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EVALUATION OF ANALGESIC ACTIVITY OF SOME NOVEL SUBSTITUTED L-ARGININE ANALOGUES

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ABSTRACT: A series of novel L-arginine analogues were synthesized characterized and screened for analgesic activity by acetic acid induced analgesia model. The purity of the synthesized compounds has been characterized by various analytical techniques such as UV, FTIR and TLC. The synthesized compounds were evaluated for analgesic activity by acetic acid induced analgesia in mice using paracetamol as the standard drug for comparing the test results. The study concluded that the compound 1, 2, 3 and 4 were found to exhibit significant anti-pyretic action.

INTRODUCTION: Analgesics are the primary need for patients to get rid of any kind of pain. Pain is one of the basic symptoms of all human ailments which is a sensorial modality and primary protective. Analgesics only relieve pain in a particular complaint without affecting its cause[1]. A prolonged painful stimulation may generate increased blood flow and inflammation, and vice versa, inflammation may lead to pain[2].The effect of Nitricoxide (NO) on peripheral nociception and with its local vasodilation, spinal pain associated with herniated discs can be classified as peripheral nociception because the spine is in this case the site of inflammation[3, 4].

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It has been concluded that pain associated with Rheumatoid arthritis (RA) is due to synovial inflammation, and that pain in RA is associated with enhanced substance P and IL-2 in the synovial fluid. Both these substances induce production of Nitric oxide. It has been shown in murine models that NO enhances the sensitivity of peripheral nociceptors and that inhibitors of Nitric oxide synthase can act as analgesic agents at the cerebral, spinal and peripheral levels[5, 6]. There is a direct evidence that NO can induce pain locally.

Intracutaneous injections of NO to humans were shown to induce dose-dependent local pain and similar findings were obtained more recently when NO was injected intravenously. Identification of N-methyl-L-Arginine (L-NMA) as the first inhibitor of NO biosynthesis led to the design of selective iNOS inhibitors[7]. Hence the present study was planned to synthesize some novel substituted L-arginine analogues and to evaluate for anti-inflammatory activity.

MATERIALS AND METHODS:

Synthetic Chemistry

STEP-1 (Synthesis of 4-benzylidine-2-phenyl oxazole – 5- ones): A mixture of benzoyl glycine, redistilled benzaldehyde, acetic acid and anhydrous sodium acetate was heated on an electric hot plate with stirring. On liquefaction it was heated for 2hrs and ethanol was added slowly and the mixture was allowed to stand overnight. The product obtained is washed with boiling water and dried at 100^{o}C. The product obtained in step-I was used in step-2 for further synthesis.

STEP-II (Synthesis of substituted L-arginine analogues):

The product obtained in step-I was reacted with unsubstituted L-Arginine and some substituted L-Arginine in alkali like NaOH and acetone which results in clear solution after 2-3hrs of reaction. The solution thus obtained was acidified by the addition of HCl. The products separated were unsubstituted and some substituted L-arginine analogues. L-Arginine analogues were washed with cold water and dried. The compounds thus obtained were used for screening analgesic activity after purification and characterization. The %yield, melting points, Rf values and molecular formula of various substituted L-arginine analogues are tabulated in **Table 1.**

TABLE: 1-PHYSICAL DATA OF SUBSTITUTED L-ARGININE ANALOGUES (1-11)

Compound Name	R	Melting Point (^{o}C)	Rf value	% yield	Molecular Formula
1	H	205	0.62	76	$C_{22}H_{25}N_5O_4$
2	4-Cl	180 – 185	0.76	66	$C_{22}H_{24}N_5O_4Cl$
3	4-OCH_3	210	0.48	65	$C_{23}H_{27}N_5O_5$
4	4-OH	190	0.66	45	$C_{22}H_{25}N_5O_5$
5	4-OH, 3-OCH_3	175 – 177	0.82	47	$C_{23}H_{27}N_5O_6$
6	5-Br, 4-OH, 3-OCH_3	170 – 172	0.72	46	$C_{23}H_{26}N_5O_6Br$
7	4-$N(CH_3)_2$	198 – 200	0.56	79	$C_{24}H_{30}N_6O_4$
8	4–$(CH_3)_2$	190	0.692	55	$C_{25}H_{31}N_5O_4$
9	4-NO_2	195	0.833	51	$C_{22}H_{24}N_6O_6$
10	4-CH_3	205	0.44	53	$C_{23}H_{27}N_5O_4$
11	5-I, 4-OH, 3-OCH_3	207	0.51	45	$C_{23}H_{26}N_5O_6I$

Preparation of the test and standard drug

The synthesized l-arginine analogues were insoluble in water. So, the test compounds and standard drug were suspended 1% carboxy methyl cellulose and prepared in the concentration of 100mg.kg body weight.

Animals

Male albino mice weighing (18-25g) were used for the evaluation of analgesic activity. They were kept in polypropylene cages at 25 ± 2^{o}C, with relative humidity of 45 – 55% under 12 hrs light and dark cycles. All the animals were acclimatized to the laboratory conditions of SreeDattha Institute of Pharmacy for a week before use. They were fed with standard animal feed and water ad libitum.

Analgesic activity

Pain is induced by injecting irritants into the peritoneal cavity of mice. 0.1ml of 0.6% solution of glacial acetic acid is injected intraperitoneally to mice with a weight between 18-25gms[8]. Test animals were divided into groups of 6 animals each and subjected to fasting overnight. Test and standard drugs were administered at various pretreatment times prior to acetic acid administration. The mice are placed individually in a glass beaker and are allowed to elapse for 5minutes. The mice were then observed for a period of ten minutes and the number of writhes was recorded for each animal. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The formula for computing % inhibition is as follows and the results are tabulated in **Table 2.**

% inhibition = Avg. writhes in control – Avg. writhes in test / Writhes in control X 100

Those compounds which inhibit writhing more than 70% were considered to possess good activity and those with less than 70% inhibition were considered to have minimal activity.

TABLE: 2 ANALGESIC ACTIVITY OF L-ARGININE ANALOGUES

Compound	Dose (mg/kg)	No. of writhes (Mean ±SEM)	% inhibition	Significance
Control	---	66.33 ± 2.29	--	---
Paracetamol	100	37.33 ± 2.41	43.72	P < 0.001
Compound 1	100	28.98 ± 2.34	56.30	P < 0.001
Compound 2	100	31.87 ± 1.98	51.95	P < 0.001
Compound 3	100	29.33 ± 1.82	55.78	P < 0.001
Compound 4	100	32.67 ± 2.36	50.74	P < 0.001
Compound 5	100	38.76 ± 2.41	41.56	P < 0.001
Compound 6	100	34.79 ± 1.76	47.55	P < 0.001
Compound 7	100	37.74 ± 2.37	43.10	P < 0.001
Compound 8	100	38.68 ± 2.46	41.68	P < 0.001
Compound 9	100	38.33 ± 1.86	42.21	P < 0.001
Compound 10	100	37.76 ± 2.32	43.07	P < 0.001
Compound 11	100	52.33 ± 3.93	21.10	NS

NS: Not significant

RESULTS AND DISCUSSION: All the L-Arginine analogues synthesized have good yield value. The melting points of all the compounds were determined in an open capillary tube using an electro thermal digital meting point apparatus and are uncorrected. The compounds were characterized using analytical techniques such as UV, TLC and FT-IR. All the Compounds were screened for analgesic activity and the results were compared with that of the standard drug. The study revealed that Compound 1, 2, 3 and 4 exhibited very significant analgesic action while compound 11 has shown very minimum anti-pyretic action. The compounds can be screened for anti-pyretic action using other screening models to assess their activity on a broader scale which is our future part of the research work.

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