; IR (KBr): $\bar{\nu} = 1299$ (C–N), 1535 (C=N), 1645 (C=O), 3349 (NH) cm^{-1}; MS (ESI): $m/z = 239$ ($[MH]^+$).

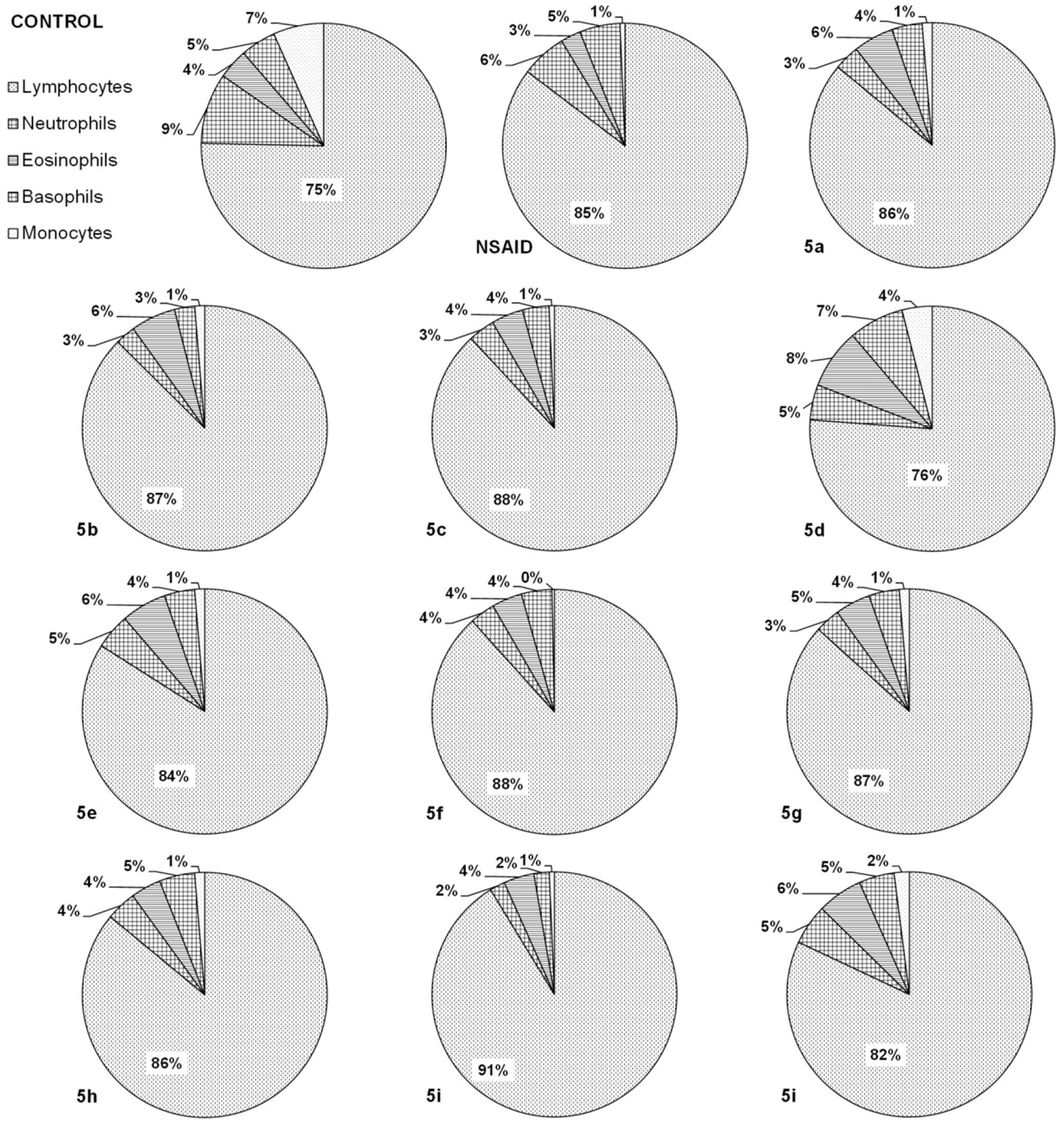


Fig. 7 Percentage counts of different types of leukocytes in mice treated with test compounds and ibuprofen in comparison to untreated control. Mice were killed 24 h after FCA injection. Blood smear was prepared and the slides were stained with Wright's stain and cells were counted under a microscope

(Z)-3-[(4-Methoxyphenyl)amino]-1-(pyridin-4-yl)prop-2-en-1-one (**3c**, $C_{15}H_{14}N_2O_2$)

Yellow solid; yield: 211 mg (83%); m.p.: 115–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.81$ (s, 3H, OCH_3), 5.95 (d, 1H, $J = 7.76$ Hz, H_α), 6.91 (d, 2H, $J = 8$ Hz, ArH), 7.09 (d, 2H, $J = 8$ Hz, ArH), 7.52 (dd, 1H, $J = 7.76$, 12.8 Hz, H_β), 7.73 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.74 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 12.34 (d, 1H, $J = 12.8$ Hz, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 55.5$ (OCH_3), 92.5 (CH-α), 115.0 (2, CH-phenyl), 118.3 (2, CH-phenyl), 120.8 (2, CH-4-pyridyl), 133.0 (Cq), 145.9 (Cq), 147.3 (Cq), 150.2 (2, CH-4-pyridyl), 156.8 (CH-β), 187.9 (CO) ppm; IR (KBr): $\bar{\nu} = 1299$ (C–N), 1535 (C=N),

Table 5 Zone of inhibition (mm) of test compounds against different bacteria, ±mean, $n = 3$

Test compounds	*Salmonella enterica*	*Escherichia coli*	*Bacillus subtilis*	*Staphylococcus aureus*
5a	–	–	–	11
5b	–	–	–	–
5c	–	–	–	–
5d	14	–	–	–
5e	–	–	–	–
5f	–	–	–	–
5g	–	–	–	–
5h	22	16	–	14
5i	–	–	–	–
5j	–	–	–	–
Ampicillin antibiotic (4 mg/cm^3)	25	22	25	26
DMSO	–	–	–	–

1645 (C=O), 3346 (NH) cm^{-1}; MS (ESI): $m/z = 255$ ($[MH]^+$).

(Z)-3-[(4-Chlorophenyl)amino]-1-(pyridin-4-yl)prop-2-en-1-one (**3d**, $C_{14}H_{11}ClN_2O$)
Yellow solid; yield: 245 mg (95%); m.p.: 122–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.01$ (d, 1H, $J = 7.8$ Hz, H_α), 7.07 (d, 2H, $J = 8$ Hz, ArH), 7.33 (d, 2H, $J = 8$ Hz, ArH), 7.54 (dd, 1H, CH, J = 7.8, 12.4 Hz, H_β), 7.74 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.76 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 12.23 (d, 1H, $J = 12.4$ Hz, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 93.7$ (CH-α), 117.9 (2, CH-phenyl), 121.0 (2, CH-phenyl), 129.6 (2, CH-4-pyridyl), 129.9 (2, CH-4-pyridyl), 138.3 (Cq), 145.7 (Cq), 146.2 (Cq), 150.0 (CH-β), 188.7 (CO) ppm; IR (KBr): $\bar{\nu} = 1299$ (C–N), 1535 (C=N), 1645 (C=O), 3353 (NH) cm^{-1}; MS (ESI): $m/z = 259$ ($[MH]^+$).

(Z)-3-[(4-Bromophenyl)amino]-1-(pyridin-4-yl)prop-2-en-1-one (**3e**, $C_{14}H_{11}BrN_2O$)
Yellow solid; yield: 291 mg (96%); m.p.: 140–141 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.02$ (d, 1H, $J = 7.8$ Hz, H_α), 7.01 (d, 2H, $J = 8$ Hz, ArH), 7.48 (d, 2H, $J = 8$ Hz, ArH), 7.53 (dd, 1H, $J = 7.8$,12.4 Hz, H_β), 7.71 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.76 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 12.21 (d, 1H, $J = 12.4$ Hz, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 93.8$ (CH-α), 117.0 (Cq), 118.2 (2, CH-phenyl), 120.7 (2, CH-phenyl), 132.8 (2, CH-4-pyridyl), 138.8 (Cq), 145.3 (Cq), 145.9 (CH-β), 150.5 (2, CH-4-pyridyl), 189.0 (CO) ppm; IR (KBr): $\bar{\nu} = 1291$ (C–N), 1568 (C=N), 1630 (C=O), 3347 (NH) cm^{-1}; MS (ESI): $m/z = 303$ (M^+).

(Z)-3-[(3-Hydroxyphenyl)amino]-1-(pyridin-4-yl)prop-2-en-1-one (**3f**, $C_{14}H_{12}N_2O_2$)
Yellow solid; yield: 226 mg (94%); m.p.: 217–218 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.12$ (d, 1H, $J = 7.8$ Hz, H_α), 6.45 (d, 1H, $J = 9.6$ Hz, ArH), 6.60 (d, 1H, $J = 8.2$ Hz, ArH), 6.71 (s, br, 1H, OH), 6.78 (s, 1H, ArH), 7.08–7.12 (m, 1H, ArH), 7.69 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 7.81 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 7.95 (dd, 1H, $J = 7.8$, 12.8 Hz, H_β), 12.03 (d, 1H, $J = 12.8$ Hz, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 93.3$ (CH-α), 104.1 (CH-phenyl), 108.0 (CH-phenyl), 111.9 (CH-phenyl), 131.1 (2, CH-4-pyridyl), 141.2 (CH-phenyl), 145.4 (Cq), 146.4 (Cq), 148.0 (CH-β), 150.9 (2, CH-4-pyridyl), 159.1 (Cq), 187.9 (CO) ppm; IR (KBr): $\bar{\nu} = 1273$ (C–N), 1528 (C=N), 1630 (C=O), 3382 (NH) cm^{-1}.

(Z)-3-[(4-Nitrophenyl)amino]-1-(pyridin-4-yl)prop-2-en-1-one (**3g**, $C_{14}H_{11}N_3O_3$)
Yellow solid; yield: 227 mg (85%); m.p.: >280 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.30$ (d, 1H, $J = 7.8$ Hz, H_α), 7.34 (d, 2H, $J = 8$ Hz, ArH), 7.70 (d, 2H, $J = 8$ Hz, ArH), 7.84 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.18 (dd, 1H, $J = 7.8$, 12.8 Hz, H_β), 8.73 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 12.0 (d, 1H, $J = 12.8$ Hz, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 101.5$ (CH-α), 116.0 (2, CH-phenyl), 117.0 (Cq), 121.5 (2, CH-phenyl), 126.3 (2, CH-4-pyridyl), 144.6 (Cq), 145.6 (Cq), 147.3 (CH-β), 151.1 (2, CH-4-pyridyl), 187.6 (CO) ppm; IR (KBr): $\bar{\nu} = 1243$ (C–N), 1584 (C=N), 1645 (C=O), 3367 (NH) cm^{-1}.

General procedure for the synthesis of (Z)-3-(aralkylamino)-1-(pyridin-4-yl)prop-2-en-1-ones ***3h–3j***
To a solution of **2** (1 mmol) in 3 cm^3 ethanol, aliphatic amines (1.5 mmol in case of phenylethyl amine and 3 mmol in case of methyl and ethyl amine) was added and the resulting mixture was refluxed for 50–68 h. After completion of the reaction (monitored by thin-layer chromatography), ethanol was distilled off to give a gum, which was dissolved in 3 cm^3 chloroform. This solution was washed with water (2 × 2 cm^3), dried over anhydrous sodium sulfate and, after removal of chloroform, the

practically pure products **3h** and **3j** (gum) were obtained in 81–92% overall yields. These were further purified by column chromatography (silica gel, 20% EtOAc–hexane). In case of **3i**, the gum on trituration with hexane gave a practically pure solid mass in 91% yield which was further purified by column chromatography (silica gel, 20% EtOAc–hexane).

(Z)-3-(Ethylamino)-1-(pyridin-4-yl)prop-2-en-1-one (**3i**, $C_{10}H_{12}N_2O$)
Yellow solid; yield: 160 mg (91%); m.p.: 66–67 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.25$–1.32 (t, 3H, CH_3), 3.36–3.41 (m, 2H, CH_2), 5.67 (d, 1H, $J = 7.4$ Hz, H_α), 7.06 (dd, 1H, $J = 7.4$, 13.2 Hz, H_β), 7.67 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.67 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 10.55 (s, brd, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.2$ (CH_3), 44.1 (CH_2), 89.9 (CH-α), 120.9 (2, CH-4-pyridyl), 146.7 (Cq), 149.7 (2, CH-4-pyridyl), 155.2 (CH-*β*), 186.9 (CO) ppm; IR (KBr): $\bar{\nu} = 1289$ (C–N), 1562 (C=N), 1630 (C=O), 3436 (NH) cm^{-1}; MS (ESI): $m/z = 177$ ($[MH]^+$).

(Z)-3-(Phenethylamino)-1-(pyridin-4-yl)prop-2-en-1-one (**3j**, $C_{16}H_{16}N_2O$)
Yellow gum; yield: 237 mg (94%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.90$–2.93 (t, 2H, CH_2), 3.54–3.58 (t, 2H, CH_2), 5.61 (d, 1H, $J = 6.9$ Hz, H_α), 6.87 (dd, 1H, $J = 6.9$, 13.2 Hz, H_β), 7.19 (d, 2H, $J = 8$ Hz, ArH), 7.25–7.27 (m, 1H, ArH), 7.30–7.32 (m, 2H, ArH), 7.67 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.68 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 10.58 (s, brd, NH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 37.6$ (CH_2), 51.1 (CH_2), 90.1 (CH-α), 121.1 (2, CH-phenyl), 126.9 (2, CH-phenyl), 128.8 (CH-phenyl), 128.9 (2, CH-4-pyridyl), 137.8 (Cq), 146.8 (Cq), 149.7 (2, CH-4-pyridyl), 155.6 (CH-β), 187.1 (CO) ppm; IR (KBr): $\bar{\nu} = 1284$ (C–N), 1571 (C=N), 1632 (C=O), 3456 (NH) cm^{-1}; MS (ESI): $m/z = 252$ (M^+).

*General procedure for the synthesis of [3-(adamantan-1-yl)-1-alkyl/aralkyl/aryl-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanones **5a–5j***
A mixture of 1-adamantanamine (**4**, 1 mmol) and formaldehyde (2 mmol) in 1 cm^3 of methanol was stirred at room temperature for 5–10 min. To this was added a solution of the enaminone **3** (1 mmol) in 4 cm^3 of methanol and the resulting solution was refluxed for 2–10 h. On completion of the reaction (monitored by thin-layer chromatography, TLC), methanol was distilled off to give a gum, which was dissolved in dichloromethane, dried over sodium sulfate and then the solvent was removed again to give a gum. This gum, on trituration with hexane, gave a solid which was collected by filtration. A practically pure product was thus obtained in 91–98% overall yields. It was further purified using column chromatography (silica gel, 20% EtOAc–hexane).

[3-(Adamantan-1-yl)-1-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone (**5a**, $C_{26}H_{29}N_3O$)
Yellow solid; yield: 391 mg (98%); m.p.: 118–120 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.58$–1.65 (m, 6H, adam), 1.68–1.80 (m, 6H, adam), 2.18 (s, 3H, adam), 3.92 (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 6.95 (d, 2H, $J = 8$ Hz, ArH), 7.12–7.16 (t, 1H, ArH), 7.33(d, 2H, $J = 6$ Hz, 4-pyridyl-H), 7.36–7.40 (m, 3H, 2H-ArH, 1H-C_6H), 8.68 (d, 2H, $J = 6$ Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 29.7$ (3, CH, adam), 36.5 (3, CH, adam), 40.1 (3, CH, adam), 41.0 (Cq, adam), 54.9 (CH_2), 62.5 (CH_2), 113.3 (Cq), 118.6 (2, CH-phenyl), 122.4 (2, CH-phenyl), 124.7 (CH-phenyl), 129.8 (2, CH-4-pyridyl), 134.5 (Cq), 143.6 (Cq), 147.3 (CH-tetrahydropyrimidine), 149.9 (2, CH-4-pyridyl), 190.5 (CO) ppm; IR (KBr): $\bar{\nu} = 1276$ (C–N), 1498 (C=N), 1574 (CO), 2849 (adam), 2906 (adam) cm^{-1}; MS (ESI): $m/z = 399$ (M^+).

[3-(Adamantan-1-yl)-1-(4-methoxyphenyl)-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone (**5b**, $C_{27}H_{31}N_3O_2$)
Pale yellow solid; yield: 420 mg (98%); m.p.: 112–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.58$–1.68 (m, 6H, adam), 1.80–1.89 (m, 6H, adam), 2.10 (s, 3H, adam), 3.78 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2), 4.60 (s, 2H, CH_2), 6.87 (d, 2H, $J = 8$ Hz, ArH), 6.91 (d, 2H, $J = 8$ Hz, ArH), 7.28 (s, 1H, C_6H), 7.38 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.65 (d, 2H, $J = 6$ Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 29.6$ (3, CH, adam), 36.5 (3, CH, adam), 40.0 (3, CH, adam), 40.8 (Cq, adam), 55.0 (OCH_3), 55.6 (CH_2), 63.1 (CH_2), 114.6 (Cq), 115.0 (2, CH-phenyl), 120.9 (2, CH-phenyl), 121.4 (Cq), 122.4 (2, CH-4-pyridyl), 137.2 (Cq), 147.5 (Cq), 148.2 (CH-tetrahydropyrimidine), 149.9 (2, CH-4-pyridyl), 190.1 (CO) ppm; IR (KBr): $\bar{\nu} = 1271$ (C–N), 1511 (C=N), 1574 (CO, 2849 (adam), 2906 (adam) cm^{-1}; MS (ESI): $m/z = 429$ (M^+).

[3-(Adamantan-1-yl)-1-(4-chlorophenyl)-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone (**5c**, $C_{26}H_{28}ClN_3O$)
Pale yellow solid; yield: 416 mg (96%); m.p.: 165–166 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.60$–1.68 (m, 6H, adam), 1.77–1.78 (m, 6H, adam), 2.10 (s, 3H, adam), 3.91 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 6.86 (d, 2H, $J = 8$ Hz, ArH), 7.28–7.31 (m, 3H, 2H-ArH, 1H-C_6H), 7.38 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.68 (d, 2H, $J = 6$ Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 29.6$ (3, CH, adam), 36.5 (3, CH, adam), 40.2 (3, CH, adam), 40.9 (Cq, adam), 54.9 (CH_2), 62.6 (CH_2), 114.0 (Cq), 119.6 (2, CH-phenyl), 122.3 (2, CH-phenyl), 129.9 (Cq), 130.0 (2, CH-4-pyridyl), 142.2 (Cq), 146.6 (CH-tetrahydropyrimidine),

147.1 (Cq), 150.0 (2, CH-4-pyridyl), 190.6 (CO) ppm; IR (KBr): $\bar{\nu}$ = 1271 (C-N), 1496 (C=N), 1580 (CO), 2849 (adam), 2906 (adam) cm^{-1}; MS (ESI): m/z = 433 (M^+).

[3-(Adamantan-1-yl)-1-(4-bromophenyl)-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone
(**5d**, $C_{26}H_{28}$ BrN_3O)
Pale yellow solid; yield: 459 mg (96%); m.p.: 170 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.58–1.65 (m, 6H, adam), 1.69–1.79 (m, 6H, adam), 2.11 (s, 3H, adam), 3.92 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 6.82 (d, 2H, J = 8 Hz, ArH), 7.31 (s, 1H, C_6H), 7.38 (d, 2H, J = 6 Hz, 4-pyridyl-H), 7.45 (d, 2H, J = 8 Hz), 8.68 (d, 2H, J = 6 Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.6 (3, CH, adam), 36.4 (3, CH, adam), 40.1 (3, CH, adam), 41.0 (Cq, adam), 54.7 (CH_2), 62.4 (CH_2), 114.1 (Cq), 118.0 (Cq), 120.1 (2, CH-phenyl), 122.3 (2, CH-phenyl), 132.9 (2, CH-4-pyridyl), 142.6 (Cq), 146.4 (CH-tetrahydropyrimidine), 146.9 (Cq), 150.0 (2, CH-4-pyridyl), 190.7 (CO) ppm; IR (KBr): $\bar{\nu}$ = 1272 (C–N), 1493 (C=N), 1578 (CO), 2848 (adam), 2907 (adam) cm^{-1}; MS (ESI): m/z = 478 (M^+).

[3-(Adamantan-1-yl)-1-(p-tolyl)-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone (**5e**, $C_{27}H_{31}N_3O$)
Yellow solid; yield: 401 mg (97%); m.p.: 150–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.53–1.65 (m, 6H, adam), 1.68–1.81 (m, 6H, adam), 2.10 (s, 3H, adam), 2.31 (s, 3H, CH_3), 3.92 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 6.83 (d, 2H, J = 8 Hz, ArH), 7.12 (d, 2H, J = 8 Hz, ArH), 7.32 (s, 1H, C_6H), 7.36 (d, 2H, J = 6 Hz, 4-pyridyl-H), 8.66 (d, 2H, J = 6 Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.8 (CH_3), 29.7 (3, CH, adam), 36.5 (3, CH, adam), 40.1 (3, CH, adam), 40.9 (Cq, adam), 55.0 (CH_2), 62.7 (CH_2), 112.6 (Cq), 118.8 (2, CH-phenyl), 122.4 (2, CH-phenyl), 130.3 (2, CH-4-pyridyl), 134.7 (Cq), 141.3 (Cq), 147.3 (Cq), 147.6 (CH-tetrahydropyrimidine), 149.9 (2, CH-4-pyridyl), 190.3 (CO) ppm; IR (KBr): $\bar{\nu}$ = 1268 (C–N), 1513 (C=N), 1571 (CO), 2850 (adam), 2902 (adam) cm^{-1}; MS (ESI): m/z = 413 (M^+).

[3-(Adamantan-1-yl)-1-(4-nitrophenyl)-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone
(**5f**, $C_{26}H_{28}N_4O_3$)
Yellow solid; yield: 400 mg (90%); m.p.: 205–207 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.49–1.53 (m, 6H, adam), 1.64 (s, 6H, adam), 1.98 (s, 3H, adam), 3.72 (s, 2H, CH_2), 4.70 (s, 2H, CH_2), 7.32 (d, 2H, J = 8 Hz, ArH), 7.47 (d, 2H, J = 6 Hz, 4-pyridyl-H), 7.51 (s, 1H, C_6H), 8.14 (d, 2H, J = 8, ArH), 8.65 (d, 2H, J = 6 Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.6 (3, CH, adam), 36.5 (3, CH, adam), 39.8 (3, CH, adam), 40.4 (Cq, adam), 52.3 (CH_2), 64.6 (CH_2), 115.2 (Cq), 118.0 (Cq), 122.9 (2, CH-phenyl), 124.7 (2, CH-phenyl), 136.2 (2, CH-4-pyridyl), 141.6 (Cq), 144.5 (Cq), 148.0 (CH-tetrahydropyrimidine), 150.5 (2, CH-4-pyridyl), 190.5 (CO) ppm; IR (KBr): $\bar{\nu}$ = 1257 (C–N), 1497 (C=N), 1580 (CO), 2852 (adam), 2906 (adam) cm^{-1}; MS (ESI): m/z = 444 (M^+).

[3-(Adamantan-1-yl)-1-methyl-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone (**5g**, $C_{21}H_{27}N_3O$)
Light brown solid; yield: 307 mg (91%); m.p.: 136–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.65–1.68 (m, 6H, adam), 1.82–1.83 (m, 6H, adam), 2.13 (s, 3H, adam), 2.96 (s, 3H, CH_3), 3.75 (s, 2H, CH_2), 4.06 (s, 2H, CH_2), 6.88 (s, 1H, C_6H), 7.32 (d, 2H, J = 6 Hz, 4-pyridyl-H), 8.64 (d, 2H, J = 6 Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7 (3, CH, adam), 36.6 (3, CH, adam), 39.6 (CH_3), 39.9 (3, CH, adam), 40.8 (Cq, adam), 54.6 (CH_2), 62.7 (CH_2), 108.6 (Cq), 122.4 (2, CH-4-pyridyl), 148.0 (Cq), 149.7 (CH-tetrahydropyrimidine), 152.1 (2, CH-4-pyridyl), 188.6 (CO) ppm; IR (KBr): $\bar{\nu}$ = 1284 (C–N), 1559 (C=N), 1622 (CO), 2851 (adam), 2909 (adam) cm^{-1}; MS (ESI): m/z = 337 (M^+).

[3-(Adamantan-1-yl)-1-ethyl-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone (**5h**, $C_{22}H_{29}N_3O$)
Light brown solid; yield: 337 mg (96%); m.p.: 112–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.18–1.25 (t, 3H, CH_3), 1.66–1.72 (m, 6H, adam), 1.85–2.02 (m, 6H, adam), 2.14 (s, 3H, adam), 3.20–3.26 (q, 2H, CH_2), 3.77 (s, 2H, CH_2), 4.11 (s, 2H, CH_2), 6.96 (s, 1H, C_6H), 7.33 (d, 2H, J = 6 Hz, 4-pyridyl-H), 8.66 (d, 2H, J = 6 Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1 (CH_3), 29.6 (3, CH, adam), 35.4 (3, CH, adam), 36.5 (3, CH, adam), 39.3 (Cq, adam), 49.1 (CH_2), 54.7 (CH_2), 62.8 (CH_2), 122.5 (Cq), 123.3 (2, CH-4-pyridyl), 141.3 (Cq), 149.5 (CH-tetrahydropyrimidine), 152.6 (2, CH-4-pyridyl), 190.7 (CO) ppm; IR (KBr): $\bar{\nu}$ = 1261 (C–N), 1559 (C=N), 1638 (CO), 2851 (adam), 2911 (adam) cm^{-1}; MS (ESI): m/z = 351 (M^+).

[3-(Adamantan-1-yl)-1-phenethyl-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone (**5i**, $C_{28}H_{33}N_3O$)
White solid; yield: 397 mg (93%); m.p.: 187–188 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.62–1.68 (m, 6H, adam), 1.71–1.82 (m, 6H, adam), 2.13 (s, 3H, adam), 2.81–2.85 (t, 2H, CH_2), 3.42–3.45 (t, 2H, CH_2), 3.70 (s, 2H, CH_2), 4.11 (s, 2H, CH_2), 6.58 (s, 1H, C_6H), 7.01 (d, 2H, J = 6 Hz, 4-pyridyl-H), 7.16 (d, 2H, J = 8 Hz, ArH), 7.32–7.34 (m, 3H, ArH), 8.54 (d, 2H, J = 6 Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9 (CH_2), 29.4 (CH_2), 29.6 (3, CH, adam), 36.1 (3, CH, adam), 43.4 (3, CH, adam), 53.5 (Cq, adam), 55.3 (CH_2), 62.4 (CH_2), 92.7 (CH-phenyl), 108.5 (Cq), 113.3 (2, CH-phenyl), 118.7 (2, CH-phenyl), 128.6 (2, CH-4-pyridyl), 133.3 (Cq), 149.6 (2, CH-4-pyridyl), 161.4 (Cq), 164.3 (CH-tetrahydropyrimidine), 186.2 (CO) ppm; IR (KBr): $\bar{\nu}$ = 1224 (C–N), 1557

(C=N), 1618 (CO), 2852 (adam), 2901 (adam) cm^{-1}; MS (ESI): $m/z = 427$ (M^+).

[3-(Adamantan-1-yl)-1-(3-hydroxyphenyl)-1,2,3,4-tetrahydropyrimidin-5-yl](pyridin-4-yl)methanone
(**5j**, $C_{26}H_{29}N_3O_2$)
Yellow solid; yield: 390 mg (94%); m.p.: 116–118 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.60$–1.67 (m, 6H, adam), 1.78–2.02 (m, 6H, adam), 2.17 (s, 3H, adam), 3.89 (s, 2H, CH_2), 4.19 (s, 1H, OH), 4.62 (s, 2H, CH_2), 6.45 (d, 1H, $J = 8$, ArH), 6.52 (s, 1H, C_6–H), 6.63 (d, 1H, $J = 8$ Hz, ArH), 7.12–7.18 (m, 1H, ArH), 7.34 (s, 1H, ArH), 7.40 (d, 2H, $J = 6$ Hz, 4-pyridyl-H), 8.61 (d, 2H, $J = 6$ Hz, 4-pyridyl-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 29.5$ (3, CH, adam), 36.4 (3, CH, adam), 39.9 (3, CH, adam), 40.8 (Cq, adam), 54.8 (CH_2), 62.4 (CH_2), 105.9 (CH-phenyl), 108.4 (CH-phenyl), 112.0 (CH-phenyl), 113.3 (Cq), 122.7 (2, CH-4-pyridyl), 130.6 (Cq), 144.2 (CH-phenyl), 147.6 (Cq), 147.7 (CH-tetrahydropyrimidine), 149.3 (2, CH-4-pyridyl), 158.3 (Cq, C–OH), 190.5 (CO) ppm; IR (KBr): $\bar{\nu} = 1296$ (C–N), 1542 (C=N), 1574 (CO), 2851 (adam), 2911 (adam) cm^{-1}; MS (ESI): $m/z = 415$ (M^+).

Anti-inflammatory assay

Materials
Griess reagent system was procured from Promega (USA); Wright stain, anhydrous potassium dihydrogen phosphate, potassium chloride, disodium hydrogen phosphate, sodium-EDTA, and dimethyl sulfoxide (DMSO) were purchased from Hi-MEDIA. Methanol was purchased from Merck and Freund's Complete Adjuvant (FCA) from Genei.

Methodology
Anti-inflammatory activities of the test compounds were studied by measuring paw diameter, NO assay in blood and in paw exudates and by performing a differential WBC count in mice carrying FCA-induced paw edema and subsequently treating with test compounds.

1. *Induction of paw edema* The anti-inflammatory activities of the test compounds were determined by following the method of Lai et al. [50] with little modification. Swiss Albino mice aged between 8 and 10 weeks of either sex (3 per group) maintained at controlled temperature with 12 h light/12 h dark conditions, provided with standard mice feed and common tap drinking water, were used in all experiments. About 50 mm^3 of the Fruend's Complete Adjuvant (FCA) was injected into the plantar side of the left hind paws of the mice [51].

The paw diameter of the FCA-induced edema of mice was measured at 0, 1, 3, and 24 h after the administration of the FCA using calipers. Test compounds (dissolved in 10% DMSO) were administered 1 h after FCA injection. The percentage increase/decrease of the paw edema is calculated by the formula $\frac{b-a}{a} \times 100$, where 'a' is the paw diameter at 0 h and 'b' is the paw diameter at different time intervals.

After 24 h, blood was collected by retro-orbital bleeding and used for estimating NO and preparing blood smear. Mice were then killed by cervical dislocation. The left hind paw tissue was excised, rinsed with ice-cold normal saline and homogenized in 1 cm^3 of cold normal saline. The homogenate was then centrifuged at 12,000 rpm for 5 min and the supernatant thus obtained was used for NO assay. Same protocol was followed for positive control (ibuprofen).

2. *NO assay* NO^{2-} was measured by using the Griess reaction. The assay of NO^{2-} was performed according to the manufacturer's instruction. Three columns in the 96-well plate were designated for the nitrite standard reference curve. Six serial twofold dilution of 100 μM nitrite solution (50 mm^3/well) in triplicate was performed to generate the nitrite standard reference curve.

50 mm^3 of the experimental sample was taken in triplicate in test wells. To all wells, 50 mm^3 of sulfanilamide solution was added and incubated for 5–10 min at room temperature protected from light. Thereafter, 50 mm^3 of the *N*-1 naphthyl ethylenediamine dihydrochloride (NED) solution was dispensed to all wells. The plate was incubated at room temperature for 5–10 min, protected from light. A purple/magenta color began to form immediately. The absorbance was measured within 30 min in a plate reader at 520 nm.

The concentration of NO in experimental samples was calculated from the standard curve obtained from above.

3. *Differential WBC count* The differential WBC count was performed according to the method described by Houwen [52]. The blood film was prepared on glass slides by the wedge method and air dried. The blood films were fixed for 30 s in absolute methanol. Slides were stained for 2 min with Wright's stain and an aliquot of Sorensen's buffer was added, mixed and allowed to stand for 3 min. Slides were rinsed with distilled water and air dried. The prepared slides were viewed under a microscope and WBCs were counted.

Antibacterial assay

Materials
Four strains of bacteria (*B. subtilis* MTCC Code-121, *S. aureus* MTCC Code-9886, *E. coli* MTCC Code-1302) and

S. enterica MTCC Code-3232) were obtained from the Institute of Microbial Type Culture (IMTECH), Chandigarh, India. Muellar Hinton Agar, Nutrient Broth, HiAntibiotic ZoneScale™, and Agar–Agar were purchased from Hi-Media and ampicillin was obtained from an indigenous source.

Methodology

The antibacterial activities of the tested compounds **3a–3j** and **5a–5j** were determined against four clinical strains such as *B. subtilis* (MTCC Code-121), *S. aureus* (MTCC Code-9886), *E. coli* (MTCC Code-1302), and *S. enterica* (MTCC Code-3232). The lyophilized cultures were obtained from the Institute of Microbial Type Culture (IMTECH). A loopful of each of the lyophilized strains was inoculated in 50 cm^3 Nutrient Broth in a conical flask and incubated on a rotatory shaker for 24 h to activate them. The test was performed using the cup and saucer method [53]. Muellar Hinton Agar was prepared and sterilized for the study. The experiment was performed under strict aseptic conditions. The sterilized media was poured into Petri dishes (Hi-Media) and allowed to solidify. The agar solution (0.7%) was prepared and sterilized, and the test strains were inoculated into the agar solution at around 40 °C. Care was taken to ensure proper homogenization. The suspension was poured over the solidified media. Once this layer solidified, wells were punched with the help of a gel puncher (0.6 cm). The test compounds were diluted to 4 mg/cm^3 in filter-sterilized 10% dimethyl sulfoxide (DMSO). The diluted test compounds were introduced into the wells and the Petri dishes were incubated overnight in an upright position at 37 °C. The antibiotic ampicillin was used as a positive control in the concentration of 4 mg/cm^3 in 10% DMSO. As a negative control, 10% DMSO was also used. The inhibition zone was measured by using HiAntibiotic ZoneScale and the mean value was calculated.

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