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Synthesis, Pharmacological Screening of Ethyl (5-Substitutedacetamido)-3-Methylthio-1-Phenyl-1h-Pyrazole-4-Carboxylate As Anti-Inflammatory And Analgesic Agents

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Abstract: Several novel ethyl (5-substitutedacetamido)-3-methylthio-1-phenyl-1H-pyrazole-4-carboxylate were synthesized and screened for their anti-inflammatory at the dose of 25mg/kg and analgesic activity at the dose of 50mg/kg. The compound **5a** and **5f** are prominent candidate which showed good anti-inflammatory activity among the series. The compound **5a, 5g** and **5h** showed 60% analgesic activity to that of standard aspirin by acetic acid induced writhing method.
Keywords: Anti-inflammatory, Analgesic, Pyrazole, NSAIDS.

1. Introduction

Nonsteriodal anti-inflammatory drugs (NSAIDs) have been recognized as important class of therapeutic agents for the alleviation of pain and anti-inflammatory associated with a number of pathological condition.[1]However long term use of (NSAIDs) has been associated with several side effect such as gastrointestinal mucosal damage, bleeding, intolerance and renal toxicity. The discovery of the inducible isoform of cycloxygenase enzyme (COX-2) spurred the search for anti-inflammatory agents devoid of the undesirable effect associated with classical NSAIDs.[2-4] Recently, a novel class of selective COX-2 inhibitor has been discovered. Among this class, celecoxib was shown to be a potent and gastrointestinal safe anti-inflammatory and analgesic agents.[5-6] It is considered a typical model of pyrazole containing, diaryl-hetrocyclic template that is known to selectively inhibit COX-2 . Futhermore, several other compound containing pyrazole functionally were also reported to exihibit anti-inflammatory activity.[7-8]

2. Experimental Work

Chemicals and Reagents

All chemicals were obtained from Research Lab and Merck. The reactions were carried out by conventional method. Melting points were determined in open capillaries using melting point apparatus and are uncorrected. The purity of synthesized compound was ascertained by TLC using silica gel-G coated plate using benzene: methanol (4:1) and $CHCl_3$: methanol (4.5:0.5) solvent system. The IR spectra were recorded on JASCO FTIR-4100, NMR spectra were recorded on BRUKER AVANCE II 400 spectrometer using $CDCl_3$ and DMSO as solvent with TMS as internal standard and Mass spectra were recorded on 1200L Varian LC/MS instrument.

Chemistry

Ethyl -5-amino-3-methylthio-1-phenyl-2H-pyrazole-4-carboxylate (3)

In 100 ml RBF, ethyl bismethylthio-2-cyanoacrylate **(1)** (2.17gm, 0.01moles) and phenylhydrazine **(2)** **(**1.08gm, 0.01moles) was dissolved in dimethylformamide. To this a catalytic amount of anhydrous K_2CO_3 was added and the reaction mixture was refluxed gently for 2 hour. The reaction mixture was cooled and poured in ice cold water, the product obtained was filtered, washed with water and recrystallised from rectified spirit to yield 1.86 gm of creamy colour product.

Ethyl 5-(2-chloroacetamido)-3-methylthio-1-phenyl-1*H*-pyrazole-4-carboxylate (4)

In 100 ml RBF, ethyl-5-amino-3-methylthio-1-phenyl-2H-pyrazole)-4-carboxylate **(3),** (0.001 mole, 0.278g) and chloro acetyl chloride (0.001mole,0.102ml) was dissolved in chloroform. It was refluxed in water bath at 80^0C for 2 hours. The separated crystals were filtered and recrystallised from rectified spirit to yield 0.239 gm of yellow crystal product

Ethyl (5-substitutedacetamido)-3-methylthio-1-phenyl-1H-pyrazole-4-carboxylate (5a-i)

Ethyl-5-(2-chloroacetamido)-3-methylthio-1-phenyl-1*H*-pyrazole-4-carboxylate **(4)** **(**0.001moles) amines (0.001moles)and acetone (5ml) was transferred to 100ml conical flask, to this triethylamine (0.00125moles,0.126ml) was added drop wise with stirring, a clear solution was obtained. The stirring was continued for 3hours. The reaction mixture was poured in petridish and scratched with petroleum ether. The solid was recrystallised from rectified spirit.

Spectral Data

Analysis Compound 5a:

IR(KBr)=3330.7(m)[2^0NH],1735(s) [CONH] ,1665(s) [$COOC_2H_5$], 3074(s) [Ar-H], 2860(m,s) [C-H of aliphatic].

1H-NMR ($CHCl_3$) : δ1.4 (t ,3H, CH_3 of CH_2-CH_3 at 4^{th} position), δ2.6(t,4H, $(CH_2)_2$ of $N(CH2)_2$ of morpholine), δ2.6(s, 3H, H of SCH3 at 3 position merged), δ3.00 (s, 2H, CH_2 of amide) 3.8(t, 4H, -O(CH2) of morpholine) , δ4.4 (q,2H, CH_2 of CH_2-CH_3 at 4^{th} position), δ7.3-7.6(m ,5H C_6H_5 at 1^{st} position), δ9.6(s(b), (1H, NH of $CONH_2$);

MS(M/z) = M^+ calculated 404.58, found 405

Analysis Compound 5b:

IR (KBr)= 3340(m) [2^0 NH], 1745(s) [C=O of $CONH_2$], 1665(s) [C=O of $COOC_2H_5$], 3074(s) [Ar-C-H] .

1H-NMR ($CHCl_3$): δ1.32 (t, 3H CH_3 of CH_2-CH_3 at 4^{th} position) , δ2,30(s, 6H, $(CH_3)_2$ of $N(CH_3)_2$, δ2.6(s,3H, SCH3 at 3^{rd} position), δ3.00(s, 2H,-CH_2 of amide), δ4.36 (q, 2H, CH_2 of CH_2-CH_3 at 4^{th} position), δ7.3-7.6(m, 5H,C_6H_5 at 1^{st} position(s(b),1H, NH of amide); **MS(m/z): M+** Calculated 362.48 ,found 363

Analysis Compound 5c:

IR (KBr)= 3375(m) [2^0 NH], 1740(s) [C=O of $CONH_2$], 1660(s) [C=O of $COOC_2H_5$], 3034(s) [Ar-C-H];

1H-NMR ($CHCl_3$) :δ1.34(t, 3H, CH_3 of CH_2-CH_3 at 4^{th} position), δ1.8(t,4H, $(CH_2)_2$ of pyrrolidine), δ2.6(s,3H, SCH3 at 3^{rd} position), δ2.65(t, 4H,N-$(CH_2)_2$ of pyrrolidine), δ3.2(s,2H, CH_2 of amide), δ4.36 (q, 2H, CH_2 of CH_2-CH_3 at 4^{th} position), δ7.3-7.6(m, 5H,C_6H_5 at 1^{st} position), 9.6 (s(b),1H, NH of amide);

MS(m/z): M+ Calculated **388.48** , **found 389**

Analysis Compound 5d:

IR (KBr)= 3430(m) [2^0 NH stretching],1742

(s)[C=O of $CONH_2$), 1662(s) [C=O of $COOC_2H_5$], 3034(s) [Ar-C-H];

1H-NMR ($CHCl_3$): δ1.34(t, 3H, CH_3 of CH_2-CH_3 at 4th position), δ2.0(s,1H,NH of amine), δ2.6(s,3H, SCH3 at 3rd position), δ3.2(s,2H, CH_2 of amide), δ3.97(q,3H, N-CH_3), δ4.36 (q, 2H, CH_2 of CH_2-CH_3 at 4th position), δ7.3-7.6(m, 5H,C_6H_5 at 1st position), 9.6 (s(b),1H, NH of amide);

MS(m/z)= M^+ calculated 347.56, found 348

Analysis Compound 5e:

IR (KBr) :3460(m) [2^0 NH], 1765(s) [C=O of $CONH_2$], 1635(s) [C=O of $COOC_2H_5$], 3034(s)[Ar-C-H];

1H-NMR ($CHCl_3$) : δ1.34(t, 3H, CH_3 of CH_2-CH_3 at 4th position), δ2.48(t,4H,H of $N(CH_2)_2$, δ2.6(s,3H, SCH3 at 3rd position), 3.15(t,4H,H of $N(CH_2)_2$, , δ3.2(s,2H, CH_2 of amide), δ4.36 (q, 2H, CH_2 of CH_2-CH_3 at 4th position), 6.93-8.45(m,4H,C_5H_4N), δ7.3-7.6(m, 5H,C_6H_5 at 1st position), 9.6 (s(b),1H, NH of amide);
Mass=348

Analysis Compound 5f:

IR (KBr): 3220(m)) [2^0 NH], 1758(s)[C=O of $CONH_2$], 1631(s) [C=O of $COOC_2H_5$], 3034(s)[Ar-C-H];

^{1}H($CHCl_3$) δ1.32 (t, 3H CH_3 of CH_2-CH_3 at 4th position), δ2.26(s,3H, N-CH_3), 2.35(t,4H, H of $N(CH_2)_2$), δ2.6(s,3H, SCH3 at 3rd position) δ3.2(s,2H, CH_2 of amide), δ4.36 (q, 2H, CH_2 of CH_2-CH_3 at 4th position), δ7.3-7.6(m, 5H,C_6H_5 at 1st position), 9.6 (s(b),1H, NH of amide);

Mass= M+ calculated 416.68, found 417

Analysis Compound 5g:

IR (KBr): 3480(m)[O-H stretching], 3230(m) [2^0 NH], 1740(s) [C=O of $CONH_2$], 1672(s) [C=O of $COOC_2H_5$], 3034(s)[Ar-C-H];

1H-NMR ($CHCl_3$) :δ1.32 (t, 3H CH_3 of CH_2-CH_3 at 4th position), δ1.77(m,2H, N-CH_2CH_2), 2.51(d,2H,N-CH_2), δ2.6(s,3H, SCH3 at 3rd position), δ3.2(s,2H, CH_2 of amide), δ4.36 (q, 2H, CH_2 of CH_2-CH_3 at 4th position), δ7.3-7.6(m, 5H,C_6H_5 at 1st position), 9.6 (s(b),1H, NH of amide);

Ms (m/z)=418

Analysis Compound 5h:

IR(KBr) : 3540(m)[O-H stretching], 3435(m) [2^0 NH stretching], 1784(s) [C=0 of $CONH_2$1652(s))[C=O of $COOC_2H_5$],3074(s) [Ar-C-H];

1H-NMR ($CHCl_3$) :δ1.32 (t, 3H CH_3 of CH_2-CH_3 at 4th position) δ1.44 & 3.8(t, 2H, H of CH_2), 1.53(m,2H, N-CH_2CH_2), 2.33(d,2H,N-CH_2),) δ3.2(s,2H, CH_2 of amide),, δ2.6(s,3H, SCH3 at 3rd position) , , δ3.2(s,2H, CH_2 of amide), 3.65(s,H of OH)δ4.36 (q, 2H, CH_2 of CH_2-CH_3 at 4th), δ7.3-7.6(m, 5H,C_6H_5 at 1st position), 9.6 (s(b),1H, NH of amide);

Ms (m/z)= M+ calculated 446.68 ,found 447

Analysis Compound 5i:

IR (KBR) :3375(m) [2^0 NH], 1740(s) [C=0 of $CONH_2$, 1660(s)) C=O[C=O of $COOC_2H_5$, 2960(m,s) [C-H of aliphatic], 3034(s) [Ar-C-H];

1H-NMR ($CHCl_3$) :δ1.32 (t, 3H CH_3 of CH_2-CH_3 at 4th position), δ1.59(m, 6H,$N(CH_2)_3$, δ 2.45(q, 4H,$N(CH_2)_2$, δ3.2(s,2H, CH_2 of amide),, δ2.6(s,3H, SCH3 at 3rd position) , , δ3.2(s,2H, CH_2 of amide), 3.65(s,H of OH)δ4.36 (q, 2H, CH_2 of CH_2-CH_3 at 4th position), δ7.3-7.6(m, 5H,C_6H_5 at 1st position), 9.6 (s(b),1H, NH of amide);

Ms (m/z)= M+ calculated 401.78 ,found 402

3. Biological Activity

Animals

All experiments were performed in Y. B. Chavan College of Pharmacy , Auranabad. Wistor albino rats of either sex weighing 150-180gm were used for the experiment. The rats were housed comfortably in a group of six in a single clean plastic cage with a metal frame lid on its top. The animal house is maintained under RT (25±2^{o}C) relative humidity 60-70% in a 12:12 hr natural light dark cycle. The animals were given standard laboratory food and water. Food was withdrawn 12 hr before and during experimental hr. All the experiments used for pharmacological activity of drugs and synthesized derivatives had prior approval of the Institutional Animal Ethical Committee, Y. B. Chavan College of Pharmacy, Aurangabad.

Experimental Conditions

The animal were divided into several group of six each The control group recived Carrageenan 0.1 ml 1% w/v in the planter aponeurosis region of right paw. The test and standard drugs aqueous suspension prepared with 1 ml 1% w/v solution of tween 80 at dose of 25mg/kg.One hr after oral administration of the drug acute inflammation was produced injected 0.1 ml of 1% w/v suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats. The standard drug aspirin as suspension, prepared in water with sufficient quantity of 1ml 1%w/v solution of tween 80. After 60 minutes of administration of test compound and aspirin, the mice were given intraperitonial injection of 0.3 ml of 0.6% v/v acetic acid.

Statistical Analysis

The data was then analyzed statistically using ANOVA followed by Dunnett's test. All the results are expressed as mean±S.E.M.

4. Pharmacological Studies

Anti-inflammatory activity

Carageenan induced paw edema was employed for evaluating the anti-inflammatory activity of the compound. A mark was applied on the leg at the malleous to facilliate subsequent reading. The paw volume was measured plethysmometrically (Ugo Basile, Italy). The paw volume of the all groups of rats before injection of carrageenan for 0 minutes paw volume and at the end of 1, 2, 3 hours after carrageenan challenge.

The percentage of inhibition of inflammation in the drug treated animals was recorded and calculated using formula:

Where,

$$\% \text{ Inhibition} = \frac{\triangle Vc - \triangle Vt}{\triangle Vc}$$

ΔVc is arithmetic mean of the increase in paw volume in the control group.

Analgesic activity

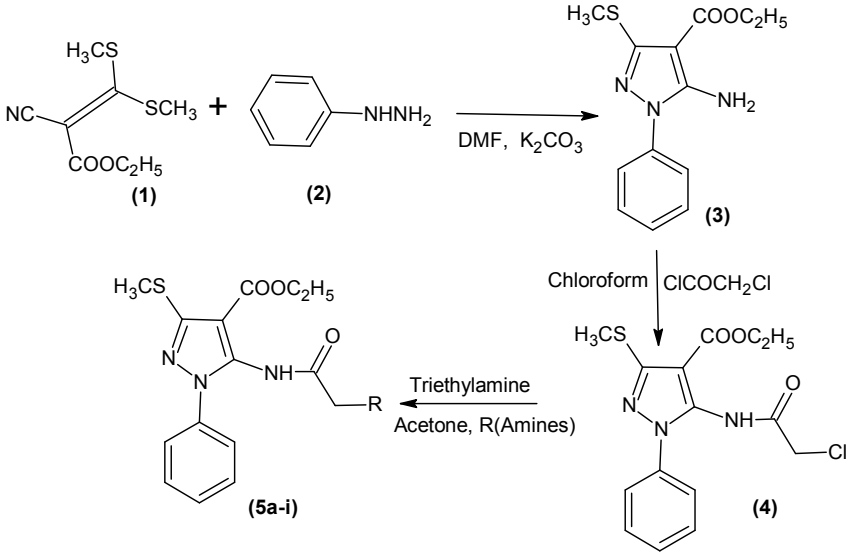
Analgesic activity was evaluated against acetic acid induced writhing assay. Mice were observed for total number of writhes for 10 minutes from the 5 minutes after the acetic acid injection. The mean value for each group were calculated and compared with control. The results were expressed as the percent reduction in number of writhes from the control group as shown in the **(TableIII).**

5. Results and Disscussion

The synthesis of the title compound was affected as outlined in the **Scheme I**. The desired target compounds **(5a-i)** were synthesized by reacting ethyl bismethylthio-2-cyanoacrylate [9] **(1)** and phenyl hydrazine. The reaction was carried out in dimethyl formamide/ethanol in presence of anhydrous K_2CO_3 for 2 hour. The product ethyl-5-amino-3-methylthio-1-phenyl-1H-pyrazole-4-carboxylate **(3)** on reflux with chloroacetyl-

chloride in chloroform for 2 hours to yields substituted pyazolo chloro acetamide **(4)**. The compounds **(4)** on treatment of with various amines like morpholine, dimethylamine, methylamine, pyrrolidine, piperidine, pyrimidylpiperazine, 4-hydroxypiperidine, 4-methylpiperazine, 3-piperidine ethanol gave the target compounds having general structure as **(5a-i).**[10] The reactions were carried out by conventional method and the compounds were purified by recrystallization. During each synthesis solubility of compounds were checked in various organic solvents and performed the TLC to acertain completion of reaction.

Scheme 1

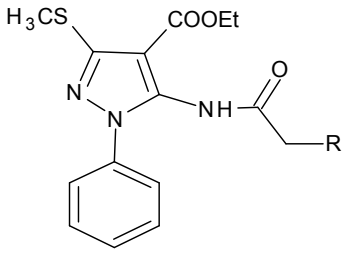


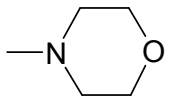
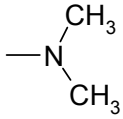
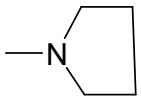
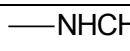
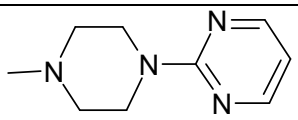
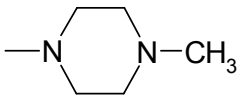
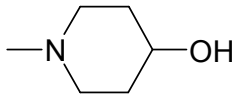
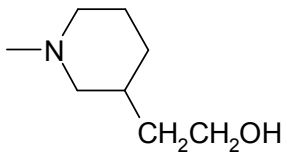
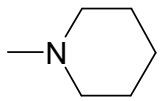
The pyrazole compounds (3) was having melting point in the of 90-100^{0}C. It is soluble in DMSO, DMF, $CHCl_3$ and hot ethanol and insoluble in water and methanol. The compound ethyl-5-[(-2-chloroacetyl)amino]-3-methylthio-1-phenyl-1*H*-pyrazole-4-carboxylate (4) characteristically yellow crystals compounds melting in the range of 150-155^{0}C. The target compound **(5a-l)** substituted with various amines having melting point in the range of 105-130^{0}C. Among the series compounds having the light cream to yellow.

The I.R spectra of compound **(5a-5i)** showed the absence of 1°NH_2 group which indicate that chloroacetylation took place at $–NH_2$ group of pyrazole at 5 position. The presence of NH group at around 3300 cm^{-1}(s) as single peak was also seen in the spectra. The peak at 3000 cm^{-1}(s) due to aromatic stretching. The C=O stretching of ester appeared at 1750 cm^{-1}(s) where as C=0 stretching of amide appeared at1650 cm^{-1}(s). The compound **5g** and **5h** have the characteristic peak of OH at 3400-3500 cm^{-1}(b).

NMR spectra of compound **(5a-i)** showed that triplet at δ 1.32-1.40 due to CH_3 of CH_2-CH_3 at 4th position. A singlet peak was appeared at δ 2.6-2.7 due to -SCH_3.A singlet peak of $–CH_2$ was appeared at around δ 3.00. A quartet peak at δ 4.36-4.50 due CH_2 of CH_2-CH_3 at 4th position. Aromatic proton appeared at δ 7.3-7.6 ppm as a multiplet. A broad singlet peak of δ 9.5-9.7 due to NH proton of amide was identified. The mass spectrum shows intense molecular ion peak (M^+) exactly at the molecular weight of the compound. The elemental analysis of compound showed that the compounds were pure. The theoretical values are comparable to that of observed values. Physical and elemental analysis data of **(5a-i)** are listed in **(Table 1)**.

Table 1: Physical charterisation of pyrazole acetamido derivatives (5a-i)



Comp	R	Mol.for	m.p	yield	R_f
5a		$C_{19}H_{24}O_4N_4S$	110-112	65	0.34
5b		$C_{17}H_{22}O_3N_4S$	105-106	80	0.36
5c		$C_{19}H_{20}O_3N_4S$	123-124	64	0.31
5d		$C_{16}H_{20}O_3N_4S$	115-116	60	0.33
5e		$C_{23}H_{27}O_3N_5S$	123-124	52	0.32
5f		$C_{20}H_{27}O_3N_5S$	118-120	56	0.26
5g		$C_{20}H_{26}O_4N_4S$	125-126	48	0.29
5h		$C_{22}H_{31}O_4N_4S$	117-118	52	0.35
5i		$C_{20}H_{26}O_3N_4S$	122-123	45	0.37

The anti-inflammatory activity of the compound **(5a-i)** were evaluated by carrageenan-induced paw edema method. The compound were tested at 25mg/kg dose and the results were compared with that of diclofenac sodium as a reference. The results were summarized in (**Table-II)**. The % inhibition is plotted against the test compound to compare anti-inflammatory with standard diclofenac sodium. It was observed that all the compounds showed the anti-inflammatory. Among the all compound, the **5a and 5f** showed activity equivalent to that of the standard diclofenac in first hour at the dose of 25mg/kg.The compound **5b, 5c, 5d and 5h** are the compound which show the moderate activity. The compound substituted with amines have greater activity than the parents compound **4**. Morpholine and methyl Piperazine derivative have greater activity among the series.

Table 2: Increasing Mean Paw Volume and % of inhibition of compound

Increasing in Mean Paw Volume (ml) ± SEM			
Comp. Code	**1st hours**	**2nd hours**	**3rd hours**
Control	0.84 ± 0.010*	1.01± 0.010*	0.1.21 ± 0.010*
Standard	0.636 ± 0.006*(73.73)	0.76 ± 0.007**(54.34)	0.88 ± 0.007**(49.76)
4	0.731 ± 0.003*(46.12)	0.80 ± 0.005**(32.30)	1.06 ± 0.007**(26.08)
5a	0.65 ± 0.005*(66.16)	0.838 ± 0.004**(46.29)	0.92 ± 0.005**(44.76)
5b	0.835 ± 0.0O8*(56.14)	0.91 ± 0.0O5**(45.12)	1.02 ± 0.0O73**(42.08
5c	0.668± 0.007*(53.32)	0.856± 0.007**(30.4)	1.03± 0.0085**(22.38)
5d	0.786 ± 0.0057*(50.23)	0.946 ± 0.005*(34.04)	1.18± 0.025**(17.91)
5e	0.745 ± 0s.004*(40.23)	0.936 ± 0.009**(23.41)	1.16 ± 0.005**(10.41)
5f	0.72 ± 0.005*(71.12)	0.835 ± 0.006**(50.42)	0.90 ± 0.0051**(39.10)
5g	0.72 ± 0.005*(36.6)	0.90± 0.004**(29.14)	1.1 7± 0.0057**(19.40)
5h	0.74± 0.006*(43.33)	0.84 ± 0.005**(32.60)	1.096 ± 0.0067**(20.8)
5i	0.734±0.03*(26.62)	0.925±0.06*(29.41)	1.09±0.007**(22.38)

n=6, the observation are mean ± SEM, P < 0.001andP < 0.005* as compared to control (ANOVA followed by dunnett's test), dose of std. and test 25mg/kg.**

In the second hour of inhibition the activity is the similar to that of 1st hour but the overall activity was reduced

In the third hour of inhibition, the activity of all the compounds get further reduced but compound **5a, 5b** showed prominent activity & equivalent to that of diclofenac sodium at the dose of 25mg/kg The compound **4** is found to show less activity as compared to its derivative **(5a-i)**. It indicate that the amide group which was found in the derivative play a major role in the activity. Overall result of anti-inflammatory activity showed that the pyrazole ring is seems to be pharmacophore and other substituents may play a role in enhancing the activity.

Table 3: Analgesic activity by acetic acid induced writhing method

Compound	% of reduction of writhing
Control	0
Std	70
4	24
5a	46
5b	20
5c	18
5d	20
5e	22
5f	36
5g	40
5h	44
5i	26

The analgesic activity of test compound **4** and **5a-i** was performed by acetic acid induced writhing method.. The compound were tested at 50mg/kg dose and the results were compared with that of asiprin as a reference. The results were summarized in **(Table-III)**. The compound **5a, 5b and 5h** showed 60% in reduction of writhing to that of std aspirin, where as other compound showed less than 50% to std aspirin. Overall the analgesic activity is less than anti-inflammatory activity i.e. the compound are potent anti-inflammatory agents.

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