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Synthesis, Characterization, Antibacterial & Anti-Inflammatory Effects Of Substituted Tetrazole Derivatives Based On Different Types Of Carbazone And Benzaldehyde

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Abstract: A new series of novel some substituted tetrazole derivatives (Compounds I-V) were synthesized by tetrazole react with different types of carbazone derivatives and various substituted type of benzaldehyde. The entire resulting compounds were characterized and confirmed by IR, 1H NMR, 13C NMR, mass and elemental analysis data. The antimicrobial activity of synthesized substituted tetrazole derivatives (compounds I-V) possess moderate specific activity (inhibition) against *E-coli* and are inactive against *Staphylococcus aureus*. Further, these compounds were screened for the anti-inflammatory activity by carrageenan induced paw oedema method in rats at a dose of 50 mg/kg body weight. The compounds (I-V) showed moderate enhancement of the activity. Among the tested compound, compound V [1,1-dimethyl-3-(phenyl (1H- tetrazol-1-yl) methyl amino urea] exhibited potential anti-inflammatory activity when compared to standard phenylbutazone (PBZ) at 5 mg/kg/po). From the above results, the relationship between the functional group variation and the biological activity of the evaluated compounds is discussed and the compound V was determined to be the most active.
Keywords: Tetrazoles, anti-inflammatory activity, phenylbutazone, carragennan**.**

INTRODUCTION

Hetrocyclic compounds are considered as the most promising molecules for the design of new drug. 1,3-dipolarocycloaddition reaction is an efficient synthetic tool for constructing biologically potent five membered heterocyclic compounds[1,2]. Tetrazole and their derivatives are attracted much more attention because of their unique structure and application as antihypertensive, antiallergic, antibiotic and anticonvulsant agents. Synthesis of tetrazole derivatives is obviously an important task in modern medicinal chemistry. Although a number of synthetic methods are available, there still exists a demand for improved protocol which allows an

effective transformation in the presence of wide range of functional groups. The development of teterazole derivatives has been largely associated with wide scale of applications of these classes of compounds in medicine, biochemistry agriculture and also large number of medicinally important teterazole heterocyclic incorporated drugs approved by FDA. The teterazole functionality plays an important role in medicinal chemistry, primarily due to its ability to serve as bioequivalent of the carboxylic acid group. Heterocyclic derivatives are widely used as antibacterial agents in human and veterinary medicines[3]. Teterozoles and its derivatives have been reported to posses' antinociceptive antiinflamatory antimicrobial and anticonvulsant properties. Benzotriazole moiety is a versatile lead molecule in pharmaceutical development and more favorable pharmacokinetic profile, wide range of biological activities. These observations led to the conception that a series of some different 5-substitutional teterazole were synthesized obtained by the addition of azide ion to organic nitriles and many methods and their chemical structure were confirmed by IR, 1H-NMR, 13C-NMR, and mass spectroscopy. Further, these compounds were screened for their antibacterial & anti-inflammatory properties.

EXPERIMENTAL

Synthesis of 2-Phenyl 1H Tetrazole-1-yl) Methyl hydrazinecarbothioamide (1) (PTMHC)

The reaction mixture [tetrazole (0.1 mol, 7.0 g), thiosemicarbazone (0.1mol, 9.1g) and benzaldehyde (0.1mol, 10.0mL) in ethanol (30 mL)] was taken in rounded bottom flask (R.B) and heated with stirred for 2 h with help of magnetic stirrer. The reaction mixture was cooled and poured into crushed ice. The resulting separated solid precipitate was filtered, dried and recrystallized from ethanol. The physical properties of the product are listed in Table 1.

Synthesis of 2-[(4-hydroxy phenyl -1H Tetrazole -1-yl) methyl] hydrozinecarbothioamide(2) (HPHMH)

The reaction mixture like tetrazole (0.1mol, 7.0g), thiosemicarbozone (0.1mol, 9.1g) and 4-hydroxy benzaldehyde (0.1mol, 12.2mL) in ethanol (30mL), was transferred to R.B flask. Further the reaction mixture was heated and stirred for 2h with help of magnetic stirrer. After cooling the reaction mixture poured into crushed ice and precipitate was filtered. The resulting filtered solid dried and recrystallized from ethanol. The physical properties of the product are listed in Table 1.

Synthesis of 2-[(4-chlorphenyl (1H-Tetrazole-1-yl)methyl] hydrazinecarbothioamide (3) (CTMHCT)

The compound of 2-[(4-chlorphenyl (1H-tetrazole-1-yl) methyl]hydrazinecarbothioamide was synthesized by addition of tetrazole (0.1mol, 7.0g), thiosemicarbozone (0.1mol, 9.1g) with 4-chlorobenzaldehyde (0.1 mol, 14.0mL) in ethanol (30mL). Further, the reaction mixture was heated and stirred for 2h with the help of magnetic stirrer. The reaction mixture cooled and poured into crushed ice. The resulting solid was filtered dried and recrystallized from ethanol. The physical properties of the product are listed in Table 1.

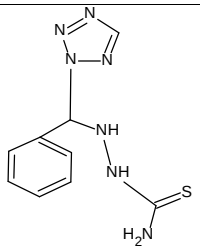
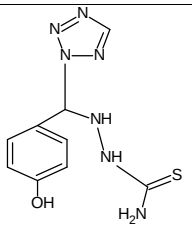
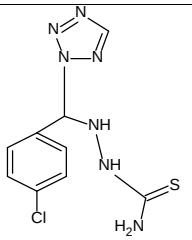
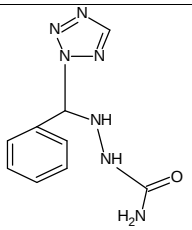
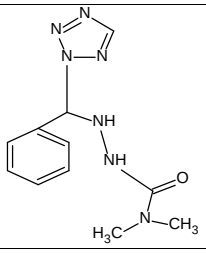
Synthesis of 2-(phenyl (1H-Tetrazole-1-yl) methyl) hydrazine carboxamide (4). (PTMHC)

Compound of 2-(phenyl (1H-Tetrazole-1-yl) methyl) hydrazine carboxamide was prepared by the mixture of tetrazole (0.1mol, 7.0g) and hydrazinecarbozone (0.1mol, 7.5g) treated with benzaldehyde (0.1 mol, 14.0mL) in ethanol (30mL). Reaction mixture was heated in R.B flask and stirred for 2h with the help of magnetic stirrer. The reaction mixture cooled and poured into crushed ice. The resulting solid was filtered dried and recrystallized from ethanol. The physical properties of the product are **listed in Table 1.**

Synthesis 1,1-dimethyl-3-(phenyl (1H- tetrazol-1-yl) methyl amino urea (5) (DPMU)

A mixture of tetrazole (0.1mol, 7.0g) and N,N –dimethyl hydrazinecarbozone (0.1mol, 8.8g) was mixed with benzaldehyde (0.1 mol) in ethanol (30mL) and heated in R.B flask & stirred with the help of magnetic stirrer for 2 hrs. The entire mixture was cooled and poured into crushed ice. The resulting solid was filtered, dried and recrystallized from ethanol. The physical properties of the product are listed in Table 1.

Table 1. Physical properties of the prepared compounds (1 – 5)

No.	Molecular formula	Compound structure	Molecular Weight	Yeild (%)	Recrystalised solvent
1	$C_9H_{11}N_7S$		249.29	78	Ethanol
2.	$C_9H_{11}N_7OS$		265.29	81	Ethanol
3.	$C_9H_{10}N_7SCl$		283.70	84	Ethanol
4	$C_9H_{11}N_7O$		233.23	80	Ethanol
5	$C_{11}H_{14}N_6O$		246.12	76	Ethanol

Antibacterial assay by agar plate diffusion method

Gram positive *S. aureus* and gram negative organism like *Escherichia coli* (E. coli) were used for antimicrobiological study by Agar plate diffusion method. Microbial cultures were collected from institute of microbial technology, Chandigarh, India. In this procedure antibiotics and test samples diffuses into a sheet of agar from a well, to produce a circular zone of inhibition in the growth of the indicator organism growing in the agar or on its surface. Agar should be prepared in batches and bottled in sufficient amounts for single plates (100 ml), the pH of the agar must be adjusted to that of the samples. Select and prepare the correct indicator organism. Pour the molten agar (typically100 ml for a 25x 25 cm dish) into the dish and spread evenly while it was still fluid by quickly, but gently, tipping the dish in each direction. Then leave undisturbed, with lid partially off, to set at room temperature until fully firm. Before use, dry the plate in an incubator for 45-60 min at 37°c with its lid off. The correct density of inoculum in 20 ml sterile water was dip it on to the plate, quickly distributing the suspension evenly over the whole surface of the plate. Then re-dry for the 10 min at 37°c with the lid off. Choose a clean, sharp cork borer of a diameter appropriate for the method in use and flame and cool it before use. With single movement exactly perpendicular to the plate cut a well with the cork borer to the base

of the plate in each position. Remove each plug with the tip of small scalpel. Draw up enough of a dose for all the replicates of the doses of samples and standard usually 3, (about 0.6ml) squeeze the pipette so as the lower meniscus was at its tip and there was no air below the fluid, put the tip on the base of the plate in the well to be filled and gently fill the well from the bottom. All the plates were kept for pre-diffusion of 1-2 hours in the refrigerator, then incubated at 35-37^{o}C for 18 hr (or) 24 hr. Zone of inhibition was measured by using a optical zone reader. Microorganisms: *Staph.aureus* and *E.coli*. Concentration used 50 mg/50 ml (10 µl per disc). Solvent used: aqueous solution. A modified agar diffusion method was used to determine antibacterial activity against *Staph.aureus* and *E.coli*. Nutrient agar was inoculated with bacterial cell (0.2 ml of bacterial cell suspension in 2 ml medium) and poured into petridishes to give a solid plate. 100 µl of test sample were applied on sterile paper discs (6 mm diameter). The discs were placed on the surface on inoculated agar plates. The plates were incubated for 24 hrs at 37^{o}C distilled water was used as a negative control. Inhibition zone diameter around each of the disc were measured and recorded at the end of the incubation time. An average zone of inhibition was calculated for the three replicates.

Acute toxicity study

Male Swiss albino rats (150 –200 g) were used and maintained at room temperature of 23−25^{o}C, respectively with alternating 12-h light and dark cycles. The animals were fed with a standard pellet diet and clean drinking water. All procedures were carried out in accord with the guidelines for care and use of laboratory animals and protocols were approved by the local ethical committee on experimental animals. Acute toxicity study was conducted according to Organization for Economic Cooperation and Development (OECD) guidelines (No. 423). Three mice [age range, 5–6 weeks and 25–30 g weight were selected for the study. A single highest dose of *teterazole* (2,000 mg/kg body weight (b.w.) per intraperitoneal (i.p)] was given to each animal. The animal was observed for 1 hour continuously for any mortality or gross behavioral changes then hourly for 4 hours and finally after every 24 hours up to 15 days.

Anti-inflammatory activity

The experimental animals were grouped into seven groups consisting of six animals in each. Group I served as carrageenan induced animals; group II rats received standard phenylbutazone (PBZ) 5 mg/kg/po) mg/kg and was treated as the standard, group III rats were treated with PTMHC (50 mg/kg. b.w. p.o) group IV rats were treated HPHMH at the dose of 50 mg/kg b.w. p.o. Group V treated CTMHCT (50.0 mg/kg b.w. p.o.), group VI received PTMHC (50.0 mg/kg b.w. p.o.), group VII treated DPMU (50.0 mg/kg b.w. p.o.). The animal dose injected into intraperitonially (i.p.). An hour after treated compounds animals in 1% solution of carrageenan in saline into the plantar aponeurosis of the left hind paw of the rats. The volume of edema in carrageenan injected and contra lateral paws were measured in 30 min, 60 min, 120 min, 240 min, 15 hrs and 24 hrs after induction of inflammation measured using a plethysmometer[4] and percentage of anti-inflammatory activity was calculated.

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism software (version 4.03). Values are expressed as mean (S.D followed by Students Paired‘t’ Test. comparison test. The data are presented as mean ± SEM. The level of statistical significance was set at $p \leq 0.01$.

RESULTS

Interpretation data of substituted tetrazole derivatives

The formation of the compound (1) was confirmed by recording the IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. The ^{1}HNMR spectra of the compound were shown in Table 3. Singlet absorption at δ 6.321, 8.721 and 9.606 corresponding to –CH-, Tetrazole ring and NH_2 proton respectively, the ^{H}NMR values were shown table 3. ^{13}C NMR spectrum of the compound (1) shown 182.86 and 74.11 corresponding to C=S and CH carbons respectable. IR spectra shown absorption at v(lamda) cm^{-1}(3408.22, 3002.47 for NH_2, NH) group; 1660.27 for C=S; 701.37 for Ar-H; 2926.03 for CH group. The EI-MS contain molecular ion peak at m/z 249.97 (M^{+}) and has 5% relative abundance value which coincide with the molecular weight of expected compound (1).

The IR spectrum (2) showed absorption bands at 3408.72, 1660.27 and 2909.59 corresponding to the NH_2, C=S and –CH- groups respectively. The ^{1}H NMR spectrum of compound (2) shown singlet observed at δ6.321 (s, 1H), 8.321 (s, 1H) and 9.21 (s, 2H) corresponding to –CH-, tetrazole -CH group and NH_2 protons respectively. ^{13}C NMR spectrum of compounds (2) showed 181.21 and 72.11 corresponding to C=S and CH carbon respectively. EI-MS contains molecular ion peak at m/z 265.87 (M^+) and has 25% relative abundance value which coincide with molecular weight of the expected compound (2), the mass spectrum of the compound (2).

The IR spectrum of compound (3) showed absorption bands at 3377.97, 1653.79, 2932.12 and 646.79 corresponding to the NH2,C=S,-CH and Ar-Cl respectively. The ^{1}H NMR spectrum of the compounds (3) showed singlet observed at δ6.436(s,1H), 8.821(s,1H) and 9.522(s,2H) corresponding to –CH-, tetrazole ring and NH_2 protons respectively. ^{13}C NMR spectrum of compounds (3) showed 181.12 and 71.11 corresponding to C=S and CH carbon respectively. EI-MS contains molecular ion peak at m/z 283.11 (M^+) and has 36% relative abundance value which coincide with molecular weight of the expected compound (3), the mass spectrum of the compound (3).

The IR spectrum of compound (4) showed absorption bands at 3345.22, 2986.26, 1694.69. 942.47, 919.99, 1512.87, 1376.30 and 2936.24 corresponding to the NH2, NH, C=O, NH, Ar, N=N, C=N and CH group proton respectively. The ^{1}H NMR spectrum of the compounds (4) showed singlet observed at δ6.422(s,1H), 7.364 – 7.212(m,5H), 8.921(s,1H), 6.422(s,2H), 6.121(s,dH) and 2.167(d,1H) corresponding to –CH-, phenyl ring, tetrazole – CH, NH2, NH, NH protons respectively. ^{13}C NMR spectrum of compounds (4) showed 154.07, 147.67, 72.11, 126.21 – 137.86 corresponding to C=O, Tetrazole, - CH, CH, Ph ring carbons respectively. EI-MS contains molecular ion peak at m/z 233.45 (M^+) and has 10% relative abundance value which coincide with molecular weight of the expected compound (2), the mass spectrum of the compound (4).

The IR spectrum of compound (5) showed absorption bands at 3297.05, 1683.76, 936.08, 1565.35, 1377.76 and 2934.75 corresponding to the CH_3, CONH, Ar, N=N, C=N and CH group proton respectively. The 1H NMR spectrum of t compounds (5) showed singlet observed at δ 7.153(s,^{1}H), 7.213 – 7.314(m,5H), 8.851(s,1H), 6.136(s,1H) and 2.837(s,1H) corresponding to –CH-,Phenyl ring, tetrazole – CH, NH, and –$N(CH_3)_2$ protons respectively. ^{13}C NMR spectrum of compounds (5) showed 156.67, 143.30, 66.21, 128.30 – 131.86 and 36.12 corresponding to C=O, Tetrazole, -CH, CH, Ph ring –N $(CH_3)_2$ carbons respectively. EI-MS contains molecular ion peak at m/z 246.87 (M^+) and has 15% relative abundance value which coincide with molecular weight of the expected compound (5), the mass spectrum of the compound (5).

Antimicrobial activity

The antimicrobial activity of some substituted tetrazole derivatives was shown in Table 6. The results of activities from the prepared compounds (Comp I, II, III, IV and V) possess moderate specific activity (inhibition) against *E-coli* and are inactive against *Staphylococcus aureus*.

Anti-inflammatory activity of substituted tetrazole derivatives

Anti-inflammatory activity in carrageenan-induced rat paw edema test at the dose of 100 mg/kg/b.w.(Table1) showed that 2-Phenyl ^{1}H Teterazole-1-yl) Methyl hydrazinecarbothioamide (PTMHC) and 2-[(4-hydroxy phenyl -1Hteterazole -1-yl) methyl] hydrozinecarbothiomide (HPHMH) showed better activity as compared to the 2-[(4-chlorphenyl (1H-teterazole-1-yl)methyl] hydrazinecarbothioamide (CTMHCT), Synthesis of 2-(phenyl (1H-teterazole-1-yl) methyl) hydrazinecarboxamide (PTMHC) and 1,1-dimethyl-3-(phenyl (1H- tetrazol-1-yl) methyl amino urea (DPMU). The percentage inhibitions produced by PTMHC, HPHMH, CTMHCT, PTMHC and DPMU were compared with that of phenylbutazone (table 5). The results were significantly normal as compared with standard.

Table 2. IR absorption spectra data (cm^{-1}) of the prepared compounds

No.	IR (KBr) Cm^{-1}
1.	3408.22(NH_2);3002.47(NH);1660.27(C=S);947.95(NH);701.37(Ar-H);1512.33(N=N);1315.07(C=N);2926.03(CH)
2	3408.72(NH_2);2997.26(NH);1660.27(C=S);942.47(NH);942.47(Ar-OH);1315.07(C=N);2909.59(CH)
3	3377.97(NH_2);30232.21(NH);1653.79(C=S);936.08(NH);646.79(Ar-Cl);1577.36(N=N);2932.12(CH)
4	3345.22(NH_2);2986.26(NH);1694.69(C=O);942.47(NH);919.99(Ar);1512.87(N=N);1376.30(C=N);2936.24(CH)
5	3297.05(CH_3);1683.76(CONH);936.08(Ar);1565.35(N=N);1377.76(C=N);2934.75(CH)

Table 3. ^{1}H NMR spectral data for prepared compounds.

No.	^{1}H NMR (δ ppm) in DMSO-d6 solvent
1.	6.321(s,1H,-CH-);7.304-7.262(m,4H,Phenylring);8.721(s,1H,Teterzole-CH);9.606(s,2H,NH_2);2.221(s,1H,NH)
2	6.321(s,1H,-CH-);7.231-7.221(dd,4H,Phenylring);9.832(s,1H,Ph-OH);8.321(s,1H,Teterzole-CH);9.212(s,2H,NH_2);2.431(s,1H,NH)
3	6.436(s,1H,-CH-);7.753-7.443(dd,4H,Phenylring);8.721(s,1H,Teterzole-CH);9.522(s,2H,NH_2);2.437(s,1H,NH);2.146(s,1H,NH)
4	6.422(s,1H,-CH-);7.364-7.212(m,5H,Phenylring);8.921(s,1H,Teterzole-CH);6.422(s,2H,NH_2);6..121(s,dH,NH);2.167(d,1H,NH).
5	7.153(s,1H,-CH-);7.213-7.314(m,5H,Phenylring);8.851(s,1H,Teterzole-CH);6.136(s,1H,NH);2.837(s,1H,-N($CH_{3)2}$)

Table 4. ^{13}C NMR Spectral data of the prepared compounds.

No	^{13}C NMR (DMSO-d_6) , δ (ppm)
1.	144.87(Teterzole-CH);182.86(C=S);74.11(CH);138.26-126.67(Ph ring)
2.	143.67(Teterzole-CH);181.21(C=S);72.11(CH);138.26-126.67(Ph ring);154.07(Ph-OH)
3.	147.67(Teterzole-CH);181.12(C=S);131.86(C-Cl);71.11(CH);128.30-131.86(Ph ring)
4.	154.07(C=O);147.67(Teterzole-CH);72.11(CH);126.21-137.86(Ph ring)
5.	156.67(C=O);143.30(Teterzole-CH);66.21(CH);128.30-131.86(Ph ring);36.12(-N($CH_3)_2$)

Table 5. Anti inflammatory activity of Tetrazole induced end paw edema in rats.

Groups	**Paw volume (ml) by mercury Displacement at regular interval of time**						
	0 min	30 min	60 min	120 min	240 min	15 Hrs	24 Hrs
CGN Induced	1.13±0.23	1.45±0.34	2.17±0.49*	2.48±0.48**	2.65±0.57**	2.85±0.59***	2.91±0.67***
STD+CGN	0.8 ± 0.05	1.35±0.06	1.13±0.04	1.13±0.04	1.01±0.06	0.89± 0.04	0.85± 0.05
PTMHC	1.2 ± 0.38	1.4 ± 0.26	2.3 ± 0.23	1.96± 0.22^{a}	1.76± 0.22^{a}	1.63 ±0.22^{b}	1.18± 0.08^{b}
HPHMH	1.3±0.42	1.31±0.25	2.7±0.36	2.23±0.41^{a}	2.01±0.42^{a}	1.35±0.34^{b}	1.21±0.25^{b}
CTMHCT	0.92±0.09	1.05±0.2	1.71±0.36	1.89±0.39^{a}	1.51±0.39^{a}	1.42±0.28^{b}	1.18±0.31^{b}
PTMHC	1.32±0.25	1.48±0.1	1.92±0.37	1.85±0.39^{a}	1.76±0.32^{a}	1.26±0.29^{b}	1.07±0.24^{b}
DPMU	0.84±0.24	1.35±0.2	1.64±0.35	1.73±0.41^{a}	1.36±0.31^{a}	1.06±0.27^{b}	1.01±0.24^{b}

P - values (calculated as compared to control using student's t-test): $p < 0.001$; STD - diclofenac ns - Non significant as compared with STD; $^{a}P< 0.01$ (**), $^{b}P< 0.001$ (*******) as compared with control.

Table 6. Antibacterial activity of the prepared compounds.

Compounds	Staphylococcus aureus	E.coli
Tetrazole	10	10
Compound I	12	10
Compound II	15	10
Compound III	17	14
Compound IV	9	12
Compound V	5	10
Ciprofloxacin (STD)	**25**	**27**

Concentration - *100µg/mL;* ***Standard (STD) –*** *Ciprofloxacin;*
Zone of inhibition – micro mole (mm).

DISCUSSION

The 5-substitutional teterazole are usually obtained by the addition of azide ion to organic nitriles and many methods[5,6&7]. Unfortunately each of those protocols suffers from some disadvantages. In toxic metals and expensive reagents, drastic reaction condition, water sensitivity and possible presence of dangerous hydrazoic acid or other explosive sublimates. The Huisgen 1.3-dipolar cyclo-addition is the reaction is the reaction of alkyne to azides to form 1,4-regioisomers of 1,2,3-triazoles as azole products[8]. Huisegen was first to understand the scope of this organic reaction. The azide and alkyne functional groups are largely inert towards biological molecules and aqueous environments, which allows the use of the Huisgen 1,3-dipolar cycloaddition in target guided synthesis[9] and activity based protein profiling. The results of the triazole has similar to the ubiquitous amide moiety found in nature, but unlike amides, is not susceptible to cleavage. Tetrazole contains cyclic secondary amino group. All secondary amine undergo acetylation reaction with acetic anhydride and conc.H_2SO_4. 5-phenyl-1,2,3,4- tetrazoles being a secondary amine was acetylated to compound 2 by acetic anhydride and concentrated H_2SO_4. The yield of compound I was found to be quantitative and it was readily converted to chalcones by treating them with different aromatic aldehydes and pottasium hydroxide and hence nine different derivatives are synthesized. The formations of these compounds were confirmed by identifying IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis data given by many authors for alkylation and acylation reactions[10,11,12&13].

The IR spectrum (1, 2, 3 and 4) showed absorption bands at 3408.72, 1660.27 and 2926.03 corresponding to the NH_2 proton in compound (2), The compound (3) 3408.72, 1660.27, 2926.03 and 646.47, 3345.22 corresponding to the NH2, C=S and C-C_l groups, compound (4) 3345.22, 2986.26, 1694.69. 942.47, 919.99, 1512.87, 1376.30 and 2936.24, corresponding to the CH3, CONH, Ar, N=N, C=N and CH group proton respectively, compounds (5) 3297.05, 1683.76, 936.08, 1565.35, 1377.76 and 2934.75. Characteristic absorption bands were observed for chloro, nitro group, bromo group, dimethylamino group, methyl group, methoxyl group and aromatic region of the synthesized compounds. The compound (3) with 2-[(4-chlorphenyl (1H-teterazole-1-yl)methyl] hydrazinecarbothioamide substituted derivative was found to be more active. In fact, the hydrazinecarbothioamide substituted tetrazole were found to have encouraging sensitivity to microbial activity compared to other compounds.

^{1}H-NMR spectra of the synthesized compounds showed multiples in the range of d 6.4– 9.50 for aromatic protons. The expected signals with appropriate multiplicities for different types of protons such as methyl, methoxy groups were observed for the derivatives within the range. Tetrazole derivatives were synthesized from thiosemicarbozone (0.1mol, 8.8g) and 4- chlorobenzoldehyde (0.1 mol, 14.0mL) in ethanol which was synthesized from benzonitrile and sodium azide in good yields.1,5-disubstituted tetrazole containing substituted pyrazolyl derivatives at first position by simple, rapid and high yield synthetic route and are found to possess good anti-inflammatory activity by denaturation of proteins mechanism. Also, the ^{13}C NMR spectrum of compound 4 gives further confirmation for the assigned structure since it presence of a NH and NH_2 group. Also the 1H NMR spectrum revealed signals at (δ, ppm): The ^{1}HNMR spectra of the compounds showed singlet observed at δ 6.321, 8.721 and 9.606 corresponding to –CH-, Tetrazole ring and NH_2 proton respectively, the HNMR values was not summarized.

^{13}C NMR spectrum of compounds (1) showed 182.86 and 74.11 corresponding to C=S and CH carbons respectable. ^{13}C NMR values summarized table 3. The EI-MS contain molecular ion peak at m/z 249.97 (M^+) and has 5% relative abundance value which coincide with the molecular weight of expected compound (1). ^{13}C NMR spectrum of compounds (2) showed 181.21 and 72.11 corresponding to C=S and CH carbon respectively. EI-MS contains molecular ion peak at m/z 265.87 (M^+) and has 25% relative abundance value which coincide with molecular weight of the expected compound (2), The ^1H NMR spectrum of t compounds (2) showed singlet observed at δ6.436, 8.821 and 9.51 corresponding to –CH-, tetrazole ring and NH_2 protons respectively. ^{13}C NMR spectrum of compounds (2) showed 181.12 and 71.11 corresponding to C=S and CH carbon respectively. The compound 1, 2 and 5 and 4-methoxy substitution has possessed a potent anti-inflammatory activity as compared with standard. The compounds 3, 4 containing 4-Cl, 4-Br substitution product produces moderate anti-inflammatory activity.

The antimicrobial activity of some substituted tetrazole derivatives (Comp I, II, III, IV and V) possess moderate specific activity (inhibition) against *E-coli* and are inactive against *Staphylococcus aureus*. Anti-inflammatory drugs, several newer templates or leads were selected which include indole nucleus, arylalkyl acid nucleus, pyrazolone, indan etc. were attempt has been taken to establish novel anti-inflammatory agent without or less gastrointestinal effects[14]. Acyclo compounds of tetrazole are one of the potent antiviral drugs by Schaeffer et al[15] and many attempts have been directed by nucleoside to produce a similar compounds with various side chain and glycons[16&17], however, acyclonucleosides of pyrazolo pyranopyrimidine derivatives were not reported in the literature. Thus, and in continuation of our previous work[18,19&20] in preparing various cyclic and acyclic nucleosides of different heterocyclic compounds, we describe here for the synthesis of some acyclonucleosides of pyrazolopyranopyrimidines by treating the sodium salts of compounds generated with 2-chloroethyl methyl ether to give corresponding acyclonucleosides. The methyl groups containing tetrazole derivatives were effective against anti-inflamatory actions. The compounds containing methyl hydrazinecarbothioamide substitution produces more anti-inflammatory activity. Some of the tetrozole derivatives have been used for both anticancer as well as antimicrobial activity, substituted tetrazole derivatives are widely used antibiotic and optically active tetrazole containing antifungal preparation of azole type[21]. The prime reason for scarcity of practical applications for these sophisticated tetrazole derivatives are lack of appealing synthetic routes to key intermediates 5-substituted tetrazoles. Tetrazoles readily tolerate in a wide range of chemical environment and uses for this unique family of heterocyclic continue to emerge in both materials science and pharmaceutical applications. 5-substituted tetrazole that contain a free N-H bond are frequently referred to as tetrazolic acids and exist in two tautomeric forms[22]. In this prime type tetrazole derivatives contains antimicrobial activities and also used for treating inflammation to control panic diseases.

CONCLUSION

In the present study concluded that synthesized tetrazole derivatives exhibited pivotal anti- inflammatory activity against carrageenan induced paw edema rats. The activity of 1,1-dimethyl-3-(phenyl (1H- tetrazol-1-yl) methyl amino urea (DPMU) was more promising than others. The study related to synthesis newer templates of anti-inflammatory agents. Overall, source of prepared compounds contains anti-inflamatory action that can be vital role for prevention of disease, health preservation and promotion of longevity promoter.

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