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Green synthesis, antiinflammatory and antimicrobial evaluation of novel isoxazole carboxamide derivatives

Gollapalli Naga Raju*, Angirekula Naga Jyothi, Jonnakuti Poojitha and Rama Rao Nadendla

Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Guntur, India

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ABSTRACT

Isoxazole derivatives possess antibacterial, antiviral, anti-fungal, anti-inflammatory insecticidal activities. The novel series of isoxazole carboxamide derivatives were prepared by the various ketene dithioacetals condensed with hydroxyl amine hydrochloride, potassium hydroxide under microwave irradiation. All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were tested for their antibacterial and antifungal activity in vitro by broth dilution method with two Gram-positive bacteria, two Gram-negative bacteria and two fungal strains. The biological activities of the synthesized compounds have been compared with standard drugs Streptomycin and Greseofulvin. The compounds exhibited significant antibacterial and moderate antifungal activities. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis antimicrobial and anti-inflammatory screening of the new compounds are reported.

Keywords: Isoxazole, Anti-inflammatory, Antibacterial activity, Antifungal activity.

INTRODUCTION

The dramatically rising prevalence of multidrug-resistant microbial infection in the past few decades has become a serious health care problem. In order to prevent this serious medical problem, the elaboration of the new types of the previously known drugs is a very actual task. In recent years, the synthesis of novel isoxazole derivatives remains a main focus of medicinal research. Isoxazole is a five membered heterocyclic compound. Derivatives of isoxazole have played a crucial role in the history of heterocyclic chemistry and been used extensively important pharmacophores and synthons in the field of organic chemistry. Owing to their versatile chemotherapeutic importance, a significant amount of research effort has been focused on these nuclei. Isoxazole derivatives exhibit various biological activities such as antibacterial [1,2], anticonvulsant [3,4], anticancer [5-7], anthelmintics [8], anti-inflammatory [9-11], adenosine antagonist [12], fungicidal [13-15], herbicidal [16], hypoglycemic [17], muscle relaxant [18], nematocidal [19, 20], insecticidal [21], antiviral [22] and antimicrobial [23].

Considering the above observations and in connection to previous publications involving the synthesis of new biologically active isoxazoles. Therefore, this work deals with the synthesis of isoxazole carboxamide derivatives and screening their biological activities.

MATERIALS AND METHODS

All the compounds are synthesized by using RAAGA Microwave synthesizer. Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on BROOKER-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a BRUKER Ac 400 MHz spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Purity of the synthesized compounds was checked by HPLC AGILENT. The results are in agreements with the structures assigned. All chemicals were reagent grade and used without further purification, and all solvents were freshly distilled before use.

General synthesis of 3-cyclopropyl-3-oxo-*N*-arylpropanamide: A mixture containing the primary amine (10 mmol), methyl-3-cyclopropyl-3-oxopropanoate (10 mmol), and catalytic amount of sodium or potassium hydroxide (10 %) in toluene (50 ml) was reflux at 110^{0}C for the approximately 12-15 h. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under *vaccuo* and the solid or oil was crystallized from methanol which afforded pure products.

General synthesis of ketene dithioacetals: A 100ml conical flask equipped with magnetic stirrer and septum was charged with a solution of 3-cyclopropyl-3-oxo-*N*-arylpropanamide (10 mmol) in DMF (10 ml). Dried K_2CO_3 (20 mmol) was added and the mixture was stirred for 2 h at room temperature. CS_2 (10 mmol) was added and the mixture was stirred for an additional 2 h at room temperature. Methyl iodide (20 mmol) was added at 0-5^{0}C and the mixture was stirred for 4 h at room temperature. The reaction was monitored by TLC. After completion, the mixture poured into water (40 ml). The precipitated crude product was purified by filtration followed by crystallization from ethanol. When the product was oil, the organic phase was extracted with Diethyl ether (3 × 10 ml). The combined organic extracts were washed with H_2O (2 × 10 ml), dried ($MgSO_4$), and concentrated in *vaccuo*to afford ketene dithioacetals directly used for the next step.

General procedure for the synthesis of novel isoxazole derivatives (ISD-1 to ISD-10):
Conventional method: A 25ml conical flask equipped with magnetic stirrer and septum was charged with hydroxyl amine hydrochloride (15 mmol), potassium hydroxide (15 mmol) and various ketene dithioacetals (10mmol) and heated up to 75-85^{0}C for appropriate times (Table-1). After completion of the reaction, the reaction mixtures were cooled to room temperature and add cold water (50 ml). The separated solid was filtered, washed with water, dried and crystallized from methanol to afford analytically pure products with 80-90% yield.

Microwave assisted method: A one neck flat bottom flask charged with hydroxyl amine hydrochloride (15mmol), potassium hydroxide (15 mmol) and various ketene dithioacetals (10mmol) and reaction mixture heated at 90^{0}C under microwave irradiation for appropriate time (Table-1). After completion of the reaction, the reaction mixture was allowed to come to room temperature and add cold water (50 ml). The separated solid was filtered, washed with water, dried and crystallized from methanol to afford analytically pure products with 85-95% yield. Comparative study of reaction time and yield in conventional and microwave irradiation methods are summarized in Table-1.

3-Cyclopropyl-5-(methylthio)-*N*-p-tolylisoxazole-4-carboxamide (ISD-1): White solid; *Rf*0.61 (8:2 hexane-EtOAc); M.P 144-146^{0}C; IR (KBr): 3282, 3149, 3081, 3033, 2978, 2839, 1630, 1584, 1410, 1236, 1039, 883, 837 cm^{-1}; MS (*m/z*): 288 (M^+); Anal. Calcd for $C_{14}H_{13}F_2N_3OS$: C, 62.48; H, 5.59; N, 9.71; Found: C, 62.49; H, 5.57; N, 9.72.

***N*-(4-Chlorophenyl)-3-cyclopropyl-5-(methylthio)isoxazole-4-carboxamide(ISD-2):** White solid; *Rf*0.70 (8:2 hexane-EtOAc); M.P 125-127^{0}C; IR (KBr): 3294, 3149, 3018, 2895, 1695, 1591, 1496, 1253, 1058, 810, 759, 715, 690 cm^{-1}; ^{1}H NMR (300MHz, CDCl3): δ 1.14-1.16 (m, 2H, CH_2), 1.25 (m, 2H, CH_2), 2.16-2.20 (m, 1H, CH), 2.68 (s, 3H, SCH_3), 7.26-7.33 (m, 1H, Ar-H), 7.53-7.56 (d, 1H, *j*=8.7*Hz*, Ar-H), 8.19 (broad, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$): 6.85, 13.66, 22.72, 29.18, 110.84, 121.05, 129.13, 136.09, 159.32, 162.66, 172.03; MS (*m/z*): 308 (M^+); Anal. Calcd for $C_{14}H_{13}ClFN_2OS$: C, 54.46; H, 4.24; N, 9.07; Found: C, 54.44; H, 4.25; N, 9.06.

3-Cyclopropyl-*N*-(4-fluorophenyl)-5-(methylthio)isoxazole-4-carboxamide (ISD-3): White solid; *Rf*0.59 (8:2 hexane-EtOAc); M.P 133-138^{0}C; IR (KBr): 3289, 3139, 3083, 3017, 2966, 1643, 1410, 1249, 885, 836 cm^{-1}; MS (*m/z*): 292 (M^+); Anal. Calcd for $C_{14}H_{13}FN_2OS$: C, 57.52; H, 4.48; N, 9.58; Found: C, 57.49; H, 4.47; N, 9.52.

3-Cyclopropyl-*N*-(4-methoxyphenyl)-5-(methylthio)isoxazole-4-carboxamide (ISD-4): White solid; *Rf*0.67 (8:2 hexane-EtOAc); M.P 120-123^{0}C; IR (KBr): 3288, 3147, 3095, 3031, 2965, 2837, 1637, 1592, 1409, 1247, 1036, 891, 833 cm^{-1}; MS (*m/z*): 304 (M^+); Anal. Calcd for $C_{15}H_{16}N_2O_3S$: C, 59.19; H, 5.30; N, 9.20; Found: C, 59.19; H, 5.31; N, 9.22.

***N*-(3,4-dichlorophenyl)-3-cyclopropyl-5-(methylthio)isoxazole-4-carboxamide (ISD-5):** White solid; *Rf*0.62 (8:2 hexane-EtOAc); M.P 180-182^{0}C; IR (KBr): 3295, 3148, 3091, 3011, 2951, 2831, 1625, 1587, 1418, 1241, 1029, 832, 779 cm^{-1}; MS (*m/z*): 343(M^+); Anal. Calcd for $C_{14}H_{12}Cl_2N_2O_2S$: C, 48.99; H, 3.52; N, 8.16; Found: C, 48.97; H, 3.53; N, 8.12.

***N*-(3-chloro-4-fluorophenyl)-3-cyclopropyl-5-(methylthio) isoxazole-4-carboxamide (ISD-6):** White solid; *Rf*0.69 (8:2 hexane-EtOAc); M.P 197-199^{0}C; IR (KBr): 3288, 3139, 3072, 3017, 2969, 2834, 1631, 1587, 1417, 1237, 1046, 839, 791 cm^{-1}; MS (*m/z*): 327 (M^+); Anal. Calcd for $C_{14}H_{12}ClFN_2O_2S$: C, 51.46; H, 3.70; N, 8.57; Found: C, 51.47; H, 3.73; N, 8.54.

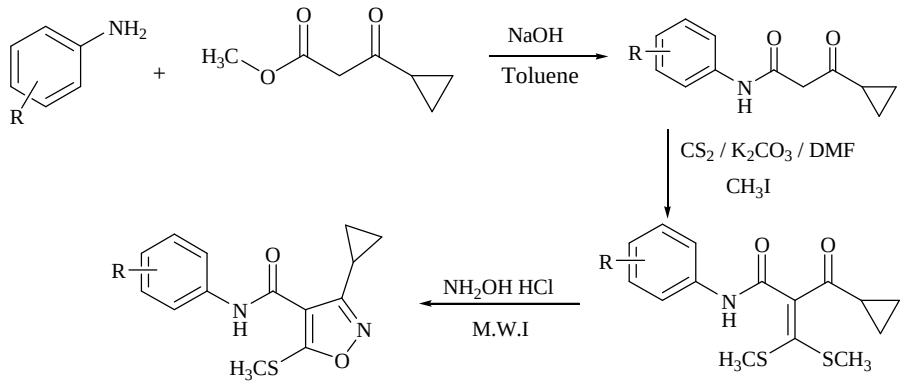
3-Cyclopropyl-*N*-(2,3-dimethylphenyl)-5-(methylthio) isoxazole-4-carboxamide (ISD-7): White solid *Rf*0.72 (8:2 hexane-EtOAc); M.P 181-183^{0}C; IR (KBr): 3297, 3138, 3089, 3015, 2952, 2823, 1631, 1591, 1421, 1237, 1025, 754, 787cm^{-1};MS (*m/z*): 302 (M^+); Anal. Calcd for $C_{16}H_{18}N_2O_2S$: C, 63.55; H, 6.00; N, 9.26; Found: C, 63.57; H, 6.03; N, 9.24.

3-Cyclopropyl-5-(methylthio)-*N*-(4-nitrophenyl)isoxazole-4-carboxamide (ISD-8): White solid *Rf*0.58 (8:2 hexane-EtOAc); M.P 177-179^{0}C; IR (KBr): 3277, 3148, 3091, 3004, 2942, 2839, 1636, 1591, 1419, 1241, 1033, 837 cm^{-1};MS (*m/z*): 319 ($M^{+)}$; Anal. Calcd for $C_{14}H_{13}N_3O_4S$: C, 52.66; H, 4.10; N, 13.16; Found: C, 52.65; H, 4.13; N, 13.14.

3-Cyclopropyl-*N*-(2-methoxyphenyl)-5-(methylthio)isoxazole-4-carboxamide (ISD-9): White solid *Rf*0.63 (8:2 hexane-EtOAc); M.P 131-133^{0}C; IR (KBr): 3285, 3142, 3083, 3013, 2964, 2833, 1637, 1591, 1414, 1246, 1028, 746 cm^{-1};MS (*m/z*): 304 (M^+); Anal. Calcd for $C_{15}H_{16}N_2O_3S$: C, 59.19; H, 5.30; N, 9.20; Found: C, 59.19; H, 5.31; N, 9.22.

3-Cyclopropyl-5-(methylthio)-*N*-o-tolylisoxazole-4-carboxamide (ISD-10): White solid; *Rf*0.67 (8:2 hexane-EtOAc); M.P 155-157^{0}C; IR (KBr): 3285, 3145, 3091, 3003, 2964, 2831, 1639, 1589, 1413, 1237, 1029, 761 cm^{-1}; MS (*m/z*): 288 (M^+); Anal. Calcd for $C_{14}H_{13}F_2N_3OS$: C, 62.48; H, 5.59; N, 9.71; Found: C, 62.49; H, 5.57; N, 9.72.

Scheme 1: Synthesis of novel isoxazole carboxamide derivatives (ISD-1 to ISD-10)



Novel isoxazole carboxamide derivatives (ISD-1 TO ISD-10)

Table-1: Comparative study of reaction time and yield in conventional and microwave irradiation methods

Compd	R	Reaction Time			
		Conventional	Yield (%)	M.W.I	Yield (%)
ISD-1	4-CH_3	2.5 hours	79	12.5min	85
ISD-2	4-Cl	2.0 hours	83	15.0min	93
ISD-3	4-F	3.0 hours	82	14.5 min	90
ISD-4	4-OCH_3	1.5 hours	87	13.0 min	92
ISD-5	3,4-diCl	2.0 hours	85	14.0 min	91
ISD-6	3-Cl,4-F	2.5 hours	82	13.0 min	87
ISD-7	2,3-diCH_3	3.0 hours	81	13.5 min	89
ISD-8	4-NO_2	2.5 hours	90	14.0 min	95
ISD-9	2-OCH_3	3.0 hours	89	15.0 min	94
ISD-10	2-CH_3	2.5 hours	87	17.5 min	92

Table-2: Physical constants of synthesized novel isoxazole carboxamide derivatives:

Compd	R	M.F	M.W	M.P (^{0}C)	R_f
ISD-1	4-CH_3	$C_{14}H_{13}F_2N_3OS$	309.33	144-146	0.61
ISD-2	4-Cl	$C_{14}H_{13}ClFN_2OS$	311.78	125-127	0.70
ISD-3	4-F	$C_{14}H_{13}FN_2OS$	276.33	133-135	0.59
ISD-4	4-OCH_3	$C_{15}H_{16}N_2O_3S$	304.36	120-122	0.67
ISD-5	3,4-diCl	$C_{14}H_{12}Cl_2N_2O_2S$	343.23	180-182	0.62
ISD-6	3-Cl,4-F	$C_{14}H_{12}ClFN_2O_2S$	326.77	197-199	0.69
ISD-7	2,3-diCH_3	$C_{16}H_{18}N_2O_2S$	302.39	181-183	0.72
ISD-8	4-NO_2	$C_{14}H_{13}N_3O_4S$	319.34	177-179	0.58
ISD-9	2-OCH_3	$C_{15}H_{16}N_2O_3S$	304.36	131-133	0.68
ISD-10	2-CH_3	$C_{14}H_{13}F_2N_3OS$	309.33	155-157	0.67

BIOLOGICAL EVALUATION [24]**:**

Preparation of Culture Media: Nutrient broth was used as growth medium for bacteria and Saubouraud dextrose broth for fungi. Nutrient broth was prepared by dissolving 13gm of dehydrated powder (HI-media) in 100ml of distilled water. Saubouraud dextrose broth was prepared by dissolving 4gm of dextrose and 1gm of peptone in 100ml of distilled water. The media were sterilized by autoclaving at 15lbs pressure for 20 minutes.

Preparation of Stock Culture: Stock cultures were obtained by aseptically transferring a loopful of test organisms to 100ml of sterile broth and incubated for 24 hours at 37^0C.

Standardization of Stock Culture: Stock cultures were placed in the incubator (37^0C for bacteria and 24^0C for fungi) and shaken well. One ml of stock cultures was aseptically transferred to 9 ml of sterile water containing 0.05% tween 80. This was mixed with using a cyclomixer and serially diluted from 10^{-1} to 10^{-10}. From each dilution, 0.2ml was taken and spread on sterile nutrient agar plates for bacteria and Sabouraud dextrose agar plates for fungi, which were incubated for 18 hours. After incubation, the numbers of colonies in the plate were counted. The number of colonies for a plate that was formed from the maximum dilute tube was noted. The number of microorganisms in stock were then calculated and expressed as colony forming units per ml (cfu/ml). By back calculation the stock culture was found to contain 15×10^8cfu/ml.

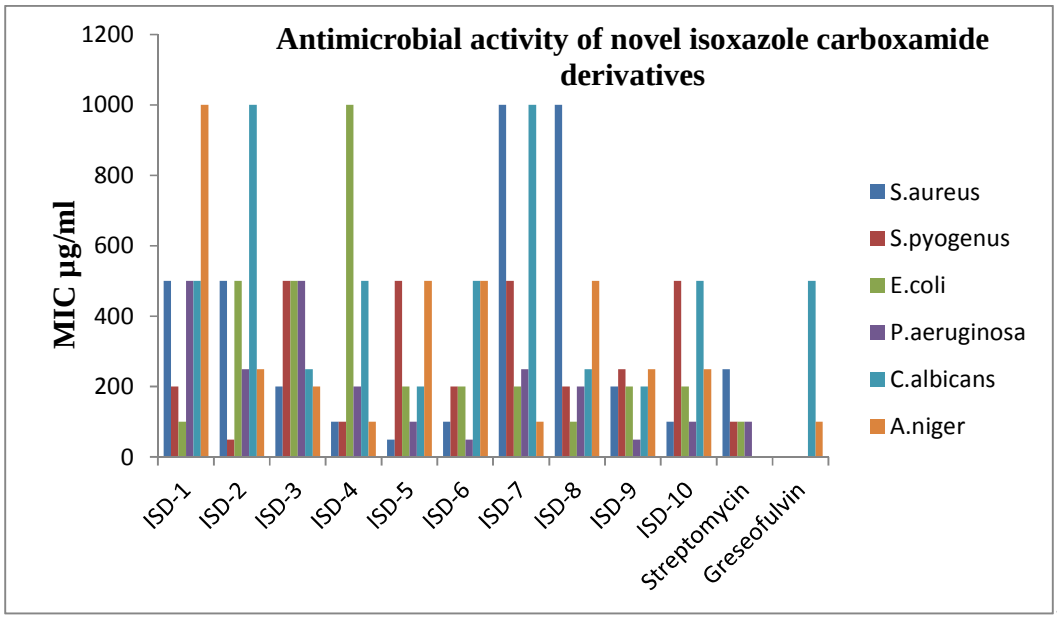
Preparation of Working Stock Culture: Stock culture (0.1ml) was diluted with nutrient broth (100ml) and Sabouraud dextrose broth (100ml) respectively to obtain 10^5cfu/ml. This was then used for further *in vitro* screening.

Preparation of Drug Dilutions: Solutions of the title compounds in DMSO (1mg/ml) were prepared and used for screening their antimicrobial activity.

Antimicrobial Screening: Synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique. Test was carried out on four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus*, *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282).

Determination of MIC: The study involved a series of six assay tubes for each title compound against each microorganism. The entire test was done in duplicate. To the first assay tube, 1.8ml of seeded broth and 0.2ml of title compound (1mg/ml) was added and mixed thoroughly and the two fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. Solvent control, negative control (growth control) and drug control were maintained during the experiment. The assay tubes were incubated at 37^0C and 25^0C respectively for 24 hours for bacteriae and fungi. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms, was considered as the minimum inhibitory concentration (MIC). The MIC values of the test compounds are recorded and given in figure 1.

Fig 1: Antimicrobial activity of novel isoxazole carboxamide derivatives (ISD-1 TO ISD-10)



ANTI-INFLAMMATORY ACTIVITY [25]**:** Carrageenan-induced rat paw edema method employing Zeitlin's apparatus was used to determine the anti-inflammatory activity of the newly synthesized isoxazole carboxamide derivatives.

Materials: Carrageenan required for inducing the inflammation was obtained from Himedia (Mumbai) whereas sodium CMC was of Merck grade and the required saline (Core Health Care) was purchased from a local supplier. Aceclofenac used as standard was supplied as a gift sample by Jagsonpal, New Delhi.

Preparation of sodium CMC suspension: 1gm of sodium CMC was triturated in 100 ml of distilled water to give the required stock suspension of sodium CMC. This stock suspension was used for suspending all the test compounds as well as the standard drug.

Experimental procedure: Albino rats of either sex, weighing between 150-200 gm, supplied by M/S Ghosh Enterprises, Kolkata were divided into twenty seven groups of six animals each. All these groups were kept for fasting overnight and only allowed water adlibitum.

0.05 ml of 1% carrageenan suspension was slowly injected subcutaneously into the subplantar region of the left hind paw to produce inflammation in all the groups. Groups III to XXVII were treated with 7-(Thiophen-2-yl)pyrido[2,3d]pyrimidin-4(3H)-one derivatives (TP-1 to TP-10) (10 mg/kg) after carrageenan administration and the time gap is at an interval of 0.5, 1, 2, 3, 4 and 6 h. Group I used as carrageenan treated control was given only 1% sodium CMC suspension (1 ml/kg) whereas group II received aceclofenac (2 mg/kg). All these doses were

administered orally and the induced paw edema in each group was measured to assess the anti-inflammatory activity.

Measurement of paw thickness: Before carrageenan injection, the thickness of both the paws of each rat was measured using Zeitlin's constant load lever method. The paws thickness was also measured in a similar way after carrageenan injection at time intervals 0, 0.5, 1, 2, 3, 4 and 6 h. The dose selection for the compound in the preliminary screening is usually 5 times the dose of the standard drug aceclofenac, which was used at a dose of 2 mg/kg.

The percent increase at each time interval was determined by using the formula: Yt-Yo / Yo×100 Yt = Paw thickness at time t hours (after injection), Yo = Paw thickness at time 0 hours (before injection)

The percent inhibition of paw oedema thickness was calculated by using the formula: Percentage inhibition = [1-Yt/Yc] × 100

Where Yt= Average increase in paw thickness in groups tested with isoxazole carboxamide derivatives and the standard.

Yc= Average increase in paw thickness in control

The results of anti-inflammatory activity of aceclofenac and the isoxazole carboxamide derivatives tested are shown in Table 4

Table 4: Percentage inhibition in paw thickness at various time intervals

Compd	Percentage inhibition in paw thickness					
	0.5hr	**1hr**	**2hr**	**3hr**	**4hr**	**6hr**
ISD-1	18±1	20±1	56±1	66±1	95±1	95±2
ISD-2	15±1	18±2*	51±1	60±1**	89±1	91±1
ISD-3	09±1	12±2	44±1	54±1*	84±1**	85±2*
ISD-4	03±1	07±1**	39±2	48±1	73±1	74±2
ISD-5	17±1	19±1	53±1*	62±1	94±1	92±2
ISD-6	15±1	17±2	50±2	60±1	85±1	90±1*
ISD-7	16±1	18±1	52±1**	62±1	85±1	89±1
ISD-8	06±1	11±1	42±1	52±1	80±1	82±2
ISD-9	18±1	20±1	54±1	62±1*	93±1	92±2
ISD-10	12±1*	13±2	46±1	55±2	82±2	85±1
Aceclofenac	21±1	24±1	57±1	66±1	96±2	96±1

Values are expressed as mean ±(n=6) P<0.05, P**<0.01 compared to control, Student t-test (Unpaired) Value for the control group in all the cases is zero*

RESULTS AND DISCUSSION

A mixture of primary amine, methyl-3-cyclopropyl-3-oxopropanoate, and catalytic amount of sodium hydroxide in toluene reacts to give 3-cyclopropyl-3-oxo-*N*-arylpropanamide. This compound with CS_2, K_2CO_3, and DMF with methyl iodide affords ketene dithioacetals. Then the hydroxyl amine hydrochloride, potassium hydroxide with ketene dithioacetals under microwave irradiation for appropriate time forms novel isoxazole carboxamide derivatives (ISD-1 TO ISD-10).All the synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique and anti-inflammatory activity by carrageenan-induced rat paw edema method.

All the synthesized compounds were evaluated for their antimicrobial and anti-inflammatory activities. Results revealed that the compounds exhibited significant in-vitro activity. Compound ISD-5 is more potent and compounds ISD- 4, ISD-6, ISD-10 are moderately potent against *S.aureus.* Compound ISD-2 is more potent and compound ISD- 4 is moderately potent against *S.pyogenus*. Compound ISD-6 is more potent and compounds ISD- 1, ISD-3, ISD-8 are moderately potent against *E.coli.* Compounds ISD-6, ISD-9 and compounds ISD-3, ISD-10 are moderately potent against *P.aeruginosa.* All these compounds are compared with the standard reference (Streptomycin) for their antibacterial activities. Compounds ISD-3, ISD-5, ISD-8 and ISD-9 are potent activity against fungal strain *Candida albicans* and compounds. Compounds ISD-2, ISD-3, ISD-9 and ISD-10 are more

potent against *Aspergilla niger* when compared with standard reference "Greseofulvin". Remaining compounds also showed moderate to weak antimicrobial activities.

The anti-inflammatory activity of the newly synthesized isoxazole carboxamide derivatives (ISD-1 to ISD-10) has been evaluated by using carrageenan-induced rat paw edema method. The results of the evaluation have been viewed by taking aceclofenac as the standard drug.

The results of anti-inflammatory activity revealed that the compounds ISD-1 to ISD-10exhibited moderate to considerable activity when compared with reference standard aceclofenac, but not at an identical dose level as the standard drug was tested at 2 mg/kg, whereas the derivatives were tested at a dose of 10 mg/kg. Isoxazole carboxamide derivatives ISD-2, ISD-5, ISD-1, and ISD-9having the electron withdrawing groups like the halogens showed maximum activity and this is consistent with the literature reports that such groups enhance the lipophilic properties of the molecule. Other compounds tested in this present study also showed some degree of anti-inflammatory activity. Some of these compounds were substituted with electron releasing substituents on the aromatic ring at different positions.

CONCLUSION

In this study, novel series of isoxazole carboxamide derivatives were synthesized in a single step with excellent yield under microwave irradiation. As a part of 'green chemistry' approach condensation reaction of various ketene dithioacetals with hydrazine hydrate in solvent free condition under microwave irradiation. The present procedure is significant over the existing methods to develop this class of molecules with excellent yield, purity and simple isolation of products. In this study isoxazole carboxamide derivatives were synthesized and evaluated for their antimicrobial & anti-inflammatory activities. Results revealed that the compounds exhibited significant in-vitro activity. All the synthesized compounds are more potent to moderate antimicrobial activities against the test organisms. Isoxazole carboxamide derivatives having the electron withdrawing groups like the halogens showed maximum activity. Other compounds tested in this present study also showed some degree of anti-inflammatory activity. The study would be a fruitful matrix for the development of novel series of isoxazole carboxamide derivatives for further bio-evaluation.

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REFERENCES

[1] E. Kumara Swamy et al, *Der Pharma Chemica*, **2011**, 3 (6):224-234.
[2] Dodia et al, *The International Journal of Science and Technoledge*2.4 (**2014**): 62-66.
[3] Ashok Kumar et al, *Int.J. ChemTech Res*.**2010**, 2(2).
[4] S. V. Manju et al, *International Bulletin of Drug Research*, **2013**, 1(1): 100-106.
[5] Yong JP, Lu CZ, Wu X et al, *Anticancer Agents Med Chem*. **2014**; 15(1):131-6. 2014; 15(1):131-6.
[6] Mostafa M. Ghorab et al, *ActaPharmaceutica*, **2013**, Volume 64, Issue 4, Pages 419–431.
[7] Canzhong Lu et al, *Med. Chem. Commun*., **2014**, 5, 968-972.
[8] P.Mondal et al, *J Young Pharm*, v.4(1); Jan-Mar **2012**
[9] B.V. Kendre, et al., *Arabian Journal of Chemistry* (**2015**), http://dx.doi.org/10.1016/j.arabjc.**2015**.01.007.
[10] EL-Hawash SA et al, *Med Chem*. **2014** May; 10(3):318-38.
[11] S.S Panda et al, *Indian J Pharm Sci*. **2009** Nov-Dec; 71(6): 684–68.
[12] Choi K et al, *Chem Biol*. **2005** Apr; 12(4):469-75.
[13] Vicentini CB et al, *Pharm Biol*. **2011** May; 49(5):545-52.
[14] Hyung Jin Kim, Kwang-Jin Hwang & Jae Hyun Lee, *Bioscience, Biotechnology, and Biochemistry*, **1994**, 58:6, 1191-1192.
[15] K. A. Parmar et al, *Der Chemica Sinica*, **2011**, 2 (1):100-110.
[16] Yuhan Zhou et al, *Journal of Heterocyclic Chemistry*, Volume 47, Issue 6, pages 1310–1316, November **2010**.
[17] Kumar A et al, *Bio org Med Chem*. **2009** Jul 15; 17(14):5285-92.
[18] Tochiro Tatee et al, *Chemical & Pharmaceutical Bulletin*; **2012**, vol.34; no.4; page.1643-1655.
[19] A. Srinivas et al, *Eur J Med Chem*. **2010** Jun; 45(6):2353-8.

[20] Singh Rajvir et al, *Pesticide Research Journal*, year **2006**, volume: 18, Issue: 2, 128-128.
[21] Qingmin Wang et al, *J. Agric. Food Chem*., **2011**, 59 (9), pp 4851–4859.
[22] Artemenko et al, *Future Med. Chem*. (**2011**) 3(1), 15–27.
[23] Gollapalli Naga Raju et al, *Der Pharma Chemica*, **2015**, 7 (6):346-352.
[24] Gollapalli Naga Raju et al, *Der Pharmacia Lettre*, **2015**, 7 (5):153-159
[25] Gollapalli Naga Raju et al, *Der Pharma Chemica*, **2016**, 8 (1):449-455