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Synthetic and biological studies on thioxoquinazolinone substituted isoxazoles

K. Lakshmi[1]*, N. Rama Rao[1] and M.V. Basaveswara Rao[2]

[1]Chalapathi Institute of Pharmaceutical Sciences, Chalapathinagar, Guntur-522 034, India
[2]Krishna University, Dr. MRAR PG Centre, Nuzvid-521 201, India
*Corresponding Author's E-mail: lakshminageswar@gmail.com

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Abstract

Eighteen novel 5-phenyl-3-(substitutedthioxoquinazolinonyl) isoxazoles (5a-r) were synthesized by cycloaddition of various chalcones with hydroxylaminehydrochloride ($NH_2OH.HCl$). All the newly synthesized compounds were confirmed by their spectral and analytical data. They were evaluated for antimicrobial, antihelminthic, analgesic and anti-inflammatory activities with an objective to evaluate the effect of thioxoquinazolinone substituted isoxazoles. Compounds 5a, 5f, 5g, 5k and 5l showed significant antimicrobial, anthelmintic, analgesic and anti-inflammatory activities. Further, isoxazoles having bromo substituted thioxoquinazolinones exhibited excellent activities than iodo substituted thioxoquinazolinones.

Key words

Analgesic, Antimicrobial, Egg white, Isoxazoles, 2-thioxo-4 (3*H*) quinazolinones

Introduction

The search of new chemotherapeutic agents showing more potency, reduced toxicity and side effects is a continuous process. Several compounds such as B complex vitamins, colouring matter of green leaves chlorophyll, blood pigment heam, nucleoproteins DNA and RNA, dyes and even most of the drugs possess one or more heterocyclic rings. Heterocyclic compounds are very useful to human being(s) in many ways (Arora *et al*., 2012; Shrivastava *et al*., 2011). Isoxazole is one of the important hetero cycle which is generally synthesized from chalcones and aromatic aldehydes. The presence of isoxazole and/or isoxazoline nucleus is observed in many synthetic drugs like sulfisoxazole, cycloserine, isocorboxazide, danazole, ibotenic acid, pleconaril, sitaxentan, leflunomide, broxaterol, parecoxib, valdecoxib, tivozanib, oxacillin, cloxacillin, diclixacillin, muscimol and muscazone etc. Compounds possessing isoxazole nucleus in their structure have been reported to possess broad spectrum of biological activities like antibacterial (Hushare and Rajput, 2012; Patel *et al*., 2013), antifungal (Manna *et al.,* 2008; Patel *et al*., 2013, Sorathiya *et al.,* 1997), antitubercular (Haripara *et al.,* 2004; Manna *et al.,* 2008), analgesic (Karabasanagouda *et al.,* 2009), antitumor (Ryng and Dec, 1994), anticonvulsant (Eddington *et al*., 2002; Jackson *et al*., 2012), anti-inflammatory (Habeeb *et al.,* 2000), anxiolytic (Becan *et al*., 2004) and antiviral (Hayden *et al.,* 2002) activities. They are also used as electron transporting materials in molecular electronics (Naka *et al.,* 2000) and liquid crystalline materials (Iglesias *et al.,* 1997; Fonseca *et al.,* 2005). Quinazolinones, with their congeners, represent another important heterocyclic system exhibiting varied biological actions (Kalken *et al.,* 1986; Parmar and Arora, 1966; Parmar *et al.,* 1969; Jiang *et al.,* 1990; Jampilek *et al.,* 2009; Musiol *et al.,* 2009; Debnath and Devanna, 2013; Ali *et al.,* 2014). Broad spectrum activities of isoxazoles and quinazolinones has created much interest in current scenario and brought into light combining these two rings for achieving better potency and efficacy. So, the main objective of the present work lies in introducing thioxoquinazolinone substituent (s) on isoxazole ring. Therefore, in the present study, synthesis of thioxoquinazolinonyl chalcones was performed initially, followed by their cyclization to synthesize thioxoquinazolinonyl isoxazoles. All the synthesized compounds were characterized for structural elucidation and then evaluated for various biological activities.

Materials and Methods

Anthranilic acid used for synthesis was of analytical grade and procured from Sigma Aldrich Ltd. Chemicals like $NH_2OH.HCl$,

aniline, para (*p*)-chloro aniline, *p*-bromo aniline, meta (*m*)-chloro aniline, *p*-fluoro aniline and *p*-methyl aniline were procured from Fischer Scientific Ltd. All other chemicals used for synthetic purpose were of reagent grade and were procured from SD Fine Chemical Ltd. and Loba Chemicals Ltd.

All the respective 2-thioxoquinazolinonylchalcones were synthesized according to the method described by Abdel Megeed *et al.* (1995) and Furniss *et al.* (1989).

General procedure for synthesis of 5-phenyl-3-[3'-(un) substituted phenyl-6'-(un) substituted-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole (5a-r) : A mixture of 2-thioxoquinazolinonylchalcones (4a-r) [0.01M] and $NH_2OH.HCl$ (0.04M) was refluxed in ethanol (25ml) containing 2 % NaOH for 6 hrs. After cooling, the reaction mixture was diluted with water. The product thus precipitated was filtered, crystallized from ethanol to get the respective compounds (5a-r). All the titled compounds were synthesized according to Scheme-1.

Characterization : Melting points (MP) of the synthesized compounds were determined by an open-end capillary tube method with an electrically heated melting point apparatus. The respective values were expressed in degree celsius and were uncorrected. Reaction progress and compounds purity were ascertained by thin layer chromatography. The percent elemental analysis (C- Carbon; H - Hydrogen; N- Nitrogen) data of synthesized compounds obtained from Carlo Erba-1108 elemental analyzer was compared with the calculated ones from Chem sketch version 11. Office software and was found to be in agreement with the molecular formula of the assigned structures. The structural elucidation of all the synthesized compounds were performed by the following methods : Fourier Transformer-Infrared Spectrum (FT-IR, Perkin Elmer 1600 series) using Potassium Bromide (KBr) Pellet method; Proton Fourier Transform- Nuclear Magnetic Resonance (^{1}H FT-NMR, BRUCKER MX 400 MHz model) spectrum using trimethylsilane (TMS) as internal standard and Mass Spectrum (LC-MS, Agilent 1100 series) for m/z analysis.

In vitro antimicrobial and anthelmintic activity

Antimicrobial activity : Whatman filter paper discs (grade-1) of 5 mm diameter were used for studies. They were auto claved at standard conditions (121 ºC,15lb pressure for 15 min) for sterilization. All test compounds were dissolved in 10% dimethyl sulfoxide (DMSO) in methanol. Sterilized discs were impregnated with different synthesized compounds to obtain concentrations 200 µg disc^{-1}, 400 µg disc^{-1} and 600 µg disc^{-1}.

Synthesized compounds, 5a-r, were screened for their *in vitro* antimicrobial (antibacterial and antifungal)activity by paper disc diffusion method as described by Forbes *et al.,* 1990. Briefly, around15 ml of nutrient agar was poured in each flat bottomed petri dish. When agar got solidified, around 4 ml of second nutrient medium, seeded with test bacteria and fungi was poured evenly on the solidified first layer (40-48 ºC). Each petri dish was checked for proper solidification of primary and secondary agar layers. Further, the test compound impregnated discs were placed on the medium, suitably spaced apart and then the plates were transferred to an incubator maintained at 37±1 ºC for 18-24 hrs in case of bacteria, 25±1 ºC for 72hr in case of fungi. The respective inhibition zones in all cases were measured in mm using digital antibiotic zone reader. Ciprofloxacin and Fluconazole 100 µg disc^{-1} were used as reference standards for comparing the antibacterial and antifungal activities of the test compounds respectively. Various strains of bacteria and fungi that were used in the study are given in Table 1.

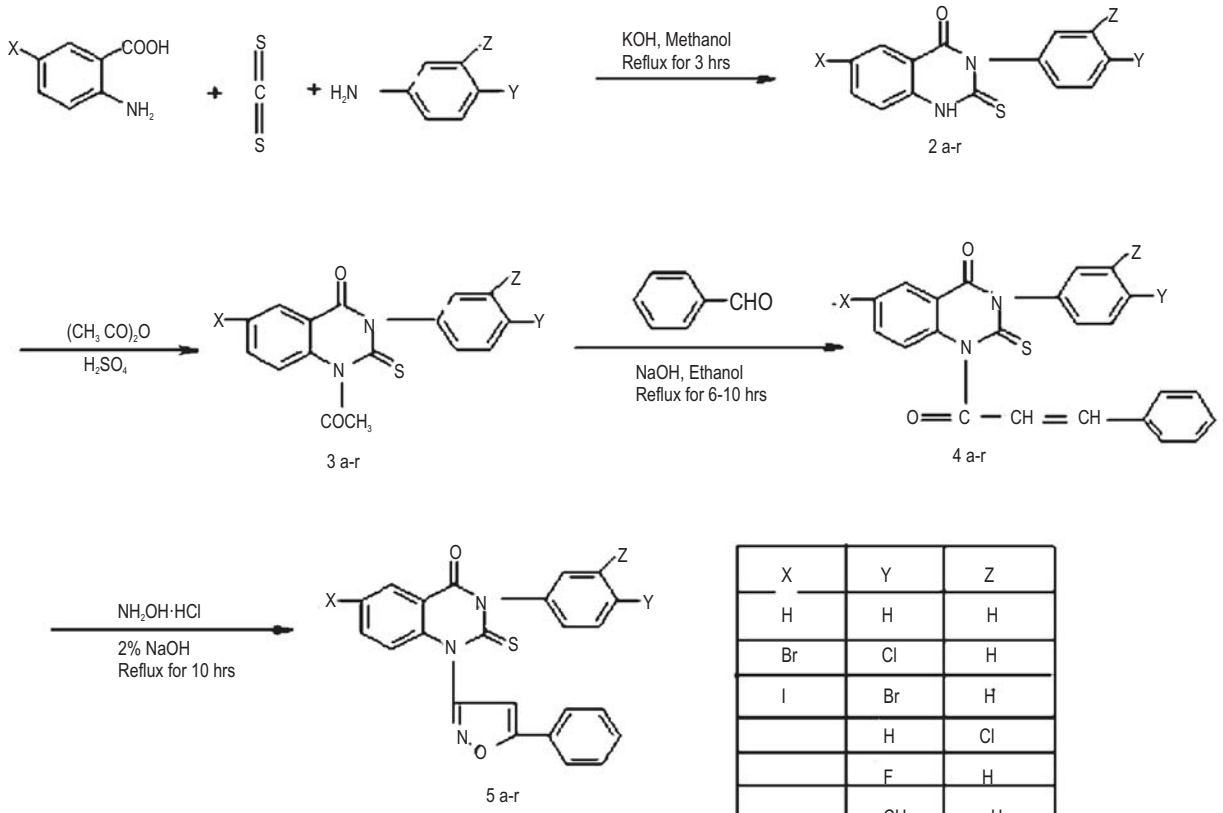
Anthelmintic activity : Synthesized compounds were tested for their *in vitro* anthelmintic activity according to the method described by Ghosh *et al.* (2003) with slight modification. Adult Indian earth worm *Peritima posthuma* of nearly equal size (9±l.5cm) was selected for study because of its anatomical and physiological resemblance with intestinal parasites in human beings. All the synthesized compounds were dissolved in 1% dimethyl for mamide (DMF) in normal saline to obtain 1 mg ml^{-1} concentration and transferred to 4 inch petri dish containing earth worms. Paralytic and lethal time taken for individual worms was observed taking 2 hrs time period as maximum time for parasite response to the drug. Paralysis was said to occur when the worms did not revive even in normal saline. Lethal time was concluded or ascertained when worms lost their motility with faded body colour. Reference standard used for comparing anthelmintic activity was Albendazole at 10 mg ml^{-1} concentration.

In vivo pharmacological studies

Animals : Animal experiments were conducted in accordance with the Principles of Laboratory Animal Care National Institutes of Health Guidelines.The study protocol was approved by the

Table 1 : List of various microbial strains used for antimicrobial activity

Microbe type	Nature	Latin names with codes	
Bacteria	Pathogenic	**Gram +ve**	*Staphylococcus aureus MTCC 737, Staphylococcus epidermidis MTCC 3086*
		Gram -ve	*Pseudomonas aerouginosa MTCC 1035, Escherichia coli MTCC 1687*
	Non-pathogenic	*Bacillus subtilis MTCC 441, Bacillus cereus MTCC 430*	
Fungi	Pathogenic	*Aspergillus niger MTCC 2638, Aspergillus foetidus MTCC 2737,Candida albicans MTCC 301* and *Candida glabrata MTCC 3019*	
	Non-pathogenic	*Saccharomyces cerevisiae MTCC 170*	



X	Y	Z
H	H	H
Br	Cl	H
I	Br	H
	H	Cl
	F	H
	CH_3	H

Scheme 1: Synthesis of novel isoxazole derivatives

Institutional Animal Ethical Committee of Chalapathi Institute of Pharmaceutical Sciences, Guntur, India (IAEC No. CIPS/ IAEC/ 02/9-4-2011). Swiss albino mice (20-25g) and Wistar albino rats (180-220g) were used for pharmacological studies. All the animals were inbreed, housed in polypropylene cages under controlled environmental conditions of 25±1 ºC, 45-55% RH and a 12:12 light/dark cycle. Experimental animals had free access to standard rodent pellets and water *ad libitum* unless stated elsewhere during experimentation.

Analgesic activity : Swiss albino mice of either sex, weighing about 20g-25g were used for this experiment. All the animals were fasted for 6hrs before starting the experiment. Animals were divided into three groups (n=6 per each group); Group I - Std. Pentazocine (30 mg kg^{-1}) in 1% v/v Tween 80 solution; Group II - Vehicle (1% v/v Tween-80) and Group III- Test compounds (5 mg kg^{-1} solubilised in 1% v/v Tween 80) dose. Group III animals were further divided into different subgroups for checking the analgesic activity (Kulkarni *et al.,* 2007; Ghosh, 2005)of all test compounds. Animals were placed on a hot plate maintained at constant temperature of 55±0.5 ºC immediately after intraperitoneal (i.p.) administration of the test compounds. Latency to exhibit the nociceptive response such as licking paws or jumping was recorded by a stop watch. A saturation cut-off time of 15 sec was selected to avoid tissue damage of the paws.

Anti-inflammatory activity : Wistar Albino rats of either sex weighing about 180g-220g were used for the study. Paw edema was induced in the plantar region of rats right hind paw. They were fasted for 6 hrs and deprived of water only during the experiment. Water deprivation was to ensure uniform hydration and to minimize variability in edematous response (Winter *et al.,* 1963). The animals were divided into three groups (n=6 per each group), Group I - Standard Diclofenac sodium (20mg kg^{-1}); Group II –Vehicle (0.2ml of 1% v/v Tween 80) and Group III - Test compounds (10 mg kg^{-1} solubilised in 1% v/v Tween 80). Group - III animals were further divided into different subgroups for checking the anti-inflammatory activity (Ekpendu *et al.,* 1994) of all test compounds. All the synthesized compounds were administered intraperitoneally 30 min before the intradermal injection of phlogistic agent (0.1ml of fresh undiluted egg albumin) in the plantar region of the right hind paw. Paw volume was measured by mercury displacement method using a Plethysmograph at 0, 0.5, 1and 2 hrs after egg-albumin injection. Anti-inflammatory activity of all the test compounds, at each time of observation was calculated as % inhibition of edema in the animals treated with substances under test, in comparison to the vehicle-treated animals.

Statistical analysis : All the results were expressed as mean± SEM. Statistical analysis was performed using Graph pad prism software version 5.01. Statistical analyses were performed using

Table 2 : Chemical details of of novel isoxazole derivatives

Comp.	X	Y	Z	Mol. Formula &Mol. Wt	IUPAC NAME
5a	H	H	H	$C_{23}H_{15}N_3O_2S$(397.00)	5-Phenyl-3-[3'-phenyl-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole
5b	H	Cl	H	$C_{23}H_{14}N_3O_2ClS$(431.45)	5-Phenyl-3-[3'-(4"-chlorophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]- isoxazole
5c	H	Br	H	$C_{23}H_{14}N_3O_2BrS$(475.90)	5-Phenyl-3-[3'-(4"-bromophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]- isoxazole
5d	H	H	Cl	$C_{23}H_{14}N_3O_2ClS$(431.45)	5-Phenyl-3-[3'-(3"-chlorophenyl)-2'-hioxo-4'(3'*H*)-quinazolinon-1'-yl]- isoxazole
5e	H	F	H	$C_{23}H_{14}N_3O_2FS$(414.90)	5-Phenyl-3-[3'-(4"-fluorophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole
5f	H	CH_3	H	$C_{24}H_{17}N_3O_2S$(411.00)	5-Phenyl-3-[3'-(4"-methylphenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole
5g	Br	H	H	$C_{23}H_{14}N_3O_2BrS$(475.90)	5-Phenyl-3-[6'-bromo- 3'-phenyl-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole
5h	Br	Cl	H	$C_{23}H_{13}N_3O_2ClBrS$(510.35)	5-Phenyl-3-[6'-bromo-3'-(4"-chlorophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole
5i	Br	Br	H	$C_{23}H_{13}N_3O_2Br_2S$(554.80)	5-Phenyl-3-[6'-bromo-3'-(4"-bromophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole
5j	Br	H	Cl	$C_{23}H_{13}N_3O_2ClBrS$(510.35)	5-Phenyl-3-[6'-bromo-3'-(3"-chlorophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole
5k	Br	F	H	$C_{23}H_{13}N_3O_2BrFS$(493.80)	5-Phenyl-3-[6'-bromo-3'-(4"-fluorophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole
5l	Br	CH_3	H	$C_{24}H_{16}N_3O_2BrS$(489.90)	5-Phenyl-3-[6'-bromo -3'-(4"-methylphenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]- isoxazole
5m	I	H	H	$C_{23}H_{14}N_3O_2IS$(522.90)	5-Phenyl-3-[6'-iodo- 3'-phenyl-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]-isoxazole
5n	I	Cl	H	$C_{23}H_{13}N_3O_2ClSI$(557.35)	5-Phenyl-3-[6'-iodo-3'-(4"-chlorophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]- isoxazole
5o	I	Br	H	$C_{23}H_{13}N_3O_2BrSI$(601.80)	5-Phenyl-3-[6'-iodo-3'-(4"-bromophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]- isoxazole
5p	I	H	Cl	$C_{23}H_{13}N_3O_2ClSI$(557.35)	5-Phenyl-3-[6'-iodo-3'-(3"-chlorophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]- isoxazole
5q	I	F	H	$C_{23}H_{13}N_3O_2ISF$(540.80)	5-Phenyl-3-[6'-iodo-3'-(4"-fluorophenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]- isoxazole
5r	I	CH_3	H	$C_{24}H_{16}N_3O_2IS$(536.90)	5-Phenyl-3-[6'-iodo -3'-(4"-methyl phenyl)-2'-thioxo-4'(3'*H*)-quinazolinon-1'-yl]- isoxazole

X – Substitutent at 6th position of quinazolinone ring; Y- Substitutent at para position of phenyl ring; Z- Substitutent at meta position of phenyl ring.

one-way ANOVA, followed by Post hoc Newman-Keuls multiple *comparison test* in case of anthelmintic activity, Dunnets test, in case of analgesic and anti-inflammatory activities. In all cases, $p < 0.05$ was considered as significant.

Results and Discussion

Reaction of anthranilic acid and its derivatives with various aromatic amines (aniline, *p*-chloro aniline, *p*-bromo aniline, *m*-chloro aniline, *p*-fluoro aniline and *p*-methyl aniline) and carbon disulfide in the presence of potassium hydroxide in methanol under reflux for 3hrs, afforded the corresponding 3-(un) substituted phenyl-6-(un) substituted-2-thioxo-4(3*H*)-quinazolinones 2a-r. When 2a-r compounds were treated with acetic anhydride, acetyl group was introduced at 1st position of the quinazolinone nucleus and yielded compounds 3a-r.Claisen Schmidt condensation (Kerher *et al*., 2003) of 3a-r with benzaldehyde in alcoholic alkali gave corresponding quinazolinonylchalcones 4a-r. IR spectrum of the quinazolinonylchalcones showed peak around 1670 cm^{-1} due to α, β-unsaturated keto functional group (Lakshmi *et al.,* 2014; Ghouili *et al.,* 2014; Hitendra *et al*., 2012; Baba *et al*., 1990). Appearance of doublets in the range of δ 6.7-6.9 parts per million (ppm) and δ 7.44-7.56 ppm, disappearance of singlet corresponds to 3 protons of N-acetyl group and confirmed 2-propen-1-one moiety of quinazolinonylchalcones (Akihisa *et al*.,2006). Cycloaddition of chalcones with $NH_2OH.HCl$ gave isoxazoles 5a-r (Table 2). Absence of C=O band and appearance of new bands in the range of 1210-1270 cm^{-1}, 1560-1610 cm^{-1} in IR spectrum of all the compounds indicated -C-O-N- and C=N of isoxazole ring respectively (Vijay kumar *et al.,*2010; Madhavi *et al*., 2010;Kumara Swamy *et al*., 2011; Vishal *et al.,* 2012; Hushare and Rajput, 2012; Peesapati *et al*., 2006; Solankee *et al*., 2011;Rajanarendar *et al*., 2013). Appearance of peek in the range of δ 5.9-6.9 ppm in ^{1}H FT-NMR spectrum correlates with IR spectrum data and confirms the formation of isoxazole ring in the respective compounds(Karabasanagouda *et al.,* 2009).

Physical characterization data : 5a: *MP (°C)*-168.*% Yield*-78.5.*E. A (%): Calcd. (Found)*- Carbon(C)69.52 (69.19), Hydrogen (H)3.78 (3.97),Nitrogen(N)10.58 (10.26); **5b:** *MP (°C)*-172.*% Yield*-71.5.*E. A (%): Calcd. (Found)*- C63.97 (63.75), H3.24 (3.10),N8.82 (9.14); **5c:** *MP (°C)*-154.*% Yield*-66.1.*E. A (%): Calcd. (Found)*- C57.99 (56.80), H2.94 (2.45),N8.82 (7.90); **5d:** *MP (°C)*-184.*% Yield*-67.4.*E. A (%): Calcd. (Found)*- C63.97 (63.71), H3.24 (3.00),N8.82 (9.12); **5e:** *MP (°C)*-117.*% Yield*-77.6.*E. A (%): Calcd. (Found)*- C66.52 (65.08), H3.37 (3.24),N10.12 (9.72); **5f:** *MP (°C)*-138.*% Yield*-66.5.*E. A (%): Calcd. (Found)*- C70.07 (69.76), H4.13 (3.97),N10.22 (10.18); **5g:** *MP (°C)*-141.*% Yield*-75.3.*E. A (%): Calcd. (Found)*- C57.99 (57.74), H2.94 (2.62),N8.82 (8.60); **5h:** *MP (°C)*-166.*% Yield*-62.9.*E. A (%): Calcd. (Found)*- C54.08 (53.92), H2.55 (2.16),N8.23 (7.95); **5i:** *MP (°C)*-158.*% Yield*-63.7.*E. A (%): Calcd. (Found)*- C49.75 (49.26), H2.34 (2.18), N7.57 (8.14); **5j:** *MP (°C)*-116.*% Yield*-58.6.*E. A (%): Calcd. (Found)*- C54.08 (53.95), H2.55 (2.10), N8.23 (7.63); **5k:** *MP (°C)*-152.*% Yield*-62.4.*E. A (%): Calcd. (Found)*- C55.89 (55.11), H2.63 (2.50), N8.50 (8.46); **5l:** *MP (°C)*-108. *% Yield*-70.1.*E. A (%): Calcd. (Found)*- C58.78 (58.64), H3.26 (2.95),N8.57 (8.16); **5m:** *MP (°C)*-130.*% Yield*-58.3. *E. A (%): Calcd. (Found)*- C52.78 (51.25), H2.67 (2.07), N8.03 (7.87); **5n:** *MP (°C)*-96.*% Yield*-70.2.*E. A (%): Calcd. (Found)*- C49.52 (48.50), H2.33 (2.10),N7.53 (6.79); **5o:** *MP (°C)*-129.*% Yield*-68.9.*E. A (%): Calcd. (Found)*- C45.86 (44.56), H2.16 (2.03),N6.98 (6.48); **5p:** *MP (°C)*-108.*% Yield*-69.1.*E. A*

Table 3 : Antibacterial activity of synthesized isoxazole derivatives

Compound (600 µg ml^{-1})	Zone of inhibition (mm)					
	B. s	*B. c*	*S. a*	*S. e*	*P. a*	*E. c*
5 a	16	9	11	11	11	12
5 b	11	10	13	12	18	14
5 c	10	9	9	12	14	9
5 d	9	9	12	9	11	10
5 e	10	12	11	12	11	13
5 f	15	12	11	13	11	12
5 g	13	12	16	14	11	10
5 h	12	11	11	14	13	12
5 i	17	14	12	11	11	13
5 j	15	13	12	16	11	12
5 k	13	12	13	13	12	11
5 l	13	12	15	15	11	16
5m	8	-	-	-	-	9
5 n	11	-	-	-	-	7
5 o	10	7	-	-	7	-
5p	11	8	19	-	9	9
5 q	10	8	-	-	8	-
5 r	10	-	15	8	7	8
Ciprofloxacin (100 µg disc^{-1})	21	17	20	20	21	20
10% DMSO in Methanol (MeOH)	-	-	-	-	-	-

1) *B.s: Bacillus subtilis, B.c: Bacillus cereus, S.a: Staphylococcus aureus, S.e: Staphylococcus epidermidis, P.s: Pseudomonas aeruginosa, E.c: Escherichia coli;* 2) (-) indicates no zone of inhibition; 3) All the above compounds were divided into various sensitive types on the basis of respective zone of inhibitions (mm); <7 mm were less active, between 8-10 mm were weakly active, between 11-13 mm were moderately active, >14 mm were highly active

Table 4 : Antifungal activity of synthesized isoxazole derivatives

Compound (600 µg ml^{-1})	Zone of inhibition (mm)				
	C. g	*C. a*	*A. n*	*A. f*	*S. c*
5a	18	10	23	20	10
5b	16	11	13	9	11
5c	12	9	-	13	7
5d	12	10	-	9	-
5e	12	11	-	-	-
5f	14	11	10	9	12
5g	13	16	13	19	9
5h	18	11	23	19	11
5i	25	20	11	10	12
5j	20	21	-	-	14
5k	21	17	13	16	16
5l	22	17	20	15	14
5m	13	13	7	-	-
5n	16	16	9	14	9
5o	15	18	-	-	-
5p	14	10	-	-	-
5q	16	15	-	-	-
5r	11	12	-	-	10
Fluconazole (100 µg disc^{-1})	21	22	16	16	13
10% DMSO in MeOH	-	-	-	-	-1)

C.g: Candida glabrata, C.a: Candida albicans, A.n: Aspergillusniger, A.f: Aspergillusfoetidus, S.c: Saccharomyces cerevisiae; 2) (-) indicates no zone of inhibition; 3)All the above compounds were divided into various sensitive types on the basis of respective zone of inhibitions (mm);<8 mm less active, between 9-12 mm were weakly active, between 13-16 mm were moderately active, >17 mm were highly active

Table 5 : Comparsion between antimicrobial activities of synthesized compounds against respective bacterial and fungal strains used in the study

Comp. ID	Bacteria Type						Fungi Type				
	B. s	*B. c*	*S. a*	*S. e*	*P. a*	*E. c*	*C. g*	*C. a*	*A. n*	*A. f*	*S. c*
5a	++++	++	+++	+++	+++	+++	++++	++	++++	++++	++
5b	+++	++	+++	+++	++++	++++	+++	++	+++	++	++
5c	++	++	++	+++	++++	++	x	x	x	x	x
5d	++	++	+++	++	+++	++	x	x	x	x	x
5e	++	+++	+++	+++	+++	+++	x	x	x	x	x
5f	++++	+++	+++	+++	+++	+++	+++	++	++	++	++
5g	+++	+++	++++	++++	+++	++	+++	+++	+++	++++	++
5h	+++	+++	+++	++++	+++	+++	++++	++	++++	++++	++
5i	++++	++++	+++	+++	+++	+++	++++	++++	++	++	++
5j	++++	+++	+++	++++	+++	+++	++++	++++	x	x	++++
5k	+++	+++	+++	+++	+++	+++	++++	++++	+++	++++	++++
5l	+++	+++	++++	++++	+++	++++	++++	++++	++++	++++	++++
5n	x	x	x	x	x	x	+++	+++	+++	++	++

1) For convenience, compounds showing an activity against all the selected organisms were only represented in the above table; 2) + - Less active; ++ - weakly active; +++ - Moderately active; ++++ - Highly active; 3) x- Not taken for consideration

(%): Calcd. (Found)- C49.52 (47.57), H2.33 (2.62),N 7.53 (6.56); **5q:** *MP (°C)*-117.*% Yield*-61.7.*E. A (%): Calcd. (Found)*- C51.03 (50.45), H2.40 (2.03),N7.76 (7.01); **5r:** *MP (°C)*-140.*% Yield*-59.6.*E. A (%): Calcd. (Found)*- C53.64 (51.38), H2.98 (2.93),N7.82 (7.37).

Spectral characterization data : 5a:*IR(KBr, $V_{max,}cm^{-1}$)*-1548.67(C=N), 1221.63(C-O-N), 1653.74(CH=CH str), 752.98 (Ar-CH str).*[1] H-NMR (δ ppm)* -6.78-8.01 (14H, m, Ar-H), 6.72 (CH=CH of isoxazole ring). *MS (m/z)* - 397.09; **5b:** *IR (KBr, $V_{max,}$-cm^{-1})* -1556.61 (C=N), 1261.00(C-O-N), 1718.63(CH=CH str), 767.69 (Ar-CH str). *[1]H-NMR (δ ppm)*-6.66-7.99(13H, m, Ar-H), 6.64 (CH=CH of isoxazole ring). *MS (m/z)*-431.05; **5c:** *IR (KBr, $V_{max,}cm^{-1}$)*-1541.18(C=N), 1259.56(C-O-N), 1622.01(CH=CH str), 750.33(Ar-CH str).*[1]H-NMR (δ ppm)*- 6.69-7.72(13H, m, Ar-H), 6.71 (CH=CH of isoxazole ring). *MS (m/z)*-475.00; **5d:** *IR (KBr, $V_{max,}cm^{-1}$)* -1622.52 (C=N), 1230.63(C-O-N), 1651.12 (CH=CH str), 761.91(Ar-CH str).*[1]H-NMR (δ ppm)*-6.51-7.95(13H, m, Ar-H), 6.70 (CH=CH of isoxazole ring). *MS (m/z)*-431.05; **5e:** *IR (KBr, $V_{max,}cm^{-1}$)*-1596.37(C=N), 1240.41(C-O-N), 1601.48(CH=CH str), 785.64(Ar-CH str). *[1]H-NMR (δ ppm)*-6.20-8.13 (13H, m, Ar-H), 6.69 (CH=CH of isoxazole ring). *MS (m/z)*-415.08; **5f:** *IR (KBr, $V_{max,}cm^{-1}$)*-1589.40(C=N), 1232.55(C-O-N), 1664.62(CH=CH str), 758.05(Ar-CH str). *[1]H-NMR (δ ppm)*-6.74-8.03 (13H, m, Ar-H), 6.74 (CH=CH of isoxazole ring), 2.31(3H, s, $-CH_3$). *MS (m/z)*-411.10; **5g:** *IR (KBr, $V_{max,}cm^{-1}$)*-1562.30(C=N), 1249.91.62(C-O-N), 1602.90(CH=CH str), 744.55(Ar-CH str). *[1]H-NMR (δ ppm)*-6.68-8.3 (13H, m, Ar-H), 5.98(CH=CH of isoxazole ring). *MS (m/z)*-475.00; **5h:** *IR (KBr, $V_{max,}cm^{-1}$)*-1548.51(C=N), 1238.19(C-O-N), 1634.73(CH=CH str), 721.36(Ar-CH str).*[1]H-NMR (δ ppm)*-6.66-7.86 (12H, m, Ar-H), 6.69 (CH=CH of isoxazole ring). *MS (m/z)*-510.96; **5i:** *IR (KBr, $V_{max,}cm^{-1}$)*-1597.11(C=N), 1261.49(C-O-N), 1664.62(CH=CH str), 752.26(Ar-CH str).*[1]H-NMR (δ ppm)*-6.56-8.24 (12H, m, Ar-H), 6.65(CH=CH of isoxazole ring). *MS (m/z)*-554.91; **5j:** *IR (KBr, $V_{max,}cm^{-1}$)*- 1557.29(C=N), 1243.89(C-O-N), 1664.54(CH=CH str), 774.56(Ar-CH str). *[1]H-NMR (δ ppm)*-6.58-7.68(12H, m, Ar-H), 6.56(CH=CH of isoxazole ring). *MS (m/z)*-510.96; **5k:** *IR (KBr, $V_{max,}cm^{-1}$)*-1575.68(C=N), 1219.98(C-O-N), 1625.13(CH=CH str), 720.08(Ar-CH str).*[1]H-NMR (δ ppm)*-6.70-8.63(12H, m, Ar-H), 6.73(CH=CH of isoxazole ring). *MS (m/z)*-494.99; **5l:** *IR (KBr, $V_{max,}cm^{-1}$)*-1560.01(C=N), 1250.49(C-O-N), 1601.57(CH=CH str), 716.16(Ar-CH str). *[1]H-NMR (δ ppm)*-6.66-8.09 (12H, m, Ar-H), 6.74(CH=CH of isoxazolering), 2.32(3H, s, $-CH_3$). *MS (m/z)*-491.01; **5m**: *IR (KBr, $V_{max,}cm^{-1}$)*-1580.97(C=N), 1234.58(C-O-N), 1658.43(CH=CH str), 717.84(Ar-CH str).*[1]H-NMR (δ ppm)*-6.57-8.05 (13H, m, Ar-H), 6.63 (CH=CH of isoxazole ring). *MS (m/z)*-522.99; **5n:** *IR (KBr, $V_{max,}cm^{-1}$)*-1586.57(C=N), 1243.57(C-O-N), 1606.83(CH=CH str), 748.39(Ar-CH str). *[1]H-NMR (δ ppm)*-6.49-8.10(12H, m, Ar-H), 6.71(CH=CH of isoxazole ring). *MS (m/z)*-556.95; **5o:** *IR (KBr, $V_{max,}cm^{-1}$)*-1608.43(C=N), 1209.78(C-O-N), 1659.01(CH=CH str), 758.89(Ar-CH str). *[1]H-NMR (δ ppm)*-6.51-8.69 (12H, m, Ar-H), 6.57(CH=CH of isoxazole ring). *MS (m/z)*-602.89; **5p:** *IR (KBr, $V_{max,}cm^{-1}$)*-1595.90(C=N), 1207.19(C-O-N), 1662.76(CH=CH str), 753.05(Ar-CH str).*[1]H-NMR (δ ppm)*-6.53-7.96(12H, m, Ar-H), 6.52(CH=CH of isoxazole ring). *MS (m/z)*-556.95; **5q:** *IR (KBr, $V_{max,}cm^{-1}$)*-1609.22(C=N), 1210.15(C-O-N), 1661.01(CH=CH str), 761.40(Ar-CH str).*[1]H-NMR (δ ppm)*-6.56-8.04 (12H, m, Ar-H), 6.70(CH=CH of isoxazole ring). *MS (m/z)*-540.98; **5r:** *IR (KBr, $V_{max,}cm^{-1}$)*-1604.88(C=N), 1213.03(C-O-N), 1662.25(CH=CH str), 755.84(Ar-CH str). *[1]H-NMR (δ ppm)*- 6.57-7.66 (12H, m, Ar-H), 6.72(CH=CH of isoxazole ring), 2.03 (3H, s, $-CH_3$). *MS (m/z)*-537.00 where O-Oxygen; Ar-Aromatic; CH_3- Methyl respectively.

All the synthesized compounds showed antimicrobial activity against majority of microbes used in the study (Table 3 and 4). Especially compounds 5a, 5b, 5c, 5d, 5e, 5f, 5g, 5h, 5i, 5j, 5k and 5l were active against all the selected Gram positive as

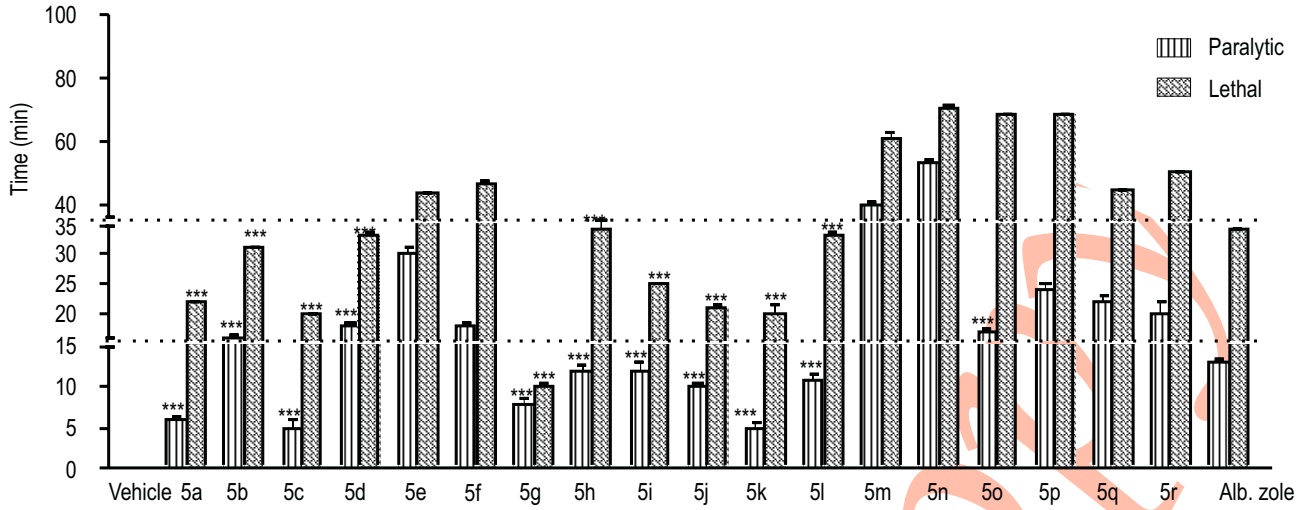


Fig. 1 : Anthelmintic activity of compounds 5a-r. Results are expressed as mean ± SEM (n=6). ***P < 0.05 compared to lethal and paralytic time (min) of albendazole

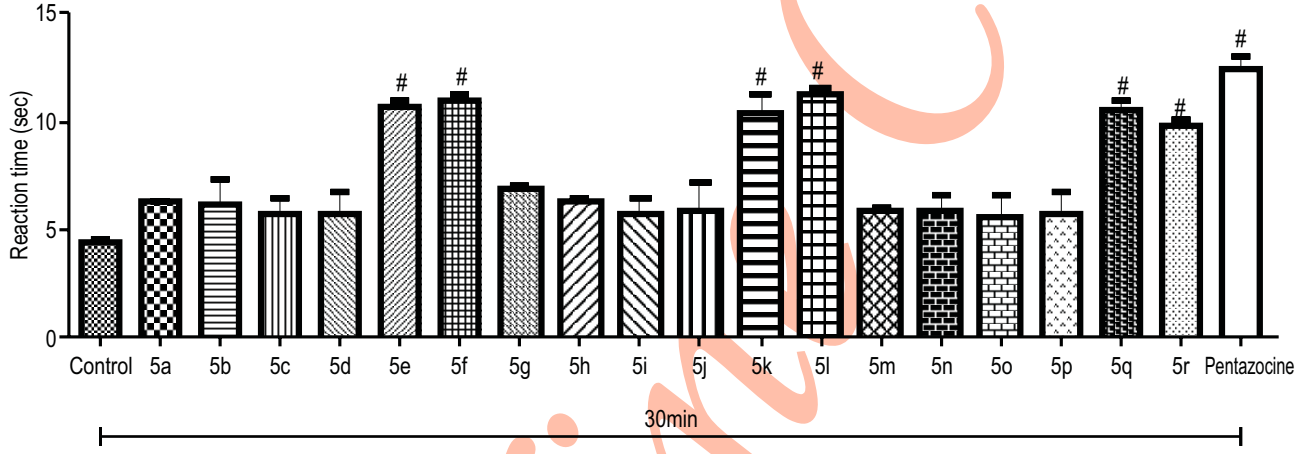


Fig. 2 : Analgesic activity of compounds 5a-r. Results are expressed as mean ± SEM (n=6). #P < 0.001 compared to reaction time (sec) of control

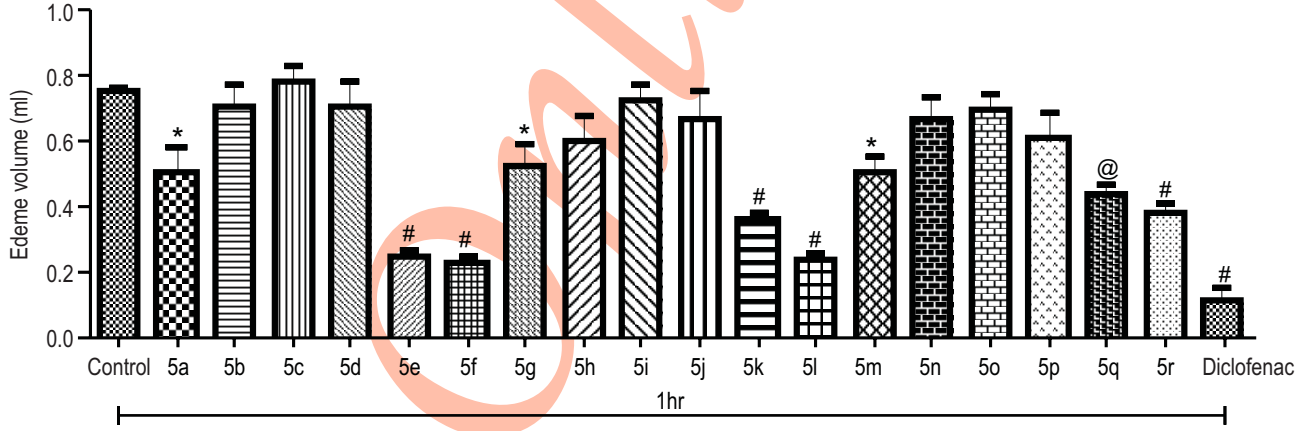


Fig. 3 : Anti-inflammatory activity of compounds 5a-r. Results are expressed as mean ± SEM (n=6). *P < 0.05 compared to edema volume (ml) of control; @P < 0.01 compared to edema volume (ml) of control; #P < 0.001 compared to edema volume (ml) of control

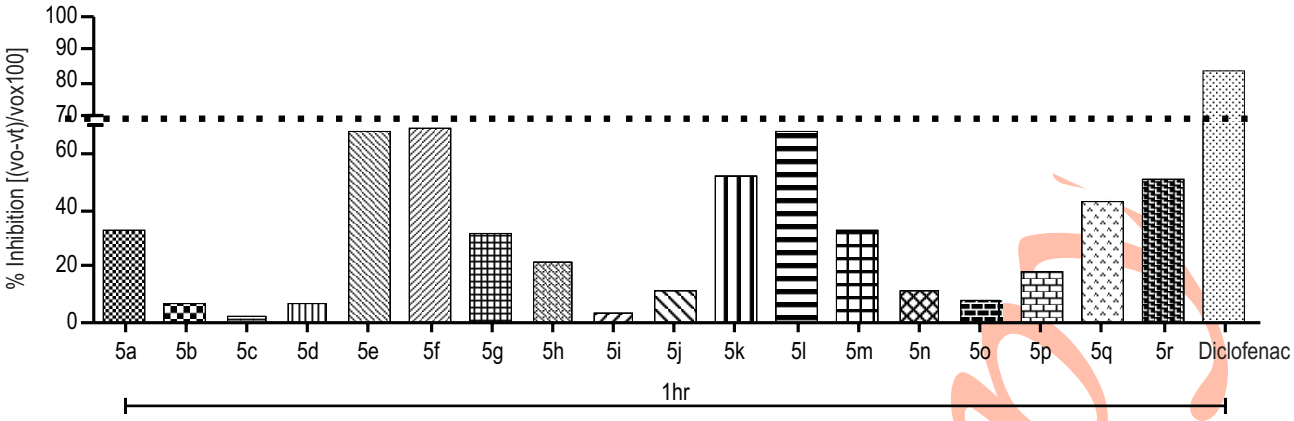


Fig. 4 : Percent inhibition $[(V_0-V_t)/V_0 \times 100]$ of synthesized isoxazole derivatives in anti- inflammatoryactivity.V_t is volume of edema at corresponding time;V_0 is the volume of edema of control rats at the same time

well as Gram negative bacteria (Table 5). Further, compounds 5i, 5j, 5k and 5l were found to be highly active against all the selected bacteria in antibacterial activity. Compounds 5m, 5q did not show activity against more than three types of bacteria. The titled compounds were active against all the selected fungal species. Particularly, compounds 5a, 5b, 5f, 5g, 5h, 5i, 5k, 5l and 5n showed good antifungal activity against all the strains of fungi when compared with other compounds. Among them 5a, 5h, 5k and 5l were found to be equal or highly active than standard fluconazole. Compounds 5c, 5d, 5e though exhibited good antibacterial activity against all selected bacterial strains, did not show good activity against fungi. Surprisingly, 5n, though not active against bacterial strains, showed good activity against all the selected fungi. Thioxoquinazolinonylisoxazoles 5a-r were tested for anthelmintic activity at 1 mg ml^{-1} concentration. Time taken by the test compounds to induce paralysis as well as lethality was recorded using a stop watch and the results are given in Fig. 1. Compounds 5a, 5b, 5c, 5d, 5g, 5h, 5i, 5j, 5k and 5l showed significant anthelmintic activity when compared with standard albendazole.

The mean basal reaction time and reaction time after 30 min of drug administration of compounds 5a-r and standard drug (Pentazocine) were recorded and is shown in Fig. 2. Compounds 5e, 5f, 5k, 5l, 5q and 5r showed significant analgesic activity when compared with control ($^{\#}P<0.001$). The presence of isoxazole ring and aryl group with electron releasing or electron withdrawing substituents at the 3rdposition of thioxoquinazolinone nucleus might be responsible for the activity. The edema volumes and % inhibition of edema after 1hr of drug administration of all compounds and standard (Diclofenac sodium) were recorded and is shown in Fig. 3 and 4. All the compounds 5a-r at a dose of 10 mg kg^{-1} body weight exhibited anti-inflammatory activity. However, compounds 5a, 5e,5f,5g,5k,5l,5m,5q and 5r exhibited significant ($^{\#}P < 0.001$, $^{@}P < 0.01$ and $^{*}P < 0.05$) edema inhibition in comparison with control. The significant activity was mainly due to isoxazole ring and the substituents of thioxoquinazolinone nucleus. Halogenated (Cl, F and Br) aryl group at 3rd position of thioxoquinazolinone gave analogues that were more lipophilic in nature (Gareth Thomas, 2004).The observed pharmacological screening results might be due to the type of halogen and their point of attachment to the thioxoquinazolinone nucleus.

In the present study, cycloaddition of substituted thioxoquinazolinonylchalcones (4a-r) with $NH_2OH.HCl$ led to the synthesis of eighteen 5-phenyl-3-(substituted thioxoquinazolinonyl) isoxazoles (5a-r). It can be one of the easiest and best way of synthesizing isoxazole derivatives with thioxoquinazolinonyl basic ring system. All the compounds were subjected to *in vitro* and *in vivo* pharmacological screening. Compounds 5a, 5f, 5g, 5k and 5l showed better activity in all the respective pharmacological screenings reflecting their broad spectrum activity. This broad spectrum activity of the above mentioned ones might be due to the improved lipophilicity and enhanced penetration to lipid membranes.

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