Synthesis and Biological Evaluation of Amide Derivatives of (6-Chloro-2,3-dihydro-1*H*-inden-1-yl)acetic Acid as Potential Anti-inflammatory Agents with Lower Gastrointestinal Toxicity

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A variety of amide derivatives of (6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid were synthesized and screened for their anti-inflammatory and related biological activities. These compounds were found to be longer acting and showed residual activity exceeding that of standard indomethacin. The studies with SKF-525A, a standard hepatic microsomal enzyme inhibitor showed that probably the test compound *per se* is the active species. The compound 6y showed best activity profile with ED_{30} of 6.45 mg/kg however this compound was found to be toxic at 100 mg/kg *p.o.* Though these compounds exhibited appreciable analgesic and antipyretic activities but they failed to prevent the development of secondary inflammation in adjuvant induced arthritis assay. The compound 6x showed 94% inhibition of acetic acid induced writhing. Studies showed that antagonism of TNF-α is not possibly involved in the mechanism of action of these compounds. However these compounds were found to have only mild ulcerogenic potential at the tested dose level of 100 mg/kg *p.o.* in comparison to indomethacin.

Key words Indene; amide derivative; anti-inflammatory; ulcerogenecity

Arylalkanoic acids are among the most widely investigated compounds in the area of non-steroidal anti-inflammatory agents. Features of a typical molecule, which are important for activity, include a carboxyl group separated by one or more carbon atoms from a flat aromatic nucleus that is further substituted by a lipophilic group. The lead compound for aryl acetic acids is ibufenac from which ibuprofen and (*S*)-(+)-2-(3-chloro-4-cyclohexylphenyl) propionic acid have come up.[2)] Ring chain modification of the aryl propionic acid has led to development of indan moiety (Fig. 1). Indan ring system has been found to act as an inert carrier, which serves to hold biologically active functional moieties in a stereospecific manner.[3)] This congener of indene is isosteric and bioisosteric with indolidine and indole respectively that are also pharmacologically active chemical nuclei.

Among the various indan-1-alkanoic acids[2,4,5)] prepared as potential anti-inflammatory agents some of the compounds not only showed anti-inflammatory activity comparable to Phenylbutazone but were also found to be much less ulcerogenic. The research in this area led to development of Clidanac,[2)] having ED_{30} of 0.85 mg/kg, which showed better gastrointestinal tolerability. An isomer of Clidanac, 6-chloro-5-(cyclopentylmethyl)indan-1-carboxylic acid was also devoid of any mucosal damage in the rat stomach upto highest tested dose of 400 mg/kg.[6)]

It has been shown that the biochemical differences between the cyclooxygenase (COX) isoforms can be exploited to improve upon the selectivity of carboxyl containing non-steroidal anti-inflammatory drugs (NSAIDs) as COX-2 inhibitors. Successful transformation of indomethacin and meclofenamic acid into selective COX-2 inhibitors by a single chemical derivatisation (amidation or esterification) has

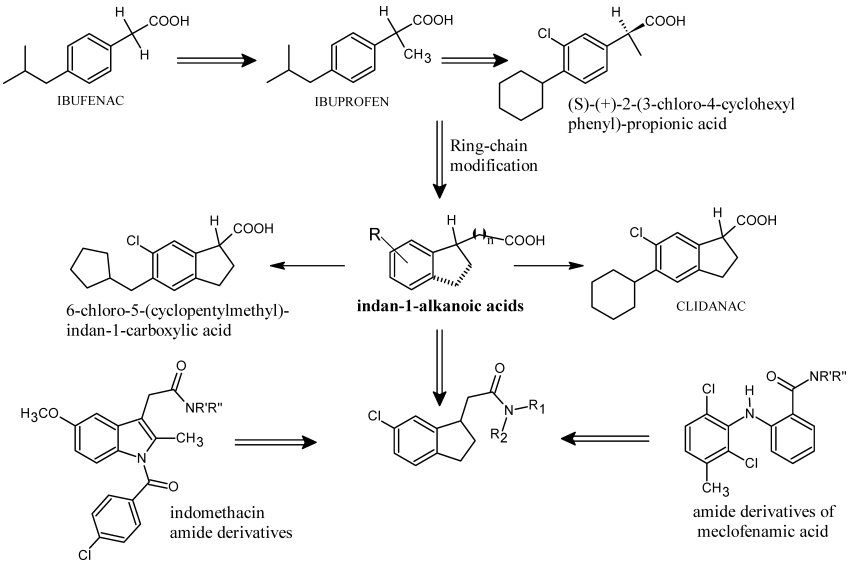

Fig. 1. Design of Amide Derivatives of (6-Chloro-2,3-dihydro-1*H*-inden-1-yl)acetic Acid

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been reported.[7] Structurally diverse indomethacin amides inhibited purified human COX-2 with IC_{50} values in the low nanomolar range but did not inhibit ovine COX-1 activity at concentrations as high as 66 mM.[8]

Keeping the above points in view, we designed (Fig. 1), synthesized and biologically evaluated some amide derivatives of 6-chloroindan-1-acetic acid for anti-inflammatory and related biological activities. The idea behind derivatisation was to further lower the side effects of gastric irritation and ulceration, which is associated with free carboxyl group. Neutralization of the carboxyl group by amidation was expected to increase the activity profile of indan-1-alkanoic acids by not only enhancing absorption due to increased lipophilicity but also by imparting COX-2 selectivity.

Chemistry The starting reagent for the preparation of the key intermediate, (6-chloro-2,3-dihydro-1*H*-inden-1-yl)-acetic acid (**5**) was 3-chlorobenzaldehyde (**1**). Compound **1** was reacted with two moles of ethylacetoacetate (EAA) in presence of catalytic amount of piperidine at room temperature to obtain the substituted phenylbisacetoacetate (**2**). Acid hydrolysis (end product is acid) of **2** was carried out with 6N potassium hydroxide (KOH) in 50% ethanol to get the diacid **3**. The IR spectra of **3** showed broad OH stretch at 3400—2200 cm^{-1} and C=O stretch at 1710 cm^{-1}. ^{1}H-NMR of **3** showed doublet of doublet at δ 2.58 and 2.70 ppm as well as quintet at 3.60 ppm for CH_2 and CH of glutaric acid side chain respectively, multiplet at δ 7.18 ppm for aromatic protons and small broad singlet at δ 10.79 ppm indicating the presence of carboxyl group.

Compound **3** was treated with aluminium chloride in the presence of catalytic amounts of sodium chloride when intramolecular Friedel-Craft's cyclization and hence ring closure took place to give the ketonic product **4**. The cyclization was also tried with polyphosphoric acid but it gave poorer yields of keto product with more impurities. Presence of doublet of doublet at 2.46 and 2.58 ppm and doublet of doublet at 2.84 and 2.99 corresponding to CH_2 of side chain and CH_2 of the indan ring respectively as well as the presence of quintet at 3.79 ppm indicates the ring closure. The presence of aromatic protons (doublets at 7.38 and 7.65 ppm and singlet at 7.59) and acid protons (broad singlet at 10.91 ppm) further supports the proposed structure of compound **4**.

The ketone group of compound **4** was finally removed using Clemmensen's reduction to give (6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid **5**. Because of the different conformational arrangement of proton present on CH of indan ring both neighbouring CH_2 peaks got split into two peaks (two doublets of doublet at 2.48, 2.81 ppm and two multiplets at 1.78, 2.42 ppm). The presence of multiplet at 2.88 ppm equivalent to two protons in addition to peaks obtained for compound **5** indicates the reduction of carbonyl group and formation of indan ring system. The presence of aromatic protons (multiplet at 7.11—7.16 ppm) and proton of COOH group (small broad singlet at 11.18 ppm) further supports the structure of compound **5**. Compound **5** was treated with oxalyl chloride in presence of catalytic amount of dimethylformamide to convert carboxyl group of **5** into acyl halide. The acyl halide thus obtained was not isolated or characterized. It was used directly in the next step. The acyl halide was reacted with various primary and secondary amines under Schotten-Baumann conditions for the formation of desired amides **6a**—**y**. The structure of amide derivatives were confirmed by the presence of two, one or no peak around 3300 cm^{-1} (NH stretch) corresponding to primary, secondary and tertiary amides. Also disappearance of broad OH stretch at 3400—2400 cm^{-1} indicates the formation of amide bond. The formations of amides were further confirmed by presence of a small singlet at 5—8 ppm in ^{1}H-NMR. In most of the cases there was an overlap of two peaks at around 2.3—2.5 ppm corresponding to doublet of doublet and multiplet. Even for most of the aromatic amides, the NH peak was mixed with the aromatic peaks at around 7.1—7.3 ppm.

Results and Discussion

Anti-inflammatory Activity The anti-inflammatory activity of the test compounds was evaluated by carrageenan-induced rat paw edema model. The results (Table 1) show that the test compounds exhibit variable anti-inflammatory activity, and a few among them have significant acute as well as residual anti-inflammatory activity at 24 h after a single oral dose of 100 mg/kg *p.o.* Most of these amide derivatives were found to have more activity than the parent acid. The compounds were slow acting and showed longer duration of action. The peak activity of the test compounds was found to be lower than that of indomethacin (10 mg/kg, *p.o.*) but their residual activity at 24 h exceeded that of the latter. The lipophilicity of acid **5** is increased by amidation which leads to better absorption of these amide derivatives. Due to high lipophilicity of the amide derivatives in comparison to parent acid **5** (Table 1) these compounds are also likely to form lipid depots which might be the cause for their longer activity profile. The compounds **6a**, **6p**, **6w** showed nearly 50% inhibition as the residual activity. The lower members in the aliphatic amides (having low log P values) showed almost negligible activity; however compound **6a** was found to have good anti-inflammatory activity. That may be due to close resemblance of this compound to centrally acting muscle relaxant (*E*)-2-(4,6-difluoro-1-indanylidene)acetamide, which also possesses potent anti-inflammatory and analgesic activity. It has been reported in literature that this muscle relaxant has been taken in phase I clinical trials.[9] The tertiary amides (**6l**, **6m**) and the cyclic amides (**6i**, **6j**) showed intermediate activity. Among the aromatic series no generalized pattern of activity was found. The *m*-chloroaniline derivative (**6p**) was found to be the most active. The *p*-nitroaniline (**6w**) and *p*-toluidine (**6t**) derivatives also showed good anti-inflammatory activity profile while *p*-acetamidoaniline (**6v**) derivative showed least activity. The 4-aminopyridine derivative (**6y**) was found to be toxic at 100 mg/kg *p.o.* where as its isomer 3-aminopyridine derivative (**6x**) didn't show any toxicity at the same dose level. However at lower dose levels the compound **6y** did not show any toxicity and in fact showed best anti-inflammatory activity with ED_{30} of 6.45 mg/kg *p.o.*

Analgesic Activity The analgesic activity of the synthesized compounds at dose of 100 mg/kg *p.o.* was determined by acetic acid induced writhing assay. The results (Table 2) reveal that the beneficial effect of increasing chain length was apparent for analgesic activity to some extent with few exceptions. The compounds **6e**, **6m**, **6s** and **6x** exhibiting poor anti-inflammatory activities showed good analgesic activities while compound **6t** exhibiting good anti-inflammatory activity showed poor analgesic activity. Among the

Table 1. Carrageenan Induced Rat Paw Edema: Anti-inflammatory Activity

Compound	Increase in paw volume (ml)±S.E.M. [% inhibition of edema]						
	1 h	2 h	3 h	4 h	6 h	24 h	Log P
6a	0.25±0.0145 [19.35]*	0.26±0.0136 [46.94]*	0.32±0.0161 [48.39]*	0.38±0.0125 [41.15]*	0.32±0.0141 [37.25]*	0.13±0.0116 [55.17]*	1.92
6b	0.29±0.0149 [6.45]ns	0.45±0.0117 [8.16]ns	0.57±0.0132 [8.06]ns	0.58±0.0132 [10.77]ns	0.45±0.0128 [11.76]ns	0.25±0.0133 [13.79]ns	2..31
6c	0.28±0.0173 [9.68]ns	0.43±0.0148 [12.24]ns	0.56±0.0273 [9.68]ns	0.59±0.0273 [9.23]ns	0.45±0.0165 [11.76]ns	0.27±0.0125 [6.90]ns	2.80
6d	0.29±0.0149 [6.45]ns	0.44±0.0117 [10.20]ns	0.55±0.0132 [11.29]ns	0.58±0.0132 [10.77]ns	0.46±0.0128 [9.80]ns	0.26±0.0133 [10.34]ns	3.27
6e	0.28±0.0118 [9.68]ns	0.37±0.0166 [24.48]*	0.48±0.0134 [22.58]*	0.50±0.0125 [23.08]*	0.44±0.0145 [13.72]*	0.26±0.0108 [10.34]ns	3.07
6f	0.27±0.0124 [12.90]ns	0.42±0.0153 [14.28]ns	0.54±0.0148 [12.90]ns	0.58±0.0132 [10.77]ns	0.45±0.0173 [11.76]ns	0.26±0.0128 [10.34]ns	3.75
6g	0.26±0.0136 [16.13]ns	0.38±0.0149 [22.45]*	0.48±0.0147 [22.58]*	0.51±0.0129 [21.54]*	0.37±0.0166 [27.45]*	0.21±0.0091 [27.59]*	4.24
6h	0.24±0.0154 [22.58]*	0.34±0.0192 [30.61]*	0.42±0.0144 [32.26]*	0.46±0.0165 [29.23]*	0.35±0.0127 [31.37]*	0.20±0.0162 [31.03]*	4.75
6i	0.25±0.0116 [19.35]*	0.38±0.0137 [22.45]*	0.44±0.0147 [29.03]*	0.46±0.0132 [29.23]*	0.35±0.0151 [31.37]*	0.19±0.0131 [34.48]*	3.74
6j	0.27±0.0143 [12.90]ns	0.40±0.0161 [18.37]*	0.48±0.0153 [22.58]*	0.50±0.0136 [23.08]*	0.41±0.0163 [19.61]*	0.20±0.0085 [31.03]*	4.24
6k	0.23±0.0155 [25.81]*	0.36±0.0154 [26.53]*	0.44±0.0148 [29.03]*	0.44±0.0164 [32.31]*	0.38±0.0135 [25.49]*	0.21±0.0113 [27.59]*	3.54
6l	0.28±0.0112 [9.62]ns	0.39±0.0121 [20.41]*	0.55±0.0134 [11.29]ns	0.56±0.0150 [13.85]ns	0.46±0.0088 [9.80]ns	0.23±0.0125 [20.69]*	3.82
6m	0.21±0.0138 [32.26]*	0.36±0.0118 [26.53]*	0.46±0.0136 [25.81]*	0.48±0.0132 [26.15]*	0.42±0.0158 [17.65]*	0.21±0.0138 [27.59]*	2.00
6n	0.29±0.0178 [6.45]ns	0.43±0.0163 [12.24]ns	0.55±0.0152 [11.29]ns	0.59±0.0186 [9.23]ns	0.47±0.0159 [7.84]ns	0.27±0.0153 [6.90]ns	1.57
6o	0.20±0.0114 [35.48]*	0.31±0.0159 [36.73]*	0.42±0.0121 [32.25]*	0.48±0.0137 [26.15]*	0.42±0.0162 [17.65]*	0.25±0.0142 [13.79]ns	3.59
6p	0.15±0.0138 [51.61]*	0.25±0.0146 [48.98]*	0.31±0.0165 [50.0]*	0.36±0.0159 [44.62]*	0.31±0.0132 [39.22]*	0.13±0.0083 [55.17]*	4.53
6q	0.26±0.0133 [16.13]ns	0.32±0.0126 [34.69]*	0.40±0.0138 [35.48]*	0.48±0.0142 [26.15]*	0.40±0.173 [21.57]*	0.21±0.0121 [27.59]*	4.37
6r	0.23±0.0125 [25.81]*	0.34±0.0161 [30.61]*	0.44±0.0178 [29.03]*	0.47±0.0136 [27.69]*	0.41±0.0179 [19.61]*	0.20±0.0130 [31.03]*	4.41
6s	0.26±0.0169 [16.13]ns	0.36±0.0141 [26.53]*	0.52±0.0177 [16.13]ns	0.55±0.0134 [15.38]*	0.43±0.0156 [15.69]*	0.26±0.0144 [10.34]ns	3.90
6t	0.19±0.0143 [38.71]*	0.26±0.0187 [46.94]*	0.36±0.0151 [41.94]*	0.39±0.0149 [40.0]*	0.34±0.0164 [33.33]*	0.21±0.0126 [27.59]*	4.00
6u	0.28±0.0186 [9.68]ns	0.42±0.0162 [14.29]ns	0.53±0.0167 [14.52]ns	0.58±0.0179 [10.77]ns	0.44±0.0206 [13.72]*	0.21±0.0165 [27.59]*	3.76
6v	0.30±0.0145 [3.22]ns	0.46±0.0134 [6.12]ns	0.56±0.0129 [9.67]ns	0.59±0.0180 [9.23]ns	0.47±0.0161 [7.80]ns	0.26±0.0114 [10.34]ns	3.14
6w	0.17±0.0157 [45.16]*	0.24±0.0149 [51.02]*	0.34±0.0176 [45.16]*	0.40±0.0136 [38.46]*	0.33±0.0182 [35.29]*	0.15±0.0142 [48.28]*	3.83
6x	0.26±0.0139 [16.34]ns	0.35±0.0156 [28.57]*	0.42±0.0162 [32.26]*	0.52±0.0148 [20.0]*	0.45±0.0151 [11.76]ns	0.23±0.0112 [20.69]*	2.74
6y	toxic at 100 mg/kg						2.78
6y (25 mg/kg)	0.24±0.0142 [22.58]*	0.33±0.0136 [32.65]*	0.29±0.0154 [53.22]*	0.12±0.0147 [81.54]*	0.14±0.0135 [72.54]*	0.08±0.0123 [72.41]*	
6y (12.5 mg/kg)	0.25±0.0133 [19.35]*	0.35±0.0148 [28.57]*	0.37±0.0156 [40.32]*	0.37±0.0141 [43.08]*	0.33±0.0146 [35.29]*	0.17±0.0135 [41.38]*	
6y (6.25 mg/kg)	0.26±0.0154 [16.31]ns	0.41±0.0144 [16.33]ns	0.48±0.00138 [22.58]*	0.51±0.0157 [21.54]*	0.43±0.0151 [15.68]*	0.22±0.0143 [24.17]*	
5	0.27±0.0165 [12.90]ns	0.40±0.0129 [18.37]*	0.50±0.0146 [19.35]*	0.52±0.0158 [20.0]*	0.43±0.0143 [15.69]*	0.22±0.0126 [24.14]*	2.71
Indomethacin	0.16±0.0145 [48.39]*	0.22±0.0125 [55.10]*	0.23±0.0131 [62.90]*	0.26±0.0113 [60.0]*	0.28±0.0154 [45.10]*	0.25±0.0141 [13.79]ns	2.98
Control	0.31±0.0163	0.49±0.0159	0.62±0.0185	0.65±0.0174	0.51±0.0166	0.29±0.0156	

Data was analyzed by one way ANOVA followed by *post-hoc* test. * Represents the significance level of $p<0.05$, ns represents not significant at $p<0.05$. Each value represents the mean±S.E.M. ($n=6$). Log P values were calculated from website www.logp.com.

cyclic analogs the cyclopentyl derivative (**6i**) was equipotent to its linear counterpart, **6g** whereas the cyclohexyl derivative (**6j**) was less active than its linear counterpart (**6h**). The cyclic piperidino derivative (**6l**) showed intermediate activity while the piperazino derivative (**6m**) exhibited good analgesic properties. Among aromatic amides there was slight

Table 2. Acetic Acid-Induced Writhing Assay: Analgesic Activity

Compound	Writhing±S.E.M.	% inhibition	Compound	Writhing±S.E.M.	% inhibition
6a	11.22±0.65*	66.82	**6m**	13.33±0.80*	60.41
6b	27.74±0.87*	17.34	**6n**	25.17±0.70*	25.24
6c	26.5±0.85*	21.29	**6o**	14.17±0.95*	57.92
6d	20.5±0.76*	39.11	**6p**	15.17±0.60*	54.94
6e	19.5±0.80*	42.08	**6q**	17.33±1.05*	48.53
6f	22.17±0.95*	34.16	**6r**	16.5±0.99*	50.99
6g	20.5±1.18*	39.11	**6s**	11.33±0.71*	66.35
6h	19.33±0.99*	42.59	**6t**	23.33±0.88*	30.71
6i	20.17±0.87*	40.10	**6u**	23.83±0.91*	29.22
6j	22.33±0.88*	33.68	**6v**	19.5±0.89*	42.08
6k	22.33±1.05*	33.68	**6w**	15.67±0.76*	53.46
6l	24.5±0.78*	27.23	**6x**	2.0±0.37*	94.06
Indomethacin	15.26±0.95*	54.68	**6y**	toxic	
Aspirin	12.18±1.13*	63.82	Control	33.67±1.12	

Data was analyzed using student's *t*-test: $*p<0.005$. Each value represents the mean±S.E.M. ($n=6$).

Table 3. Lipopolysaccharide-Induced Pyresis: Antipyretic Activity

Compound	Change in rectal temperature (°C) and temperature index				
	1 h	2 h	3 h	4 h	TI
6a	−0.06±0.0125	0.05±0.0142	0.08±0.0133	0.13±0.0126	0.17
6e	0.28±0.0161	0.18±0.0130	0.13±0.0152	0.11±0.0142	0.70
6i	0.12±0.0153	0.30±0.0148	0.62±0.0128	0.71±0.0137	1.75
6m	0.28±0.0173	0.70±0.0162	0.76±0.0141	0.84±0.0148	2.58
6p	0.20±0.0141	0.11±0.0115	0.18±0.0112	0.30±0.0120	0.79
6r	0.24±0.0144	0.15±0.0136	0.11±0.0138	0.11±0.0125	0.61
6s	0.18±0.0126	0.38±0.0152	0.44±0.0134	0.56±0.0155	1.56
6t	0.37±0.0133	0.55±0.0128	0.55±0.0147	0.64±0.0134	2.11
6w	0.23±0.0136	0.42±0.0164	0.51±0.0156	0.72±0.0145	1.88
6x	0.22±0.0121	0.11±0.0142	0.16±0.0168	0.28±0.0170	0.77
Aspirin	0.05±0.0112	−0.02±0.0131	0.03±0.0124	0.11±0.0115	0.17
Indomethacin	0.25±0.0118	0.29±0.0108	0.38±0.0125	0.59±0.0110	1.51
Control	0.32±0.0179	0.71±0.0156	0.96±0.0166	1.22±0.0143	3.21

Each value represents the mean±S.E.M. ($n=6$).

decrease in activity upon halogen substitution (**6p**, **6q**, **6r**) in comparison to aniline derivative (**6o**). Though *m*-toluidine derivative (**6s**) showed poor anti-inflammatory activity than para derivative (**6t**) the results were opposite for analgesic activity. The 3-aminopyridinyl derivative (**6x**) and the amide derivative (**6a**) showed maximum inhibition of 94% and 66% respectively and were more potent than standard drugs.

Antipyretic Activity Few selected compounds were subjected to lipopolysaccharide (LPS)-induced hyperthermia assay. It is generally accepted that the NSAIDs exhibit their anti-inflammatory effect primarily through inhibition of prostaglandin synthesis. However, relatively recent *in vitro* studies have indicated that NSAIDs also interfere with peripheral proinflammatory cytokine production.[10,11] It is well known that LPS produces a biphasic response in the rat. It shows an initial hypothermia up to 3 h of its administration. It has been shown that this hypothermia is triggered by tumour necrosis factor-α (TNF-α).[12] For determining the antipyretic activity two protocols were adopted, one in which the initial hypothermic phase was excluded and other in which the hypothermic phase was also monitored in order to determine if inhibition of TNF-α is involved in mechanism of action of these compounds. The test compounds (Table 3) exhibited significant antipyretic property at the dose level of 100 mg/kg *p.o.*, as indicated by the temperature index. The compound **6a** showed antipyretic activity comparable to that of aspirin. The isopropyl (**6e**), halogen substituted amine (**6p**, **6r**), and 3-aminopyridino (**6x**) amide derivatives showed appreciable antipyretic activity. The data reveals that the test compounds exhibited significant antipyretic property, but these compounds did not exhibit antagonism of the initial LPS-induced hypothermia as indicated by first temperature index in Table 4 thus indicating that the antagonism of TNF-α is not possibly involved in the mechanism of action of these compounds.[13]

Anti-arthritic Activity Compounds were selected for adjuvant-induced arthritis assay based on the inhibition of carrageenan-induced edema, analgesic and antipyretic activity profile. Adjuvant-induced arthritis test in rats produced a biphasic response. The initial response started immediately after adjuvant administration and reached maximum on 3rd day and then it started subsiding. The 2nd phase started from the 9th day and continued till the end of the experiment (14th day). Secondary lesions (appearance of nodules, and erythema in tail, nose and ears) started developing from the 10th day. Results of this test summarized in Table 5 show that compound **6w** has the best profile in this assay. Compound **6a** and **6w** were found to be active and the animals did gain more weight than those treated with indomethacin indicating a better toxicity profile compared to the reference drug. In

Table 4. Lipopolysaccharide-Induced Pyresis Model to Study the Inhibition of TNF-α

Compound	Change in rectal temperature (°C) and temperature index								
	1 h	2 h	3 h	TI	4 h	5 h	6 h	7 h	TI
6a	−0.51±0.011	−1.37±0.013	−0.47±0.012	−2.35	0.03±0.014	0.11±0.012	0.19±0.011	0.27±0.015	0.60
6w	−0.49±0.013	−1.25±0.012	−0.35±0.016	−2.09	0.18±0.013	0.40±0.015	0.59±0.012	0.71±0.011	1.88
Aspirin	−0.43±0.014	−1.31±0.011	−0.43±0.012	−2.17	0.01±0.013	0.09±0.012	0.18±0.014	0.31±0.011	0.59
Indo.	−0.36±0.012	−1.03±0.015	−0.11±0.013	−1.50	0.17±0.012	0.28±0.013	0.41±0.013	0.55±0.014	1.41
Control	−0.49±0.013	−1.12±0.014	−0.14±0.014	−1.75	0.23±0.015	0.63±0.013	0.94±0.016	1.18±0.012	2.98

Each value represents the mean±S.E.M. (n=6); indo stands for indomethacin.

Table 5. Adjuvant-Induced Arthritis: Anti-arthritic Activity

Compound	Increase in paw volume (ml)±S.E.M. [% inhibition of edema]				Secondary lesions	Weight change (g)
	3rd day	8th day	13th day	13th day[a)]		
6a	1.65±0.0142 [30.38]*	1.56±0.0155 [26.76]*	1.45±0.0163 [35.84]	1.09±0.0164 [33.54]	Moderate	6.67±0.144
6i	2.09±0.0162 [11.81]*	1.47±0.0182 [30.99]*	1.89±0.0171 [16.37]*	1.41±0.0158 [14.02]*	Severe	4.33±0.150
6p	1.46±0.0175 [38.40]*	1.55±0.0167 [27.23]*	2.21±0.0154 [2.21]*	1.60±0.0160 [2.44]*	Severe	3.58±0.169
6q	2.23±0.0156 [5.91]*	2.03±0.0148 [4.69]*	2.19±0.0175 [3.10]*	1.61±0.0163 [1.83]*	Severe	3.25±0.181
6t	1.82±0.0158 [23.21]*	1.79±0.0162 [15.96]*	1.56±0.0188 [30.97]*	1.21±0.0171 [26.22]*	Moderate	5.98±0.142
6w	1.68±0.0176 [29.11]*	1.14±0.0153 [46.48]*	1.38±0.0144 [38.93]*	1.06±0.0157 [35.37]*	Moderate	6.92±0.158
Indomethacin	1.48±0.0154 [37.55]*	1.16±0.0176 [45.53]*	1.23±0.0187 [45.57]*	1.07±0.0172 [34.76]*	Moderate	5.42±0.133
Control	2.37±0.0170	2.13±0.0194	2.26±0.0173	1.64±0.0166	Severe	3.14±0.141

Significance level $*p<0.05$ as compared with the control. Each value represents the mean±S.E.M. (n=6); *a*) uninjected left paw.

this biomodel though compounds **6a** and **6w** significantly reduced the primary inflammation of the right hind paw but these were not able to reduce the secondary inflammation of left hind paw as well as the development of secondary lesions. Seeing the activity profile of other tested compounds it can be said that in general these compounds exhibited poor anti-arthritic activity at tested dose level of 100 mg/kg *p.o*.

Evaluation of Ulcerogenic Potential Some selected compounds (based on anti-inflammatory and analgesic activity profile) were tested for their ulcerogenecity potential. The tested compounds developed much lesser number of ulcerogenic lesions as indicated by ulcer index in comparison to the control (Table 6). The nitro derivative (**6w**) showed no ulcer at tested dose level of 100 mg/kg *p.o*. It may, however, be noted here that the administration of the test compounds at the dose level of 100 mg/kg *p.o.* even for 14 d did not cause any ulceration of the gastric mucosa as revealed in the post mortem studies of sacrificed animals at the end of the adjuvant-induced arthritis study.

Studies with SKF-525A We hypothesized that the high residual anti-inflammatory activity of compounds could be due to higher protein binding of its active metabolite(s). These metabolites may arise *via* their hydrolytic metabolism to deamidated product. We, therefore, studied the anti-inflammatory activity of compounds **6a** and **6p** in carrageenan induced rat paw edema model using SKF-525A, a standard hepatic microsomal enzyme inhibitor,[14] pretreated rats. Examination of Table 7 reveals that there is no significant difference between data generated from this test and those generated using standard protocol. This indicates that probably the test compound *per se* is the active species.

Table 6. Evaluation of Ulcer Index

Compound	Dose (mg/kg)	Time	Ulcer index
Control		6 h	0
Indomethacin	30	6 h	30.83±2.07
6a	100	6 h	2.5±0.43
6o	100	6 h	11.17±0.70
6p	100	6 h	1.83±0.60
6t	100	6 h	7.67±0.67
6w	100	6 h	0
Control		14 d	0
Indomethacin	1	14 d	33.67±5.33
6a	100	14 d	0
6w	100	14 d	0

Each value represents the mean±S.E.M. (n=6).

Acute Toxicity Study The rats employed in anti-inflammatory screening were observed during 24 h. No mortality was present with these compounds except compound **6y** at the end of observation period. The compounds **6a** and **6p** were also studied for their toxicity profile at higher dose levels. No toxicity was observed with these compounds upto the highest tested dose of 1000 mg/kg.

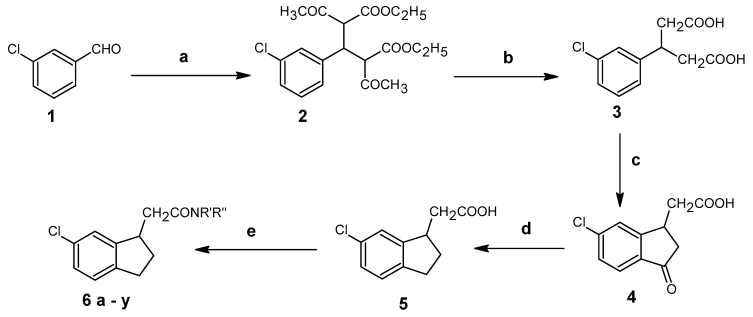
Conclusions

From the anti-inflammatory activity results it was found that compounds **6a**, **6p**, and **6w** exhibited good anti-inflammatory activity. The compound **6y** showed best anti-inflam-

Table 7. Study with Cytochrome P450 Enzyme Inhibitor

Compound	Increase in paw volume (ml)±S.E.M. [% inhibition of edema]					
	1 h	2 h	3 h	4 h	6 h	24 h
SKF-525A & **6a**	0.16±0.0135 [23.81]	0.23±0.0147 [43.90]	0.32±0.0132 [46.67]	0.34±0.0151 [40.35]	0.30±0.0142 [36.17]	0.11±0.0133 [50.00]
6a	0.17±0.0141 [19.05]	0.22±0.0138 [46.34]	0.31±0.0144 [48.33]	0.33±0.0148 [42.10]	0.29±0.0152 [38.29]	0.107±0.0128 [51.36]
SKF-525A & **6p**	0.10±0.0153 [52.38]	0.21±0.0146 [48.78]	0.30±0.0143 [50.00]	0.32±0.0154 [43.85]	0.29±0.0161 [38.30]	0.10±0.0125 [54.54]
6p	0.11±0.0147 [47.62]	0.23±0.0148 [43.90]	0.32±0.0139 [46.67]	0.34±0.0147 [40.35]	0.30±0.0158 [36.17]	0.11±0.0131 [50.00]
Control	0.21±0.0267	0.41±0.0148	0.60±0.0173	0.57±0.0203	0.47±0.0165	0.22±0.0135

Each value represents the mean±S.E.M. ($n=6$). The Results obtained was analyzed by student's *t*-test and the difference between the two groups was not found to be statistically significant.



Compound	R'	R''	Compound	R'	R''	Compound	R'	R''
6a	H	H	6j	H	cyclohexyl	6s	H	H
6b	H	methyl	6k	H	benzyl	6t	H	methyl
6c	H	ethyl	6l	--piperidino--		6u	H	ethyl
6d	H	propyl	6m	H	propyl	6v	H	propyl
6e	H	isopropyl	6n	H	isopropyl	6w	H	isopropyl
6f	H	n-butyl	6o	H	n-butyl	6x	H	n-butyl
6g	H	n-amyl	6p	H	n-amyl	6y	H	n-amyl
6h	H	n-hexyl	6q	H	n-hexyl			
6i	H	cyclopentyl	6r	H	cyclopentyl			

(a) Ethylacetoacetate/pipieridine, 4 d; rt (b) Alcoholic KOH, 1 h reflux (c) $AlCl_3$/NaCl, 180 °C, 30 min after cessation of liberation of HCl gas (d) Zn/Hg/HCl, 12 h reflux (e) i: $(COCl)_2$/DMF, 24 h rt; ii: NHR′R″/Triethylamine, 12 h, rt.

Chart 1. Reagents and Conditions

matory activity at 25 mg/kg but it was found to be toxic at 100 mg/kg. These compounds were found to be longer acting and showed residual activity exceeding that of standard indomethacin. These compounds showed moderate analgesic activity profile with some variations from the anti-inflammatory activity data. The compound **6x** was found to be most active with 94% inhibition in acetic acid induced writhings. Significant anti-pyretic activity in LPS induced pyresis was obtained and compound **6a** showed lowest temperature index equivalent to that of aspirin. However the antagonism of TNF-α is not possibly involved in the mechanism of action of these compounds. The adjuvant induced arthritis study reveals that these compounds though have long duration of action are not effective in preventing the formation of secondary lesions. The compounds showed mild gastrointestinal toxicity in comparison to standard indomethacin. Consideration of results from the battery of the screening tests employed, it can be said that the test compounds **6a**, **6p** and **6w** have the potential to turn out as drug candidates and thus they warrant further detailed studies regarding their pharmacological profile.

Experimental

Melting points were determined in open capillaries in a Buchi 530 melting point apparatus and are uncorrected. Identity and purity of the compounds were ascertained by TLC, elemental microanalysis and spectral analysis. Infrared-red spectra were recorded with a Shimadzu IR Prestige-21 FT-IR Spectrometer in KBr using powder diffraction technique. ^{1}H-NMR spectra were recorded on a 400 MHz Brucker Avance II NMR Spectrometer. Mass Spectra of the compounds were recorded in a Jeol SX 102/DA-6000 spectrometer. Elemental microanalysis was done in a Perkin-Elmer 2400 CHN analyzer. Chart 1 was followed for the synthesis of 6-chloroindan-1-acetic acid amides.

Diethyl 2,4-Diacetyl-3-(3-chlorophenyl)pentanedionate (2) 3-Chlorobenzaldehyde (**1**) (28 g, 0.2 mol) was dissolved in ethylacetoacetate (56 g, 0.43 mol) in a dry conical flask and piperidine (2.5 ml) was added slowly at ambient temperature and then kept for 3 d or more (up to seven days, depending on room temperature) with the mouth stoppered. The solid product thus obtained was crushed and then washed with solvent ether to get the desired product in 70—75% yield.[15] Recrystallisation from dil. alcohol gave the analytical product, mp 120—121 °C, IR (cm^{-1}): 1722 (C=O stretching), 657 (C–Cl stretch), ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.38 (t, CH_3, 6H), 2.03 (s, CH_3, 6H), 3.38 (d, CH, 2H), 4.02 (qr, CH_2, 4H), 4.46 (t, CH, 1H), 7.08 (m, ArH, 4H).

3-(3-Chlorophenyl)pentanedioic Acid (3) Compound **2** (40 g, 0.1 mol) was dissolved in a hot solution of KOH (160 g in 120 ml of water) and 160 ml of 50% ethanol was added. The hot reaction mixture was refluxed on a water bath for 1 h. Alcohol was then removed by distillation, and after dilution with water it was cooled and washed with solvent ether. The aqueous layer on acidification with cold conc. HCl with cooling gave crude **4** which was filtered and recrystallized from hot water.[15] Yield 80—85%; mp 147—148 °C; IR (cm^{-1}): 3400—2200 (br OH stretch), 1710 (C=O stretching), 663 (C–Cl stretch); ^{1}H-NMR: δ (ppm) ($CDCl_3$): 2.58, 2.70 (dd, CH_2, 4H), 3.60 (qn, CH, 1H), 7.18 (m, ArH, 4H), 10.79 (br s, COOH, 2H).

(6-Chloro-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic Acid (4) Cyclization of compound **3** was carried by treating the acid (14 g, 0.058 mol) with aluminium chloride (49 g, 0.37 mol) in presence of sodium chloride[16] (4.47 g, 0.08 mol) on an oil bath maintained at 180 °C for 30 min with stirring. After decomposition of the hot reaction mixture with crushed ice, the crude keto acid **4** thus obtained was filtered. The crude product was finally recrystallized from dilute acetic acid to get the pure compound. Yield 60—65%; mp 188—190 °C; IR (cm^{-1}): 3400—2400 (br OH stretch), 1680, 1713 (C=O stretching), 657 (C–Cl stretch), ^{1}H-NMR: δ (ppm) ($CDCl_3$): 2.46, 2.58 (dd, CH_2, 2H), 2.84, 2.99 (dd, CH_2, 2H), 3.79 (qn, CH, 1H), 7.38 (d, ArH, 1H), 7.59 (s, ArH, 1H), 7.65 (d, ArH, 1H), 10.91 (br s, COOH, 1H).

(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)acetic Acid (5) Compound **4** was subjected to Clemmensen's reduction.[17] 0.1 mol of compound **4** was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75 ml of water. Around 200 ml of benzene was added as a co-solvent. The reaction mixture was refluxed on steam bath for about 16 h (until the reaction mixture became keto-negative). The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was then dried over anhydrous sodium sulfate and was finally distilled off to get the reduced acid. The reduced acid obtained was a liquid and was subjected to vacuum distillation to get the pure compound. Yield 80—85%; distilled at 148 °C/0.3 mmHg; IR (cm^{-1}): 3400—2400 (br OH stretch), 1700 (C=O stretching), 657 (C–Cl stretch); ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.78, 2.42 (m, CH_2, 2H), 2.48, 2.81 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.56 (qn, CH, 1H), 7.11—7.16 (m, ArH, 3H), 11.18 (br s, COOH, 1H), MS 210.66 (M^+).

General Method for the Synthesis of Amide Derivatives (6a—y) A solution of compound **6** in dry dichloromethane and catalytic amount of dimethylformamide was treated with oxalyl chloride in 1 : 2.5 molar ratios under ice cold conditions. The solution was allowed to stand for 24 h at room temperature with occasional stirring. Excess oxalyl chloride was removed by co-distillation with dry benzene under reduced pressure. The acyl halide thus obtained was not characterized or isolated and was used directly in the next step. To a solution of the acyl halide in dry dichloromethane was added a mixture of triethylamine (1.1 mol) and the appropriate amine in dichloromethane with constant stirring under ice-cold conditions. The mixture was kept at ambient temperature for 12 h. The resulting reaction mixture was then extracted with 0.1 N HCl, water, saturated solution of $NaHCO_3$, brine and water. The organic phase was dried with anhydrous sodium sulfate and then distilled to obtain the title compounds.[18]

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)acetamide (6a) Yield 57.8% (water); mp 109—110 °C; IR: 3374, 3229 (NH), 1638 (C=O), 657 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.77, 2.43 (m, CH_2, 2H), 2.35, 2.64 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.62 (qn, CH, 1H), 5.49 (s, NH, 2H), 7.10—7.18 (m, ArH, 3H); MS (*m*/*z*): 210 [M^+]; *Anal.* Calcd for $C_{11}H_{12}ClNO$: C, 63.01; H, 5.77; N, 6.68. Found: C, 62.86; H, 5.76; N, 6.70.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-methylacetamide (6b) Yield 58.9% (dil. alcohol); mp 102—103 °C; IR: 2270 (NH), 1632 (C=O), 658 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.78, 2.44 (m, CH_2, 2H), 2.36, 2.67 (dd, CH_2, 2H), 2.74 (s, CH_3, 3H), 2.87 (m, CH_2, 2H), 3.65 (qn, CH, 1H), 5.38 (s, NH, 1H), 7.11—7.18 (m, ArH, 3H); MS (*m*/*z*): 224 [M^+]; *Anal.* Calcd for $C_{12}H_{14}ClNO$: C, 64.43; H, 6.31; N, 6.26. Found: C, 64.67; H, 6.30; N, 6.25.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-ethylacetamide (6c) Yield 60.4% (dil. alcohol); mp 98—100 °C; IR: 3281 (NH), 1630 (C=O), 657 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.28 (t, CH_3, 3H), 1.80, 2.43 (m, CH_2, 2H), 2.36, 2.66 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.31 (qr, CH_2, 2H) 3.64 (qn, CH, 1H), 5.35 (s, NH, 1H), 7.09—7.17 (m, ArH, 3H); MS (*m*/*z*): 238 [M^+]; *Anal.* Calcd for $C_{13}H_{16}ClNO$: C, 65.68; H, 6.78; N, 5.89. Found: C, 65.51; H, 6.77; N, 5.87.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-propylacetamide (6d) Yield 63.6% (dil. alcohol); mp 74—76 °C; IR: 3288 (NH), 1633 (C=O), 657 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 0.96 (t, CH3, 3H), 1.59 (m, CH_2, 2H), 1.82, 2.46 (m, CH_2, 2H), 2.35, 2.60 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.20 (t, CH_2, 2H), 3.60 (qn, CH, 1H), 5.41 (s, NH, 1H), 7.09—7.16 (m, ArH, 3H); MS (*m*/*z*): 252 [M^+]; *Anal.* Calcd for $C_{14}H_{18}ClNO$: C, 66.79; H, 7.21; N, 5.56. Found: C, 66.98; H, 7.22; N, 5.59.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-isopropylacetamide (6e) Yield 62.5% (benzene); mp 104—106 °C; IR: 3294 (NH), 1631 (C=O), 658 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.28 (d, CH_3, 6H), 1.80, 2.40 (m, CH_2, 2H), 2.33, 2.61 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.61 (qn, CH, 1H), 3.87 (m, CH, 1H), 5.26 (s, NH, 1H), 7.10—7.18 (m, ArH, 3H); MS (*m*/*z*): 252 [M^+]; *Anal.* Calcd for $C_{14}H_{18}ClNO$: C, 66.79; H, 7.21; N, 5.56. Found: C, 66.58; H, 7.23; N, 5.57.

***N*-Butyl-2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetamide (6f)** Yield 66.8% (semisolid); IR: 3275 (NH), 1635 (C=O), 660 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 0.94 (t, CH_3, 3H), 1.30 (m, CH_2, 2H), 1.54 (qn, CH_2, 2H), 1.78, 2.40 (m, CH_2, 2H), 2.33, 2.63 (dd, CH_2, 2H), 2.88 (m, CH_2. 2H), 3.17 (t, CH_2, 2H), 3.60 (qn, CH, 1H), 5.37 (s, NH, 1H), 7.11—7.19 (m, ArH, 3H); MS (*m*/*z*): 266 [M^+]; *Anal.* Calcd for $C_{15}H_{20}ClNO$: C, 67.79; H, 7.58; N, 5.27. Found: C, 68.01; H, 7.60; N, 5.28.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-pentylacetamide (6g) Yield 65.1% (semisolid); IR: 3281 (NH), 1640 (C=O), 658 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 0.97 (t, CH_3, 3H), 1.31 (m, CH_2, 4H), 1.52 (qn, CH_2, 2H), 1.79, 2.45 (m, CH_2, 2H), 2.37, 2.66 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.19 (t, CH_2, 2H), 3.64 (qn, CH, 1H), 5.40 (s, NH, 1H), 7.09—7.17 (m, ArH, 3H); MS (*m*/*z*): 280 [M^+]; *Anal.* Calcd for $C_{16}H_{22}ClNO$: C, 68.68; H, 7.93; N, 5.01. Found: C, 68.90; H, 7.91; N, 4.99.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylacetamide (6h) Yield 67.9% (semisolid); IR: 3291 (NH), 1638 (C=O), 657 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 0.95 (t, CH_3, 3H), 1.31 (m, CH_2, 6H), 1.53 (qn, CH_2, 2H), 1.77, 2.43 (m, CH_2, 2H), 2.35, 2.65 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.20 (t, CH_2, 2H), 3.65 (qn, CH, 1H), 5.38 (s, NH, 1H), 7.09—7.18 (m, ArH, 3H); MS (*m*/*z*): 294 [M^+]; *Anal.* Calcd for $C_{17}H_{24}ClNO$: C, 69.49; H, 8.23; N, 4.77. Found: C, 69.30; H, 8.25; N, 4.79.

***N*-Cyclopentyl-2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetamide (6i)** Yield 66.8% (cyclohexane); mp 136—138 °C; IR: 3310 (NH), 1640 (C=O), 658 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.52 (m, CH_2, 4H) 1.86 (m, CH_2, 4H), 1.79, 2.42 (m, CH_2, 2H), 2.33, 2.64 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.61 (qn, CH, 1H), 3.68 (qn, CH, 1H), 5.32 (s, NH, 1H), 7.11—7.18 (m, ArH, 3H); MS (*m*/*z*): 278 [M^+]; *Anal.* Calcd for $C_{16}H_{20}ClNO$: C, 69.18; H, 7.26; N, 5.04. Found: C, 69.41; H, 7.29; N, 5.06.

***N*-Cyclohexyl-2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl))acetamide (6j)** Yield 64.3% (cyclohe xane); mp 145—147 °C; IR: 3318 (NH), 1643 (C=O), 659 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.18 (qn, CH_2, 2H), 1.40 (m, CH_2, 4H), 1.74 (m, CH_2, 4H), 1.79, 2.42 (m, CH_2, 2H), 2.34, 2.64 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.61 (qn, CH, 1H), 3.68 (qn, CH, 1H), 5.29 (s, NH, 1H), 7.08—7.16 (m, ArH, 3H); MS (*m*/*z*): 292 [M^+]; *Anal.* Calcd for $C_{17}H_{22}ClNO$: C, 69.97; H, 7.60; N, 4.80. Found: C, 70.10; H, 7.61; N, 4.81.

***N*-Benzyl-2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl))acetamide (6k)** Yield 69.7% (cyclohexa ne); mp 106—108 °C; IR: 3306 (NH), 1642 (C=O), 658 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.77, 2.40 (m, CH_2, 2H), 2.32, 2.57 (dd, CH_2, 2H), 2.84 (m, CH_2, 2H), 3.65 (qn, CH, 1H), 4.46 (s, Bnz CH_2, 2H), 5.81 (s, NH, 1H), 7.09—7.17 (m, ArH, 3H), 7.31 (m, ArH, 5H); MS (*m*/*z*): 300 [M^+]; *Anal.* Calcd for $C_{18}H_{18}ClNO$: C, 72.11; H, 6.05; N, 4.67. Found: C, 72.33; H, 6.06; N, 4.69.

1-[(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)acetyl]piperidine (6l) Yield 69.1% (dil. alcohol); mp 108—110 °C; IR: 1624 (C=O), 657 (C–Cl) 663 cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.65 (m, CH_2, 6H), 1.75, 2.45 (m, CH_2, 2H), 2.72, 2.46 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.37 (t, CH_2, 4H), 3.66 (qn, CH, 1H), 7.10–7.17 (m, ArH, 3H); MS (*m*/*z*): 278 [M^+]; *Anal.* Calcd for $C_{16}H_{20}ClNO$: C, 69.18; H, 7.26; N, 5.04. Found: C, 69.31; H, 7.29; N, 5.06.

1-[(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)acetyl]piperazine (6m) Yield 57.8% (dil. alcohol); mp 215—216 °C; IR: 3347 (NH of piperazine), 1628 (C=O), 661 (C–Cl); ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.78, 2.37 (m, CH_2, 2H), 2.13 (s, NH, 1H), 2.79 (t, CH_2, 4H), 2.42, 2.69 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.32 (t, CH_2, 4H), 3.65 (qn, CH, 1H), 7.10—7.18 (m, ArH, 3H); MS (*m*/*z*): 279 [M^+]; *Anal.* Calcd for $C_{15}H_{19}ClN_2O$: C, 64.63; H, 6.87; N, 10.05. Found: C, 64.87; H, 6.85; N, 10.01.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-hydroxyethyl)acetamide (6n) Yield 60.3% (benzene); mp 60—62 °C; IR: 3278 (NH), 1632 (C=O), 657 (C–Cl), 3605 (OH) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 2.15 (s, OH, 1H), 1.79, 2.46 (m, CH_2, 2H), 2.40, 2.68 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.41 (t, CH_2, 2H), 3.65 (qn, CH, 1H), 3.79 (t, CH_2, 2H), 5.28 (s, NH, 1H), 7.11—7.18 (m, ArH, 3H); MS (*m*/*z*): 254 [M^+]; *Anal.* Calcd for $C_{13}H_{16}ClNO_2$: C, 61.45; H, 6.36; N, 5.52. Found: C, 61.36; H, 6.34; N, 5.51.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-phenylacetamide (6o) Yield 73.7% (dil. alcohol); mp 146—148 °C; IR: 3282 (NH), 1648 (C=O), 663 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.81, 2.40 (m, CH_2, 2H), 2.47, 2.75 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.70 (qn, CH, 1H), 7.24 (s, NH, 1H), 7.10—7.21 (m, ArH, 4H), 7.31—7.48 (m, ArH, 4H); MS (*m*/*z*): 286 [M^+]; *Anal.* Calcd for $C_{17}H_{16}ClNO$: C, 71.45; H, 5.64; N, 4.90. Found: C, 71.67; H, 5.66; N, 4.92.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-(3-chlorophenyl)acetamide (6p) Yield 72.8% (dil. alcohol); mp 138—140 °C; IR: 3306 (NH), 1642 (C=O), 662 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.80, 2.44 (m, CH_2,

2H), 2.47, 2.76 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.71 (qn, CH, 1H), 7.17 (s, NH, 1H), 7.08—7.33 (m, ArH, 6H), 7.64 (s, ArH, 1H); MS (*m/z*): 320 [M^+]; *Anal.* Calcd for $C_{17}H_{15}Cl_2NO$: C, 63.76; H, 4.72; N, 4.37. Found: C, 63.89; H, 4.74; N, 4.39.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-chlorophenyl)acetamide (6q) Yield 74.6% (dil. alcohol); mp 145—146 °C; IR: 3294 (NH), 1643 (C=O), 660 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.81, 2.42 (m, CH_2, 2H), 2.46, 2.76 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.68 (qn, CH, 1H), 7.16 (s, NH, 1H), 7.09—7.17 (m, ArH, 3H), 7.30—7.58 (m, ArH, 4H); MS (*m/z*): 320 [M^+]; *Anal.* Calcd for $C_{17}H_{15}Cl_2NO$: C, 63.76; H, 4.72; N, 4.37. Found: C, 63.93; H, 4.74; N, 4.38.

***N*-(4-bromophenyl)-2-(6-chloro-3-2,3-dihydro-1*H*-inden-1-yl)acetamide (6r)** Yield 65.8% (dil. alcohol); mp 127—129 °C; IR: 3300 (NH), 1644 (C=O), 661 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.80, 2.43 (m, CH_2, 2H), 2.47, 2.75 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.70 (qn, CH, 1H), 7.15 (s, NH, 1H), 7.09—7.18 (m, ArH, 3H), 7.35—7.56 (m, ArH, 4H); MS (*m/z*): 365 [M^+]; *Anal.* Calcd for $C_{17}H_{15}BrClNO$: C, 55.99; H, 4.15; N, 3.84. Found: C, 56.18; H, 4.17; N, 3.85.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-(3-methylphenyl)acetamide (6s) Yield 71.3% (dil. alcohol); mp 109—111 °C; IR: 3313 (NH), 1649 (C=O), 661 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.83, 2.39 (m, CH_2, 2H), 2.31 (s, CH_3, 3H), 2.46, 2.75 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.65 (qn, CH, 1H), 7.11 (s, NH, 1H), 6.91—7.19 (m, ArH, 5H), 7.47 (d, ArH, 2H); MS (*m/z*): 300 [M^+]; *Anal.* Calcd for $C_{18}H_{18}ClNO$: C, 72.11; H, 6.05; N, 4.67. Found: C, 72.36; H, 6.07; N, 4.69.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methylphenyl)acetamide (6t) Yield 70.4% (dil. alcohol); mp 194—195 °C; IR: 3320 (NH), 1648 (C=O), 659 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.84, 2.39 (m, CH_2, 2H), 2.30 (s, CH_3, 3H), 2.47, 2.74 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.66 (qn, CH, 1H), 7.10 (s, NH, 1H), 7.05—7.16 (m, ArH, 5H), 7.45 (d, ArH, 2H); MS (*m/z*): 300 [M^+]; *Anal.* Calcd for $C_{18}H_{18}ClNO$: C, 72.11; H, 6.05; N, 4.67. Found: C, 72.31; H, 6.03; N, 4.69.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methoxyphenyl)acetamide (6u) Yield 69.1% (dil. alcohol); mp 176—178 °C; IR: 3324 (NH), 1650 (C=O), 660 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.78, 2.38 (m, CH_2, 2H), 2.44, 2.74 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.67 (qn, CH, 1H), 3.84 (s, OCH_3, 3H), 7.14 (s, NH, 1H), 6.86—7.18 (m, ArH, 5H), 7.51 (d, ArH, 2H); MS (*m/z*): 316 [M^+]; *Anal.* Calcd for $C_{18}H_{18}ClNO_2$: C, 68.46; H, 5.75; N, 4.44. Found: C, 68.22; H, 5.77; N, 4.46.

***N*-[4-(Acetylamino)phenyl]-2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetamide (6v)** Yield 67.6% (dil. alcohol); mp 156—158 °C; IR: 3276 (NH), 1641 (C=O), 660 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.81, 2.41 (m, CH_2, 2H), 2.03 (s, CH_3, 3H), 2.46, 2.76 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.71 (qn, CH, 1H), 7.13 (s, NH, 1H), 7.15 (s, NH, 1H), 7.10—7.17 (m, ArH, 3H), 7.56—7.64 (m, ArH, 4H); MS (*m/z*): 343 [M^+]; *Anal.* Calcd for $C_{19}H_{19}ClN_2O_2$: C, 66.57; H, 5.59; N, 8.17. Found: C, 66.35; H, 5.61; N, 8.19.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-nitrophenyl)acetamide (6w) Yield 65.8% (dil. alcohol); mp 137—139 °C; IR: 3280 (NH), 1639 (C=O), 659 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.79, 2.43 (m, CH_2, 2H), 2.47, 2.77 (dd, CH_2, 2H), 2.89 (m, CH_2, 2H), 3.70 (qn, CH, 1H), 7.21 (s, NH, 1H), 7.10—7.18 (m, ArH, 3H), 7.89—8.25 (m, ArH, 4H); MS (*m/z*): 331 [M^+]; *Anal.* Calcd for $C_{17}H_{15}ClN_2O_3$: C, 61.73; H, 4.57; N, 8.47. Found: C, 61.54; H, 4.58; N, 8.49.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-3-ylacetamide (6x) Yield 60.3% (dil. alcohol); mp 196—198 °C; IR: 3321 (NH), 1650 (C=O), 658 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.83, 2.46 (m, CH_2, 2H), 2.53, 2.81 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.71 (qn, CH, 1H), 7.31 (s, NH, 1H), 7.10—7.30 (m, ArH, 4H), 8.17—8.51 (m, ArH, 3H) ; MS (*m/z*): 287 [M^+]; *Anal.* Calcd for $C_{16}H_{15}ClN_2O$: C, 67.02; H, 5.27; N, 9.77. Found: C, 67.23; H, 5.25; N, 9.73.

2-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-4-ylacetamide (6y) Yield 58.6% (dil. alcohol); mp 157—159 °C; IR: 3330 (NH), 1648 (C=O), 660 (C–Cl) cm^{-1}; ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.80, 2.42 (m, CH_2, 2H), 2.49, 2.79 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.70 (qn, CH, 1H), 7.28 (s, NH, 1H), 7.11—7.19 (m, ArH, 3H), 7.65—8.34 (m, ArH, 4H); MS (*m/z*): 287 [M^+]; *Anal.* Calcd for $C_{16}H_{15}ClN_2O$: C, 67.02; H, 5.27; N, 9.77. Found: C, 66.86; H, 5.29; N, 9.80.

Biological Evaluation All synthesized compounds were evaluated for analgesic and anti-inflammatory activity and few selected compounds were screened for antipyretic, ulcerogenecity and anti-arthritic activity. The test compounds, **6a**—**y**, and standard drugs (indomethacin and aspirin) were administered orally as suspensions in 0.5% carboxymethylcelluse sodium in distilled water. Each group consisted of six animals. The animals were maintained at temperature of 24±2 °C, relative humidity of 45% and kept under a 12 h light and dark cycle. The animals were fasted overnight for analgesic and anti-inflammatory assays and 24 h for antipyretic and ulcerogenecity studies. During fasting they had free access to water. The data obtained in pharmacological experiments was subjected to statistical analysis using student's *t*-test, one way ANOVA, *post hoc* test, and the chosen level of significance was $p<0.05$. The protocol for the animal experiments was approved by the Institutional Animal Ethics Committee (IAEC) as registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Acute Anti-inflammatory Activity The anti-inflammatory activity of the test compounds (**6a**—**y**) was determined by carrageenan-induced rat hind paw edema assay as described by Winter *et al.*[19] Female Wistar rats weighing 170±20 g were administered the test compounds and standard indomethacin orally equivalent to 100 mg/kg and 10 mg/kg respectively. One hour after dosing, 0.1 ml of 1% carrageenan was administered into the subplantar region of the right hind paw. The paw volumes were measured using an Ugo Basile plethysmometer at 0, 1, 2, 3, 4, 6 and 24 h after carrageenan injection. The results (Table 1) are expressed as percentage inhibition of edema formation. The percent inhibition of edema in each drug treated group was calculated using the formula given below:

$$\% \text{ inhibition} = 100(1 - V_t/V_c)$$

where V_c and V_t are average edema volumes in control and treated groups respectively.

Analgesic Activity The analgesic activities of the compounds (**6a**—**y**) were determined by acetic acid induced writhing test in mice as described by Collier *et al.*[20] with minor modifications. Female Swiss albino mice, 20±5 g, were administered the test drugs orally at 100 mg/kg. and the standard indomethacin and aspirin at 10 mg/kg and 100 mg/kg respectively. One hour after the oral dosing they were injected i.p. with 0.1 ml/10 g of 1% v/v acetic acid. Five minutes after the injection the writhing (full extension of hind limbs) were noted for next 15 min. Animals giving 20 or more wriths were selected for the study. The percent inhibition by individual drug as well as by the reference standard drugs were calculated using the following formula,

$$\% \text{ inhibition} = 100[1 - W_t/W_c]$$

where W_c represents the average writhing produced by the control group and W_t represents the average writhing produced by the test groups.

Antipyretic Activity The antipyretic activity of the compounds was evaluated by lipopolysaccharide induced pyrexia. Two protocols were adopted, first in which initial hypothermic phase was excluded and only antipyretic activity was evaluated and the second in which the hypothermic phase was also evaluated in order to determine if these compounds also inhibit TNF-α. The method was that of Dogan *et al.*[13] with minor modifications. Adult female Wistar rats (170±20 g) were used. Phenol extracted LPS from *Eschericia coli* serotype 0111: B4 (Difco) was used. The experiment was started at 0900 h. LPS dissolved in apyrogenic saline was injected at dose of 100 μg/kg i.p. After 3 h test compounds and aspirin were given orally at dose level of 100 mg/kg. Indomethacin was given at a dose level of 10 mg/kg. The rectal temperature was determined using telethermometer probes immediately before and 4, 5, 6, 7 h after LPS administration. For evaluation of antipyretic activity, the temperature index was calculated following the method of Winter *et al.*[21] In the second protocol after half an hour of oral administration of the drugs LPS dissolved in apyrogenic saline was injected at dose of 100 μg/kg i.p. The rectal temperature was determined using telethermometer probes immediately before and 1, 2, 3, 4, 5, 6, 7 h after LPS administration. Two temperature indexes were calculated, first at the end of 3rd hour and second at the end of 7th hour of observations.

Chronic Anti-inflammatory Activity The method used was essentially that of Newbould.[22] Only three compounds were selected for this study. Female albino rats weighing 160±10 g were chosen for the study. The animals were given the test compounds at the dose level of 100 mg/kg *p.o.* once daily for 14 d starting from the day before the administration of 0.1 ml Freund's complete adjuvant (Genie, Bangalore, India) into the subplantar surface of right hind paw of each rat. Indomethacin was taken as the standard drug and was given for 14 d at the dose level of 10 mg/kg *p.o.* Both hind paw volumes up to the fixed mark at the level of lateral malleolus were measured before and daily after adjuvant administration. The formation of nodules and appearance of erythema in tail, nose and ears were observed and graded as mild, moderate and severe for comparison. Change in body weight of the animals during the test period was also recorded for comparison. The results are presented in Table 6.

Ulcerogenecity Test Only few amides from the series **6a**—**y** were selected for ulcerogenic studies. The method used to evaluate the ulcerogenic potential of the test compounds was that of Velázquez *et al.*[23] with minor modifications. Twenty four hour fasted Wistar female rats (175±25 g) were used. The test compounds and the standard drug indomethacin were dosed orally at 100 mg/kg and 30 mg/kg respectively. After six hours of oral dosing the rats were sacrificed using cervical dislocation. The stomachs were taken out and cut along the greater curvature. After washing with saline the stomach mucosa was examined for ulcers using a hand lens. The gastric lesions were counted, and an ulcerative index (UI) for each animal was calculated according to Szelenyl and Thiemer[24];

$$UI = (\text{n lesion I}) + (\text{n lesion II})2 + (\text{n lesion III})3$$

Where:

I=presence of edema, hyperemia and single, submucosal, punctiform hemorrages (petechiae)

II=presence of submucosal, hemorrhagic lesions with small erosions;

III=presence of deep ulcer with erosions and invasive lesions

Metabolism Inhibition Study Using SKF-525A[13] The rats were pretreated with SKF 525A (50 mg/kg i.p.). One hour later, the rats were dosed orally the test drug **6a** and **6p** at 100 mg/kg. The remaining protocol followed was the same as that of anti-inflammatory screening by carrageenan induced rat paw edema model.

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