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Research Article

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SOME NOVEL SUBSTITUTED L-ARGININE ANALOGUES

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ABSTRACT

Objective: The present objective of the study is to synthesize a series of novel L-arginine analogues and to evaluate for their anti-inflammatory activity by rat paw oedema method.

Methods: A series of novel L-arginine analogues (Compounds 1 - 11) were synthesized. 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 anti-inflammatory activity by carrageenan-induced rat paw oedema model using ibuprofen as the standard drug for comparing the test results.

Results and Conclusion: The study concluded that the compounds 4 and 11 were found to exhibit significant anti-inflammatory action.

Keywords: Substituted l-Arginine analogues, Anti-inflammatory activity, Carrageenan induced rat paw oedema model, Ibuprofen.

INTRODUCTION

Nitric oxide is thought to promote a number of chronic inflammatory diseases such as arthritis, hepatitis, inflammatory bowel disease, sepsis, hemorrhagic shock and certain autoimmune disorders [1, 2, 3]. Nitric oxide has been recognized as one of the versatile players in immune function. It is known that in the presence of molecular oxygen (O_2), NO can form reactive nitrogen oxide species that can damage DNA and inhibits a variety of enzymes and initiates lipid peroxidation [4].NO is synthesized by inducible NO synthase in activated immune cells during inflammation [5]. Skin inflammatory dermatoses such as prostate lesions exhibit abnormally high levels of mRNA of iNOS which explains the local vasodilation and hypothermia associated with these disorders [6]. Therefore it could be of therapeutic value to inhibit iNOS and there is a considerable progress in the molecular design of highly potent and selective inhibitors of iNOS. A basic understanding of the physiological chemistry of Nitric Oxide may help in the design of new therapeutic strategies for the treatment of inflammatory tissue injury [7]. Identification of N-methyl-L-Arginine (L-NMA) as the first inhibitor of NO biosynthesis led to the design of selective iNOS inhibitors [8]. 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 the anti-inflammatory 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.

Characterization of the synthesized compounds

1. N-[2-(benzoylamino)-3-phenyl-1-oxo-2-propenyl]arginine

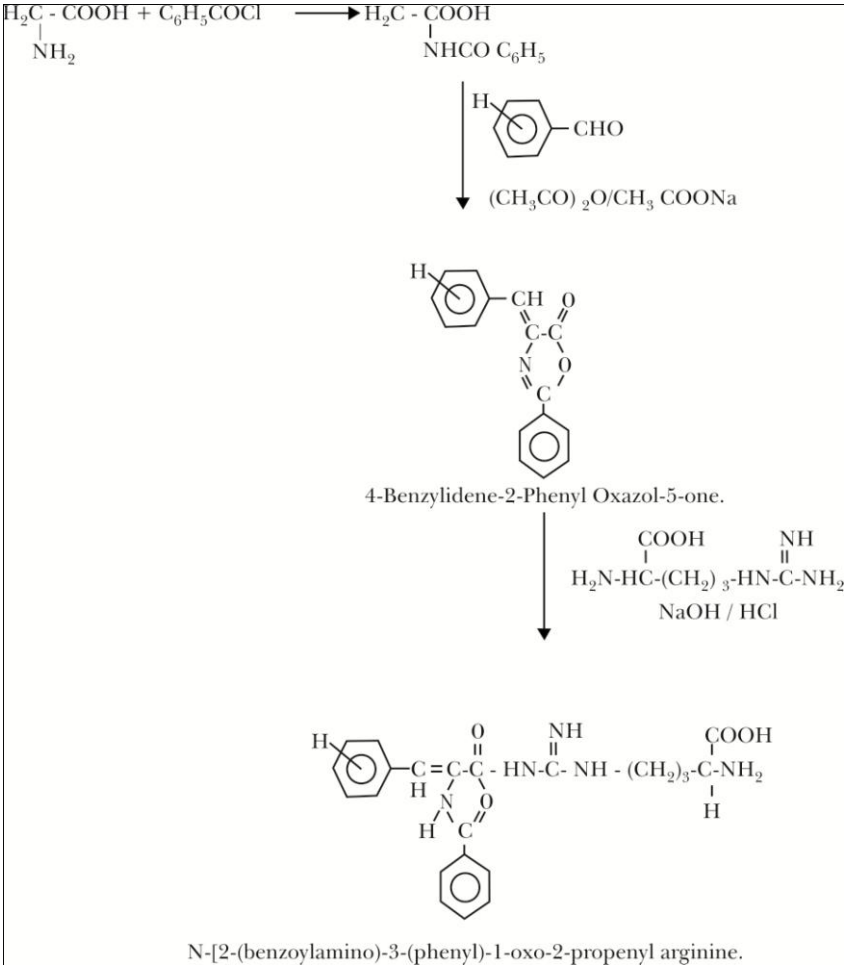
Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.62. The compound was also characterized by UV and FT-IR. The UV absorption peak of the compound was observed at 260nm. The FT-IR spectra of the compound displayed bands at 3298 cm^{-1} due to N-H stretching, 2926 cm^{-1} (O-H), 1648 cm^{-1} (C=O), 1601 cm^{-1} (C=C) and at 1578 cm^{-1} due to C=N stretching.

2. N-[2-(benzoyl amino)-3- (4-chloro phenyl)- 1-oxo-2-propenyl]arginine

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.76. The compound was also characterized by UV and FT-IR. The UV absorption peak of the compound was observed at 221nm. The FT-IR spectra of the compound displayed bands at 3298 cm^{-1} due to N-H stretching, 2926 cm^{-1} (O-H), 1648 cm^{-1} (C=O), 1601 cm^{-1} (C=C), 789.88 cm^{-1} due to C-Cl stretching and at 1578 cm^{-1} due to C=N stretching.

3. N-[2-(benzoyl amino)-3- (4-methoxy phenyl)- 1-oxo-2-propenyl]arginine

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.48. The compound was also characterized by UV and FT-IR. The UV absorption peak of the compound was observed at 223nm. The FT-IR spectra of the compound displayed bands at 3429 cm^{-1} due to N-H stretching, 3240 cm^{-1} (O-H), 1695 & at 1646 cm^{-1} (C=O), 1606 cm^{-1} (C=C) and at 1579 cm^{-1} due to C=N stretching.



Scheme representing the chemical synthesis

Table 1: Physical data of substituted L-arginine analogues (1-11)

Compound name	R	Melting point (°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	$C_{23}H_{26}N_5O_6I$

4. N-[2-(benzoyl amino)-3- (4-hydroxy phenyl)- 1-oxo-2-propenyl]arginine

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.66. The compound was also characterized by UV and FT-IR. The UV absorption peak of the compound was observed at 260nm. The FT-IR spectra of the compound displayed bands at 3426 cm^{-1} due to N-H stretching, 3238 cm^{-1} (O-H), 1698 & at 1686 cm^{-1} (C=O), 1604 cm^{-1} (C=C) and at 1575 cm^{-1} due to C=N stretching.

5. N-[2-(benzoyl amino)-3- (4-hydroxy – 3-methoxy phenyl)-1-oxo-2-propenyl]arginine

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.82. The compound was also characterized by UV and FT-IR. The UV absorption peak of the compound was observed at 364nm. The FT-IR spectra of the compound displayed bands at 3427 cm^{-1} due to N-H stretching, 2923 cm^{-1} (O-H), 1653 cm^{-1} (C=O), 1602 cm^{-1} (C=C) and at 1558cm^{-1} due to C=N stretching.

6. N-[2-(benzoyl amino)-3- (5-bromo, 4-hydroxy, – 3-methoxy phenyl)- 1-oxo-2-propenyl]arginine

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.72. The compound was also characterized by UV and FT-IR. The UV absorption peak of the compound was observed at 290nm. The FT-IR spectra of the compound displayed bands at 3437

cm^{-1} due to N-H stretching, 2928 cm^{-1} (O-H), 1653 cm^{-1} (C=O), 1642 cm^{-1} (C=C) and at 1568cm^{-1} due to C=N stretching.

7. N-[2-(benzoyl amino)-3- (4-dimethyl amino phenyl)- 1-oxo-2-propenyl]arginine

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.56. The compound was also characterized by UV. The UV absorption peak of the compound was observed at 235nm and at 452 nm.

8. N-[2-(benzoyl amino)-3- (4-dimethyl amino phenyl)- 1-oxo-2-propenyl]arginine

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.69. The compound was also characterized by UV and FT-IR. The UV absorption peak of the compound was observed at 221nm and at 282 nm.The FT-IR spectra of the compound displayed bands at 3263 cm^{-1} due to N-H stretching, 2959 cm^{-1} (O-H), 1701 & 1646cm^{-1} (C=O), 1609 cm^{-1} (C=C) and at 1581cm^{-1} due to C=N stretching.

9. N-[2-(benzoyl amino)-3- (4-nitro phenyl)- 1-oxo-2-propenyl]arginine

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.83. The compound was also characterized by UV. The UV absorption peak of the compound was observed at 207nm.

10. N-[2-(benzoyl amino)-3- (4-methyl phenyl)- 1-oxo-2-propenyl]arginine Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.44. The compound was also characterized by UV. The UV absorption peak of the compound was observed at 281nm

11. N-[2-(benzoyl amino)-3- (5iodo vanillin) - 4-hydroxy – 3-methoxy phenyl) arginine

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapors. The final compound displayed Rf value of 0.51. The compound was also characterized by UV. The UV absorption peak of the compound was observed at 323nm.

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 Wistar rats (150-180 gms) were used for the study and kept at the laboratory animal house of SreeDattha Institute of Pharmacy for acclimatization to laboratory environment. They were kept in well cross ventilated room at 27±2^{o}C for 1 week before the commencement of experiment. Animals were provided with commercial rodent pellet diet and water ad libitum.

Anti-inflammatory activity [9]

Anti-inflammatory activity of few synthesized derivatives was determined by carrageenan induced rat paw oedema model. Wistar rats (150 – 180gms) were divided into 3 groups as control, rest and standard six animals in each group. Overnight fasted animals were used and during that period only tap water was given .Ibuprofen was used as the standard drug. Both test compounds and the standard drug were administered orally through gastric lavage needle. 1% of CMC was administered in control group. After 1hr of administering the compound, 1% carrageenan by sub plantar surface of the right hind paws of animals. The initial paw volume and also the paw volume after 2nd and 3rdhr of administering carrageenan were measured. Percentage paw oedema inhibition was calculated using the formula

V_c – V_t/V_cX 100. Where V_c=volume of paw oedema in control animals, V_t=volume of pawoedema in treated animals. The results were noted and are presented in Table: 2

Table: 2 Anti-inflammatory activity of L-arginine analogues

Compound	Dose (mg/kg)	Time (hrs)	Paw volume (ml) Mean ± SEM	% inhibition	Significance
Control	--	1	0.31 ± 0.077	--	--
		2	0.51 ± 0.065		
		3	0.74 ± 0.041		
Standard	100	1	0.125 ± 0.004	59.6	P < 0.001
		2	0.10 ± 0.013	86.23	
		3	0.06 ± 0.013	88.48	
Compound 2	100	1	0.20 ± 0.023	35.48	NS
		2	0.32 ± 0.037	37.25	
		3	0.49 ± 0.068	23.78	
Compound 3	100	1	0.39 ± 0.065	23.52	P < 0.001
		2	0.19 ± 0.041	38.7	
		3	0.30 ± 0.120	59.45	
Compound 4	100	1	0.27 ± 0.053	59.6	P < 0.001
		2	0.42 ± 0.054	86.23	
		3	0.52 ± 0.053	88.48	
Compound 5	100	1	0.29 ± 0.060	6.4	NS
		2	0.38 ± 0.058	25.4	
		3	0.52 ± 0.050	29.7	
Compound 7	100	1	0.19 ± 0.056	38.7	P < 0.001
		2	0.32 ± 0.072	37.25	
		3	0.51 ± 0.029	31.08	
Compound 9	100	1	0.32 ± 0.039	37.25	P < 0.001
		2	0.19 ±0.048	38.7	
		3	0.38 ± 0.043	48.65	
Compound 11	100	1	0.29 ± 0.005	63.14	P < 0.001
		2	0.125 ± 0.05	75.4	
		3	0.07 ± 0.031	77.4	

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. Compound 2, 3, 4, 5, 7, 9, 11 were screened for anti-inflammatory activity and the results were compared with what of the standard drug. The study revealed that Compound 4 and 11 exhibited very significant anti-inflammatory action while compound 5 has shown very minimum anti-inflammatory action. The compounds can be screened for anti-inflammatory 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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