Novel Series of some phenylpiperazine substituted imidazolyl-thiazole as anti-inflammatory and antibacterial agent

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Abstract:

Novel Set of target compounds (**AR-14a to AR-54a**) were synthesized as per procedure reported by Reji et al.[1]In brief the 1-chloro-3-(2-methyl-4-nitro1H-imidazole-1-yl) propane-2-on **(a)** was added in equimolar quantity to a solution of adduct (substituted N-(phenylpiperazinyl-4-thioyl) benzamide/chlorobenzamide in acetonitrile further with different α-halo compounds.The synthesized compounds were evaluated for their in-vitro anti-inflammatory activity using human red blood cells (HRBC) membrane stabilization methodand in-vivo anti-inflammatory activity by carrageenin-induced rat paw oedema model at 20 mg/kg body weight using diclofenac sodium and ibuprofen as standard drugs. Compounds AR-14a to AR-54ashowed promising anti-inflammatory activity of 59% and 61% protection at 20 mg/kg to inflamed paw. The synthesized compounds AR-14a to AR-54awere screened for their in-vitroantibacterial activity against gram positive bacteria Staphylococcusaureus ATCC25923andEscherichia faecalis ATCC29212, furthergram-negativebacteriaEscherichiacoli ATCC8739andPseudomonasaeruginosa ATCC9027 using ciprofloxacin and cefdinir as standard drugs.

Keywords: Phenylpiperazine, imidazolyl-thiazole, anti-inflammatory; antibacterial; MIC;

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Introduction

Piperazine and derivatives used as a starting material for pharmaceutical and agrochemical applications with the potential.Selected phenyl piperazines demonstrated good inhibition of uptake by neuroblastoma cells. Of the phenyl piperazines studied, 3-iodophenylpiperazine was also shown to accumulate in neuroblastoma. Data support the concept taken up by a specific transporter which is saturable and that this uptake is strongly inhibited by the known uptake-1 inhibitor. As phenyl piperazines are known to interact with 5-HT receptors. Competition of 3-iodophenylpiperazine with 5HT was also used to characterize uptake. The weak inhibition of uptake by 5HT indicates that the transporter responsible for 3-iodophenylpiperazine uptake is more specific for IPP than 5HT. Further work is needed to characterise 3-iodophenylpiperazine and its mode of uptake fully as it has been reported that certain serotonin receptors are present on some neuroblastoma cell lines[23],[4].

In continuation to previous reports in designing and synthesizing new trisubstituted thiazole with good anti-inflammatory activity and selectivity, we report here synthesis, in-vitro and in- vivo anti-inflammatory activity of a new series of designed 1-(4-substituted)-2-(-4-phenylpiperazine-1-yl) thiazole-5-yl) 2-methyl-4-nitro-1H-imidazole-1-yl) ethanone.

Rational of this work prevails keeping all the substituents constant at fourth position,now we are further exploring group bioisosteric replacement and effect of electron density on biological activities we had modify N-$CH_3$to second position by aryl group. In this chapter detail account of target compounds 1-(4-substituted)-2-(-4-phenylpiperazine-1-yl) thiazole-5-yl) 2-methyl-4-nitro-1H-imidazole-1-yl) ethanone are systematically describe in the following manner. Moreover, second generation of imidazole-thiazole have better pharmacokinetic properties because of C-7 piperazinyl substituent. These analogues were soon followed by the third generation.

Multi-drug treatment of inflammatory conditions associated with microbial infections pose a unique problem especially for patients with impaired liver or kidney functions.Therefore, from the pharmacoeconomic and patient compliance points of view the monotherapy with a drug having both anti-inflammatory and antimicrobial activities desirable to a great extent.

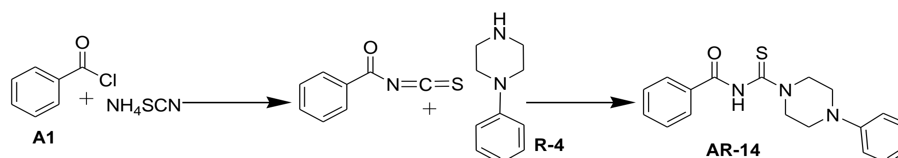
Material and Methods

All chemical reagents and solvents were used as obtained from the trader or recrystallized /redistilled if necessary.Norfloxacine(purity > 96%), Ciprofloxacine (purity > 98%), Cefdinir (purity > 97%) were purchased from Relax Pharma Ltd. Mumbai, India. Diclofenac sodium (purity > 98%), Ibuprofen (purity > 99%) were obtained as a gift from Glenmark pvt. Ltd. Mumbai, India. Carrageenan (λ) (C38895G purity > 97%) was purchased from Sigma Aldrich, USA. Acid chlorides with higher purity were obtained as gift from Cambay Organics pvtLtd,Khambhat, Gujarat, India. Alsevior reagent, ammonium thiocyanate, Mueller Hinton Agar media (Himedia-cube M173)andother chemical with high grade purity were purchased from Sahyadri scientific suppliers Mumbai, India.Thin layer chromatography (TLC) was performed on microscopic slides coated with silica gel G and toluene: acetonitrile as a mobile phase. The spots were visualized by normal thin layer chromatography(TLC) and exposure to iodine vapors. Open capillary melting point apparatus were used for recording melting points and were uncorrected. Infrared (IR) spectra were recorded in KBr on SHIMADZU Fourier Transform Infrared 8400S spectrometer, Kyoto Japan. Mass spectra were recorded on a Jeol JMS-D-300 spectrometer Electron impact (EI) 70eV. Nuclear Magnetic Resonance spectra (^1H NMR) were recorded in deuterated dimethylsulfoxide (DMSO-d6) on Bruker advance at 400 MHz using Tetramethyl silane (TMS) as internal standard and the chemical shift (δ) were reported in parts per million (ppm). Elemental analysis was determined using a Carlo-Erba 1108 instrument or Elementar's vario EL III, the vario EL microanalyzer.

Chemistry

Procedure for synthesis of phenylpiperazine novel adduct as intermediate[5]

AR-14 Adduct: N-[(4-phenylpiperazin-1-yl) carbonothioyl] benzamide

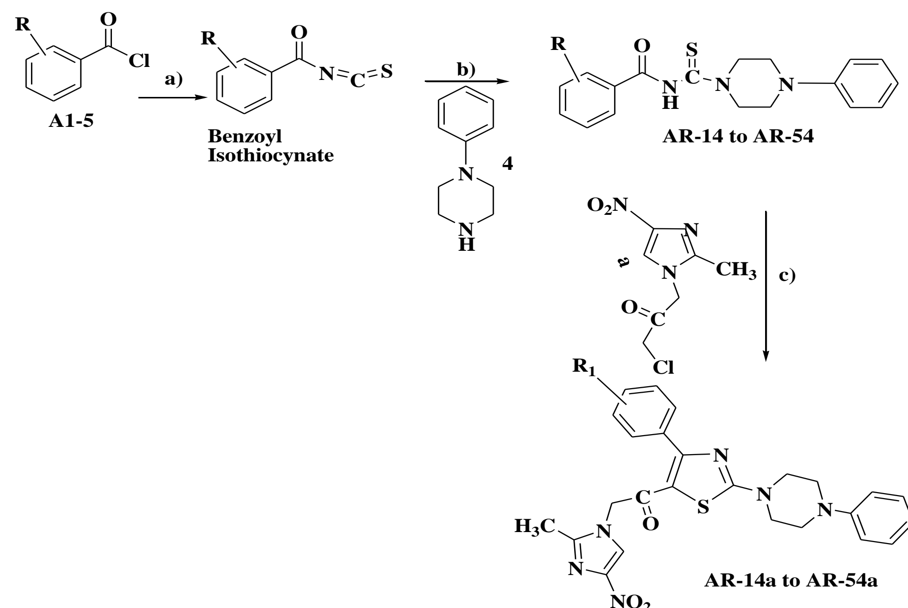


Adduct Scheme I : a) NH_4SCN, acetone, reflux 15-25 min; b) reflux for 20 min, pour reaction mixture to crushed ice .To a stirred solution of ammonium thiocyanate (10.48 g 0.13 mole) in acetone (100 mL) at room temperature was added benzoyl chloride**A1** (17.76 g 0.12 mole) in 5 min and the reaction mixture was refluxed after 15 min reflux, 1-phenyllpiperazine **R4**(17.84 g 0.11 mole) was added into reaction mixture at reflux temperature in 3 min and mixture was further refluxed for 25 min. Product was isolated by pouring reaction mixture into crushed ice and separated solid was filtered,washed with acetone and dried yielding Adduct AR-14.

Molecular Formula: $C_{18}H_{19}N_3OS$ Formula Weight: 325Melting Point: 140-142°C Yield: 84 %IR (KBr cm-1): 3043, 2962, 2848, 1615, 1581, 1474, 1443, 1406, 1267, 1223, 1168, 1112, 1026, 956, 928, 864, 843.

General method for Synthesis of target compounds (AR-14a to AR-54a)

As shown in **Scheme II**, isothiocyanates were obtained by stirring ammonium thiocyanate (0.1379 mol) in 100mLacetone at room temperature with benzoyl chloride/substituted benzoyl chloride (0.1263 mol) for 15-25 minutes followed by refluxing the reaction mixture for 15 min. Substituted N-(phenylpiperazinyl-4-thioyl) benzamide/chlorobenzamide (**AR-14 to AR- 54**) were synthesized by nucleophilic addition of benzoyl isothiocyanate /substituted benzoyl isothiocyanate and in equimolar quantity at reflux temperature as discussed above. The target compounds (**AR-14a to AR-54a**) were synthesized as per procedure reported by Rejiet al[6,7]In brief the 1-chloro-3-(2-methyl-4-nitro1H-imidazole-1-yl) propane-2-on **(a)** was added in equimolar quantity to a solution of adduct (substituted N-(phenylpiperazinyl-4-thioyl) benzamide/chlorobenzamide in 12 mL acetonitrile. The reaction mixture was heated on a water bath at 100 ^{0}C for 5-6 hr. The reaction mixture was cool and filtered. To the filtrate small amount of sodium bicarbonate was added[8,9,10,]. Off-white to yellow precipitates thus obtained were filtered, wash with water, air dried and purified by recrystallization corresponding to the (**AR-14a to AR-54a**) and characterized by spectral and elemental analysis. Melting point was determined using open glass capillary tube and represents the uncorrected values. All the reaction were monitored using thin layer chromatography (TLC) using glass plate coated with silica gel G. TLC Plates were developed in iodine and toluene: acetonitrile (7:3) taken as mobile phase represented in **Table 1**.



Scheme-II.1-(4-substituted)-2-(-4-phenyllpiperazine-1-yl) thiazole-5-yl) 2-methyl-4-nitro-1H-imidazole-1-yl) ethanone. Reagents and conditions: a) NH_4SCN, acetone, reflux 15-25 min; b) reflux for 20 min, pour reaction mixture to crushed ice; c) acetonitrile, stir at 100°C for 5-6 h, pour to crush ice

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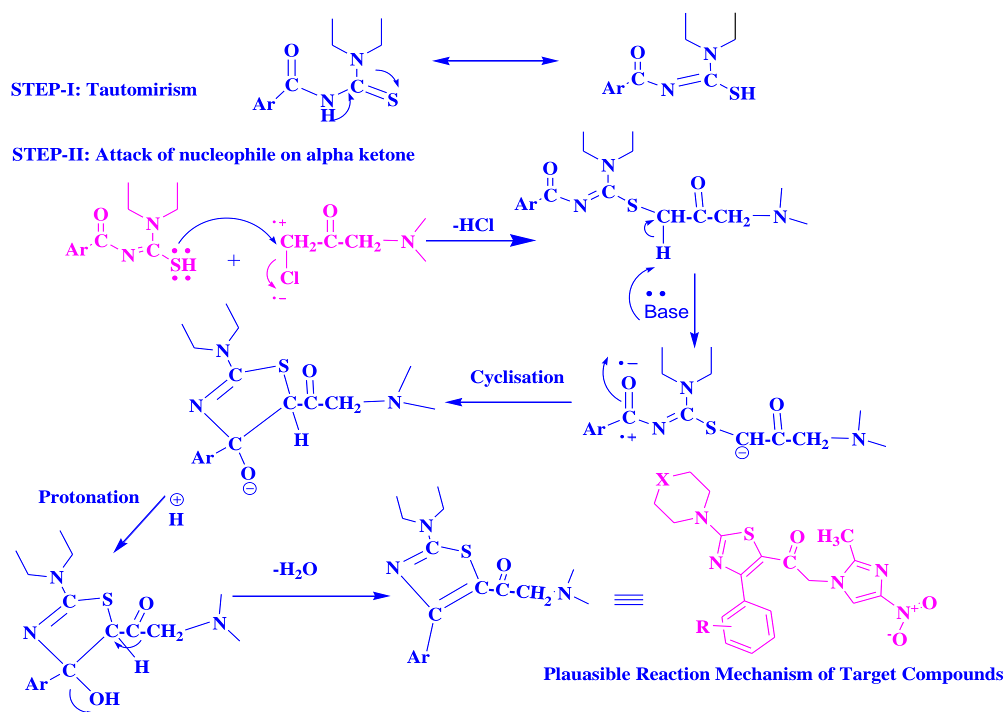

Scheme-III. General plausible reaction mechanism of target compounds of series AR-14a to AR-54a from adduct AR-14 to AR-54 correspondingly.

Table 1: Physicochemical characterization of synthesized compound AR-14a to AR-54a

Compounds	R_1	X = (N-Ph)	Melting Point* (^{0}C)	Rf Value**	% Yield
AR-14a		HN N–	178-180	0.52	71%
AR-24a	Cl–	HN N–	174-175	0.61	64%
AR-34a	Cl	HN N–	112-114	0.71	75%
AR-44a	Cl	HN N–	124-125	0.57	60%
AR-54a	O	HN N–	108-110	0.62	57%

* Melting point were determined on melting point apparatus using open glass capillary tube and represent the uncorrected values.

**** All the reaction were monitored using thin layer chromatography (TLC) using glass plate coated with silica gel G. TLC Plates were developed in iodine and a) toluene: methanol (7:3) taken as mobile phase, unless mention otherwise.

AR-14a: 2-(2-methyl-4-nitro-1H-imidazol-1-yl)-1-(4-phenyl-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) ethanone

IR (KBr cm-1): 3139 (aromatic stretching), 2862-2826 (-C-H stretching) 2812 (-CH_3stretching), 1632 (C=O stretching), 1599-1577 (C=C stretching, aromatic), 1481-1468 (NO_2- stretching), 1336-1303 (N-C of aromatic), 931-830 (-C=C out of plane for mono substituted benzene), 1158-1132 (C-N-C stretching of phenyl piperazine).^{1}H NMR:(400 MHz, DMSO) δ (ppm): 2.46 (s, 3H proton at 2nd position of nitroimidazole –CH_3) 3.27-3.46 (m, 8H, piperazine) 5.48 (s, 2H, –CH_2-C=O), 6.82-7.0 (m, 4H, aromatic proton of N-phenyl) 7.21-7.79 (m, 5H, aromatic proton at 4th position), 8.0 (s, 1H, aromatic proton at 5th position of nitroimidazole) Mass: m/z., 488, 489 (M+1) Elemental analysis for $C_{25}H_{24}N_6O_3S$: Calculated: C, 61.46; H, 4.95; N, 17.20; found: C, 60.53; H, 4.55; N, 17.20; UV Visible λ Max: 357.2.

AR-24: 1-(4-(4-chlorophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl)-2-(2-methyl-4-nitro-1H-imidazol-1-yl) ethanone

IR (KBr cm-1): 3124 (aromatic stretching), 2967-2943 (-C-H stretching) 2876-2800 (-CH3stretching), 1750-1663 (C=O stretching), 1546-15 (C=C stretching, aromatic), 1486-1446 (NO_2- stretching), 1334 (N-C of aromatic), 876-832 (-C=C out of plane for mono substituted benzene), 1142-1138 (C-N-C stretching of phenyl piperazine), 750 (-C-Cl Bending)^{1}H NMR: (400 MHz, DMSO) δ(ppm): 2.15 (s, 3H proton at 2nd position of nitroimidazole –CH_3) 3.28-3.63 (m, 8H, piperazine) 4.82 (s, 2H, –CH_2-C=O), 6.82-6.99 (m, 4H, aromatic proton of N-phenyl) 7.50-7.80 (m, 4H, aromatic proton at 4th position), 8.03 (s, 1H, aromatic proton at 5th position of nitroimidazole)Mass: m/z., 522, 523 (M+1), Elemental Analysis for $C_{25}H_{23}ClN_6O_3S$: Calculated: C, 57.41; H, 4.43; N, 16.07; found: C, 57.21; H, 4.23; N, 17.07

AR-34a: 1-(4-(2-chlorophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl)-2-(2-methyl-4-nitro-1H-imidazol-1-yl) ethanone

IR (KBr cm-1): 3140 (aromatic stretching), 2967-2943 (-C-H stretching) 2836 (-CH_3 stretching), 1736-1707 (C=O stretching), 1599-1562 (C=C stretching, aromatic), 1450-1433 (NO_2 stretching), 1349.25 (N-C of aromatic), 860- (-C=C out of plane for mono substituted benzene), 1228 (C-N-C stretching of phenyl piperazine), 750 (-C-Cl Bending) 1H NMR: (400 MHz, DMSO) δ(ppm): 2.27 (s, 3H proton at 2nd position of nitroimidazole –CH_3) 3.22-3.61 (m, 8H, piperazine N-CH2) 5.41 (s, 2H, –CH_2-C=O), 6.80-7.26 (m, 4H, aromatic proton of N-phenyl) 7.39-7.56 (m, 4H, aromatic proton at 4th position), 8.1 (s, 1H, aromatic proton at 5th position of nitroimidazole) Mass: m/z., 522, 523 (M+1) Elemental Analysis for $C_{25}H_{23}C_lN_6O_3S$: Calculated: C, 57.41; H, 4.43; N, 16.07; found: C, 56.41; H, 4.43; N, 17.07 UV Visible Max: 271.

AR-44a: 1-(4-(3-chlorophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl)-2-(2-methyl-4-nitro-1H-imidazol-1-yl) ethanone

IR (KBr cm-1): 3240 (aromatic stretching), 2967-2943 (-C-H stretching) 2836 (-CH3stretching), 1736-1727 (C=O stretching), 1544-1536 (C=C stretching, aromatic), 1450-1433 (NO_2- stretching), 1332 (N-C of aromatic), 810- (-C=C out of plane for mono substituted benzene), 1342-1228 (C-N-C stretching of phenyl piperazine), 756 (-C-Cl Bending)^{1}H NMR: (400 MHz, DMSO) δ(ppm): 2.27 (s, 3H proton at 2nd position of nitroimidazole –CH_3) 3.24-3.61 (m, 8H, piperazine N-CH_2) 5.41 (s, 2H, –CH_2-C=O), 6.82-7.24 (m, 4H, aromatic proton of N-phenyl) 7.40-7.55 (m, 4H, aromatic proton at 4th position), 8.02 (s,

1H, aromatic proton at 5th position of nitroimidazole) Mass: m/z., 522, 523 (M+1) Elemental Analysis for $C_{25}H_{23}ClN_6O_3S$: Calculated: C, 57.41; H, 4.43; N, 16.07; found: C, 57.23; H, 4.43; N, 17.12;

AR-54a: 1-(4-(furan-2-yl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl)-2-(2-methyl-4-nitro-1H-imidazol-1-yl) ethanone**.**

IR (KBr cm-1): 3136 (aromatic stretching), 2850-2835 (-CH_3stretching), 2922 (-C-H stretching) 1734 (C=O stretching), 1535 (C=C stretching aromatic), 1491-1442 (NO_2 stretching), 1390-1332 (N-C of aromatic), 991-754 (-C=C out of plane for furan or phenyl piperazine), 1122-1014 (C-N stretching of phenyl piperazine)1H NMR: (400 MHz, DMSO) δ(ppm): 2.46 (s, 3H proton at 2nd position of nitroimidazole –CH_3) 3.27-3.46 (m, 8H, piperazine N-CH2) 5.48 (s, 2H, –CH2-C=O), 6.8-6.99 (m, 4H, aromatic proton of N-phenyl) 7.47-7.61 (m, 3H, aromatic proton of furoyl 4th position), 8.0 (s, 1H, aromatic proton at 5th position of nitroimidazole) Mass: m/z., 478, 479,480 (M+1)Elemental Analysis for $C_{23}H_{22}N_6O_4S$: Calculated: C, 57.73; H, 4.63; N, 17.56; found: C, 56.43; H, 4.63; N, 17.14 UV Visible Max: 371.20.

Anti-Inflammatory Activity

In-vitro anti-inflammatory activity of synthesized compound byhuman red blood cells membrane stabilization method

Human Red Blood Cells (HRBC) membrane alike to lysosomal membrane apparatus, the prevention of hypotonicity induced human red blood cells membrane lysis is taken as a measure of anti-inflammatory activity of drugs,[11],[12] [10, 11].The human red blood cells membrane stabilization has been used as method to study the anti-inflammatory activity. Blood was collected from healthy volunteer. The collected blood was mixed with equal volume of sterilized alsevior solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) suspension was made with isosaline. The assay mixture contained the diclofenac sodium as reference drug (50, 100 and 200 µg/mL), 1 mL of phosphate buffer (0.15M, pH 7.4), 2mL of hyposaline (0.36%) and human red blood cells suspension (0.5 mL). Instead of hyposaline 2 mL of distilled water was used in the control. All the assay mixture were incubated at 37 ^{0}C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated taking optical density by Ultraviolet-visible spectrophotometer at 560 nm. The percentage hemolysis was calculated by considering the 100 percentage hemolysis produced in presence of distilled water. The percentage of human red blood cells membrane stabilization or protection was calculated using optical density (OD) of test and control by formula% Protection = 100-(OD test/OD control) x 100[13,14,15,16,17].

In-vivo methods for anti-inflammatory activity by carrageenan induced rat hind paw edema.

All the experimental procedures were approved by the Institutional Animal Ethics Committee RCPIPER/IAEC/2013-14/09 (Reg. No. 651/C/02/CPCSEA) constituted under 'Prevention of Cruelty to the Animals Act1960; Government of India. The method[18,19] adopted resembles essentially that described by Winter et al.The animals were studied for toxicity of dimethylsulfoxideup to 10% v/v in saline, and 5% dimethylsulfoxidewas selected as a vehicle to suspend the standard drugs and the test compounds. Albino rats of either sex weighing between 150 - 250 g were starved for 18 hours prior to the experiment. The animals were weighed, marked at tibiotarsal articulation for identification and divided into groups of six. The standard drug, ibuprofen (20 mg/kg body weight) and diclofenac sodium (10 mg/kg body weight) and the test compounds were given orally (20 mg/kg body weight) as a suspension using 5% dimethylsulfoxideas a vehicle. One hour later foot paw oedema was induced by injecting 0.1 mLof 1% carrageenin subcutaneously into the planter portion

of the right hind paw of each rat. Initial foot paw volume was measured immediately by mercury plethysmometer. Oedema was measured three hours after carrageenin administration. The swelling in test group animals was used to calculate the percent inhibition standard error of the mean(SEM) of oedema achieved by the compound at the test dose compared with the vehicle control group. The % protection of oedema was calculated according to the formula, % anti-inflammatory activity = 100 x (1-Vt/Vc) where, Vt and Vc were the volume of oedema in test compounds and control groups, respectively.

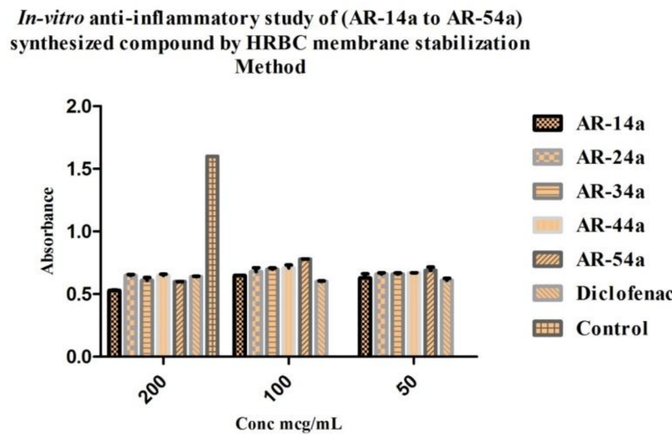

Figure 1: In vitro anti-inflammatory activity of (AR-14a-AR-54a) synthesized compound by HRBC Membrane stabilization method.

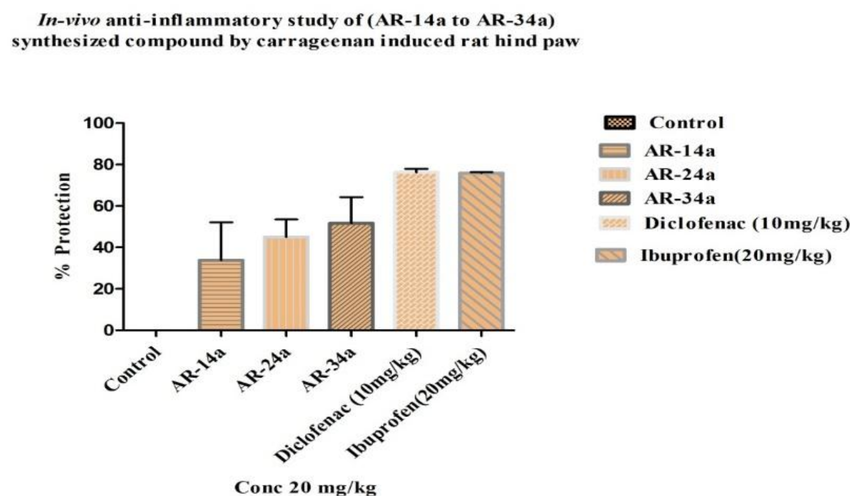

Figure 2: In-vivoanti-inflammatory activity of synthesized compounds by carrageenin-induced rat hind paws oedema % protection

Table 2: In-vitroanti-inflammatory activity of **(AR-14a to AR-54a**) synthesized compound by HRBC Membrane stabilization method.

Test compound	Conc. µg/mL	Mean±SEM	% Hemolysis	% Protection
AR-14a	50	0.53± 0.0033	33.40	66.60
	100	0.65± 0.0003	40.83	59.17
	200	0.59± 0.0047	36.67	63.33
AR-24a	50	0.65± 0.0069	40.46	59.54
	100	0.68± 0.0313	42.77	57.23
	200	0.66± 0.0107	41.52	58.48
AR-34a	50	0.61± 0.0234	38.19	61.81
	100	0.70± 0.0088	43.60	56.40
	200	0.66± 0.0087	41.19	58.81
AR-44a	50	0.65± 0.0087	40.33	59.67
	100	0.68± 0.0216	42.63	57.38
	200	0.67± 0.0025	41.81	58.19
AR-54a	50	0.60± 0.0007	37.23	62.77
	100	0.78± 0.0010	49.00	51.00
	200	0.69± 0.0257	43.31	56.69
Dicl. Sod.	50	0.61 ± 0.0160	38.06	61.94
	100	0.60 ± 0.0030	37.19	62.81
	200	0.64± 0.0051	39.83	60.17
Control	Unknown	1.60± 0.0000	100.00	0.00

***Values of Optical density or absorbance at 560 nm are expressed in Mean ± SEM

Table 3: In-vivoanti-inflammatory activity of synthesized compounds by carrageenin-induced rat hind paws oedema (% protection**)**

Compounds	Dose mg/kg	I hr	II hr	III hr
AR-14a	20	0.46 ± 0.13 (13.01)	0.57 ± 0.12 (46.47)	0.91 ± 0.15 (42.20)
AR-24a	20	0.34 ± 0.02 (35.21)	0.57 ± 0.02 (49.62)	0.92 ± 0.02 (50.32)
AR-34a	20	0.33 ± 0.094 (37.27)	0.43 ± 0.11 (59.62)	0.66 ± 0.126 (58.32)
Dicl. Sod	10	0.12 ± 0.01 (77.87)	0.27 ± 0.02 (74.64)	0.37 ± 0.05 (76.45)
Ibuprofen	20	0.13 ± 0.03 (76.34)	0.26 ± 0.05 (75.27)	0.41 ± 0.20 (74.23)
Control	-	0.53 ± 0.16 (0.00)	1.07 ± 0.24 (0.00)	1.57 ± 0.24 (0.00)

Oral administration for all test compounds, $P < 0.05$, Standard drugs, (dose and % protection) were ibuprofen (20 mg/kg, ~75%) and Diclofenac sodium(10 mg/kg, 77%).n=6, ANOVA followed by Dunnet's multiple comparison test

***p<0.001, **p<0.01 and *p<0.05 compared with control

Antibacterial Activity

The in-vitroantibacterial screening was done by agar well diffusion techniquewith Mueller Hinton Agar no.2 as the nutrient medium. The agar well diffusion method was favored to be used in this study since it was found to be improved than the disc diffusion method, [19-20].The test bacteria (0.2 mL; 108 cells / as

per McFarland standard) were then inoculated into the molten Mueller Hinton agar media, following suitablehomogenization it was poured into sterile 100 mm petri plates and allowed to solidified. After solidification of the media, 0.85 cm well was made in the plates using a sterile cork borer. Preliminary screening was conducted for all compounds at 100 µg /mL concentration, against the above mentioned microorganism. Each well was filled with 0.1mL of the test solution (100 µg/mL). The plates were incubated for 24 h in an incubator at 37 ^{o}C. For antibacterial activity ciprofloxacin and cefdinir were used as standard. The mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The controls were maintained for each bacterial strain, where pure solvent dimethylsulfoxide was inoculated into the well. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of the target synthesized compounds. Zone of inhibition produced by test compounds were measured in mm in various axis and average reading was considered and the activity index was calculated against the standard. The results are shown in **Table 4.**

The Minimum Inhibitory Concentration Assay [20,21]**against gram negative and gram positive bacteria (100** to **0.39 µg/mL)**

The minimum inhibitory concentration evaluation is a method used to determine the lowest concentration of a particular test compoundsor antibiotic needed to kill bacteria. This assay wasclassically carried out on free floating planktonic microbe's cells. Serial dilutions of the test compounds and antibiotics (representing different concentrations (100, 50, 25, 12.50, 6.25…0.39 µg/mL) of the synthesized test compounds) were added to a growth medium in separate test tubes. Stock solutions of the tested compounds, cefdinir and ciprofloxacine were prepared in dimethylsulfoxide at concentration of 100 µg/mL followed by twofold dilution at concentrations of (100, 50, 25, 12.5, 6.25…0.39 µg/mL).Each of these tubes has growth media inoculated with a standard concentration of bacteria and the respective antibiotic concentration.

Results and discussion

Novel set of phenylpiperazine substituted imidazolyl-thiazole derivatives (AR-14a-AR-54a) were synthesized, characterized and evaluated for their in-vitro andin-vivo anti-inflammatory activity.The compounds (AR-14a-AR-54a) were designed and synthesized by keeping 1-phenylpipereazin at second position of thiazole ring. The fourth position of thiazole was substituted by introducing electron withdrawing group (–Cl), at different position in phenyl moiety and (1-chloro-3-(2-methyl-4-nitro1H-imidazole-1-yl) propane-2-on at fifth position devoid of any change[22]. The chemical structures of these compounds andresults of in-vitro and in vivo anti-inflammatory activity are shown in Table 2-V and Table 3-V respectively.The infrared (IR) spectrum of series 1-(4-substituted)-2-(-1-phenylpiperazine-1-yl) thiazole-5-yl) 2-methyl-4-nitro-1h-imidazole-1-yl) ethanone (AR-14a, AR-24a AR-34a, AR-44a and -AR-54a) showed an absorption band at 3139, 3124, 3140, 3240 and 3136 cm^{1} indicate the aromatic (Ar-H stretching) respectively. Likewise, the absorption band at 1632, 1750-1663, 1736-1707, 1736-1727, and 1734 cm^{1} indicates the presence of (C=O stretching) attached to the fifth position of thiazole ring system. Further key absorption band are observed at 1158-1132, 1142-1138, 1342-1228, 1122-1014 of (C-N-C stretching of phenylpiperazine) and 1481-1468, 1486-1446, 1450-1433, 1450-1433, 1490-1442 cm^{1} of ($-NO_{2-}$ group). The ^{1}H NMR spectrum recorded at 400 MHz of compounds AR-14a-AR-54aconfirmed a singlet at δ ppm, 8.0, 8.03, 8.1, 8.02 and 8.0 integrating for one proton, which is attributed

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to aromatic proton at fifth position of nitroimidazole. The aromatic protons resonate as multiplets at δ ppm 7.21-7.79, 7.50–7.80, 7.39–7.56, 7.40-7.55, and 7.47-7.61. Additionally, evidence for the structures of compounds AR-14a-AR-54a was made by recording its mass spectra. The Mass spectrum of compounds AR-14a-AR-54aillustrated a molecular ion peak at m/z, 488, 523, 523, 523, 479 (M+1) similarly, which is in trustworthy with their molecular formula.The in-vitro anti-inflammatory activity was carried out by membrane stabilization of human red blood cells (HRBC) which was causing heat induced hemolysis of RBC as one of the mode of inflammation and all the compound were found to have significant % protection. Further in-vivo anti-inflammatory activity was performed in carrageenin-induced rat hind paw oedema model at doses employed at 20 mg/kg body weight using diclofenac sodium and ibuprofen.Results are shown in Table 2and Table 3 as % protection value. Looking at the results of in-vivopharmacological activity, it was found that AR-34aof these compounds from series relatively active and compounds AR-14aand AR-24awere exhibiting pooractivity.The computational properties of thenovel series tested by mole inspiron and PASS Inet represented in Table 5 and 6. Which strongly support the protocol.

Anti-adhesion molecule therapy and inflammatory bowel disease.

The recent explosion of information on the molecules which govern the emigration of leukocytes from blood vessels and their accumulation at loci of inflammation has completely changed our understanding of inflammation and may lead to new strategies in the treatment of acute and chronic inflammation. We would illustrate the current ideas as to the pathogenesis of inflammation [25]'the biochemistry and function of the adhesive molecules involved in inflammation, and what we recognize of their role in rheumatic disease. We also indicate how drugs presently used to treat these diseases affect adhesion molecules.Bacterial products initiate or augment gastrointestinal inflammation with the recruitment and activation of leukocytes during inflammation of colon (eg. Clostridium difficile) and stomach (eg.Helicobacter pylori), there is an accumulation of neutrophils in the lamina propria in response to an acute gastro intestinal infection with invasive bacteria. Intestinal epithelial cell play an important role in the recruitment of inflammatory cells to the site of infection through the secretion of adhesion molecule [26].The PASS Inet support the protocol having **Pa** value more than 0.5 as cell adhesion Inhibitor (Pa = 0.900), Antimycobacterial more than (Pa = 0.700), Antiprotozoal more than (Pa = 0.600) and finally antibacterial (Pa = 0.500 or less) by all the compounds; indication potent anti-inflammatory agent when tested by PASS Inet software.

Conclusions

Novel set of phenylpiperazine Imidazolyl-thiazole compounds have been synthesized and characterized. The synthesized compounds were evaluated for their in-vitro andin-vivo anti-inflammatory activity.Phenylpiperazine Imidazolyl-thiazole scaffold were also screened for its potential antibiotic activity against pathogenic bacteria, which included Staphylococcus aureus, Escherichia faecalis, Escherichia coliandPseudomonas aeruginosa. The preliminary screening results revealed that these compounds possess promising antibacterial activity.Objective of the study was achieved with few of the promising structure possess single candidate as monotherapy in the treatment of chronic inflammatory diseases and bacterial infections.

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Conflict of Interest

The authors have declared no conflict of interest.

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