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SYNTHESIS OF NEW TETRAZOLYL HYDROXYETHYL ACETAMIDES AS ANTI BACTERIAL AND ANTIPROTOZOAL AGENTS

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ABSTRACT

Present investigation describes about the synthesis of some novel tetrazolyl hydroxyacetamides. These compounds were designed, based on the structure of etanidazole, an imidazole containing antiprotozoal drug and its radio protective activity is under clinical trials. Some new *N*-(2-hydroxyethyl)-2-(5-phenyl-1*H*-tetrazol-1-yl) acetamides (5a - 5h) were synthesized by 1, 3-dipolar cycloaddition of arylnitriles with sodium azide followed by reaction with ethyl chloroactetate and ethanolamine. The structures of the newly synthesized compounds were confirmed on the basis of physical, proton NMR, mass and IR spectral data. All the synthesized compounds were screened for *in vitro* antibacterial and anti protozoal activities and the results of some of the derivatives exhibited promising activities. The MIC values of the compounds 5b, 5e and 5h showed significant activity at a concentration of 1.56 and 12.5 µg/mL. Highest antiamoebic activity was observed with compounds 5a and 5h possessing electron withdrawing substituents on phenyl ring.

Keywords: Arylnitriles; Substituted tetrazoles; Tetrazolyl acetamides; Antiprotozoal; Antibacterial.

INTRODUCTION

Tetrazole is an important heterocycle and its derivatives have attracted greater attention in recent years because of its medicinal applications in the area of medicine, biochemistry and agriculture. In recent years a large number of tetrazole incorporated heterocyclic drugs have

been synthesized [1] and some of them were approved as antihypertensive agents [2]. They have wide applications as carboxylic surrogates and bioisosteres of carboxylic acids [3, 4] and lipophilic spacers in pharmaceuticals. As per literature, they have been reported to possess anti nociceptive [5], anti viral [6], anti tumor [7, 8], anti-inflammatory [9, 10, 11], antibacterial [11], algistatic [12] and anticancer activities [13].

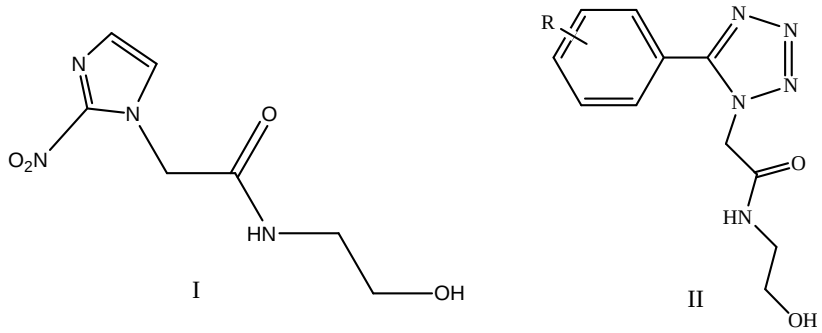

Fig 1. Structures of etanidazole (I) and designed compounds (II)

Thomas M. Beale *et al.*, [14] synthesized novel combretastatin analogues by incorporating tetrazole moiety in between two phenyl rings in place of double bond and reported improved anti cancer activity against various cell lines. Mohmmad Younis Wani *et al.*, [15] reported synthesis of tetrazole embedded 1, 3, 5-trisubstituted pyrazoline derivatives as *Entamoeba histolytica* growth inhibitors. Inspired by the outstanding properties of tetrazole moiety, here in we report novel tetrazole derivatives based on the structure of etanidazole which is an imidazole containing antiprotozoal medication with hydroxyethyl acetamide side chain and its radio protective activity is under clinical trials. In continuation of our research on synthesis of new biologically active tetrazole derivatives [16] and also based on the structure of etanidazole, in the present study, we herein report novel tetrazole analogues by substituting imidazole nucleus in etanidazole with tetrazole without modifying hydroxyethyl acetamide side chain with an aim to obtain potent anti protozoal and anti bacterial agents.

Experimental

Melting points (^{0}C) were determined on Analab melting point apparatus by open capillary method and are uncorrected. The IR spectra were recorded on Schimadzu FTIR spectrophotometer by using 1**%** KBr discs. ^{1}H NMR spectra was recorded on Varian 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. ^{13}C NMR were recorded on Varian Gemini 400 MHz spectrophotometer and mass spectra on Agilent

6430 triple quadruple LC-MS system. TLC was done using E-Merck 0.25 mm silica gel plates and visualization was accomplished with UV light (256 nm).

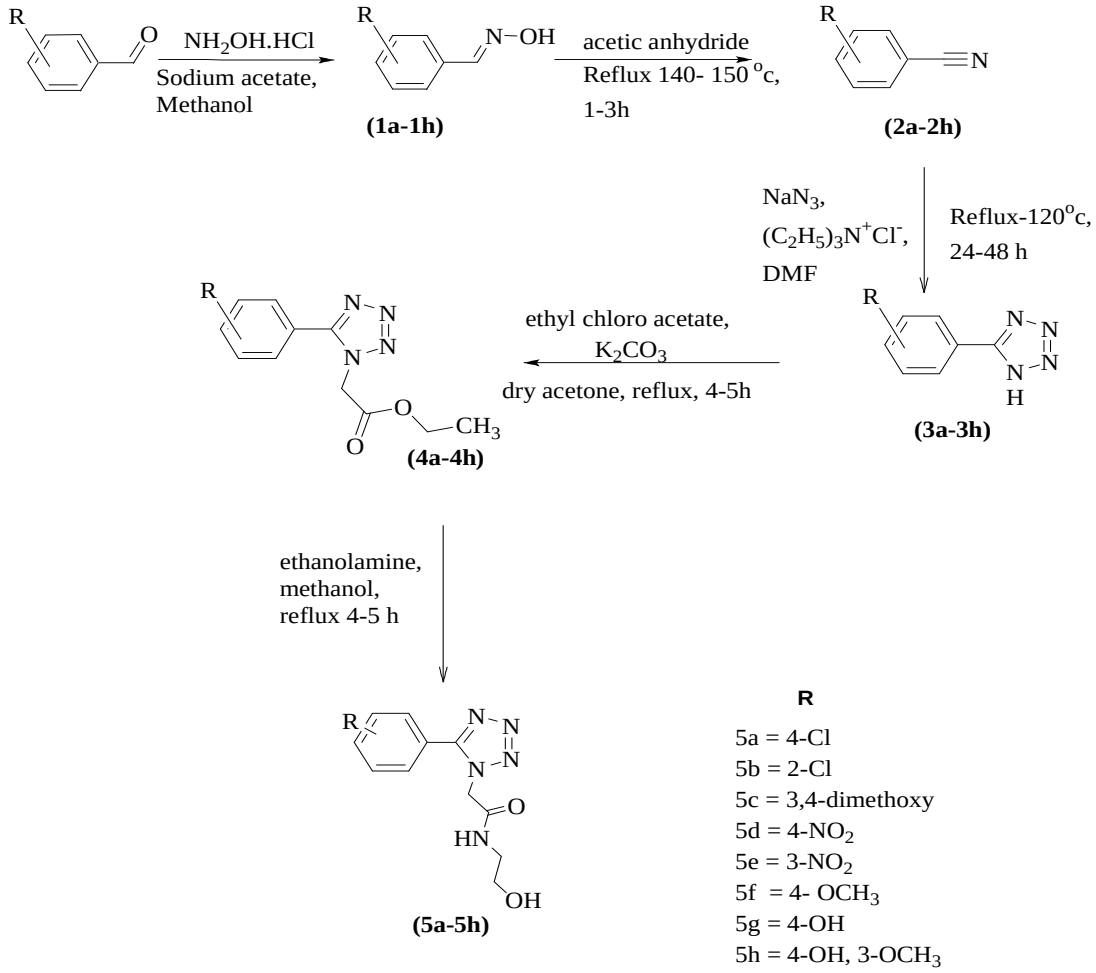

Fig. 2 Synthetic scheme for the preparation of tetrazolyl hydroxyacetamides 5a -5h

General procedure for synthesis of arylaldoximes (1a-1h)

To the solution of substituted benzaldehyde (0.01 mol) in methanol, was added hydroxylamine hydrochloride (0.02 mol, 1.4 g dissolved in 5 mL of water) and sodium acetate (0.02 mol) in portion-wise with shaking and refluxed for 4-5 h. The reaction mixture was cooled and left overnight in refrigerator. The separated precipitate was filtered under suction and recrystallized from methanol.

General procedure for synthesis of arylnitriles (2a-2h)

Substituted arylaldoximes (0.01 mol) were dissolved in redistilled acetic anhydride (0.01 mol) and strongly heated at 150 ^{o}C for 1-3 h using air condenser. The reaction mixture was then cooled and added to crushed-ice with stirring. The separated precipitate was filtered, washed thoroughly with water and recrystallized from methanol.

General procedure for synthesis of 5-phenyl-1*H*-tetrazoles (3a-3h)

A mixture of sodium azide (0.02 mol) and triethylamine hydrochloride (0.02 mol) were added to the solution of arylnitrile (0.01 mol) in 15 mL of DMF and refluxed for 24-40 h at 120 ^{o}C. The reaction mixture was cooled and added to water (30 mL) and acidified with dilute HCl. The separated solid was filtered under suction and recrystallized from 2:3 ratio of methanol and chloroform solvent system.

General procedure for synthesis of ethyl (5-phenyl-1*H*-tetrazol-1-yl) acetates (4a-4h)

Substituted phenyl tetrazole (0.01 mol) was dissolved in acetone and to this was added ethyl chloroactetate (0.01 mol, 1.22 mL) and anhydrous potassium carbonate and refluxed for 4-5 h at 50 - 60 ^{o}C. The potassium carbonate was filtered off and the filtrate was evaporated on water bath and allowed to cool. A cream colored solid was obtained and was recrystallized from methanol.

General procedure for synthesis of *N*-(2-hydroxyethyl)-2-(5-phenyl-1*H*-tetrazol-1-yl) acetamides (5a-5h)

Tetrazolyl acetate (0.01 mol) was dissolved in methanol (15 mL) and to this ethanolamine (10 mL) was added and refluxed for 4 h. The reaction mixture was cooled and distilled water (50 mL) was added to the reaction mixture and the separated precipitate was filtered and recrystallized from methanol.

2-[5-(4-chlorophenyl)-1*H*-tetrazol-1-yl]-*N*-(2-hydroxyethyl) acetamide (5a)

Cream colored amorphous powder, m.p. 298 ^{o}C, yield, 69 %. IR [KBr] cm^{-1}: 3433 (OH), 1670 (C=O of amide), 1604 (C=N), 740 (C-Cl); ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.90 (s, 1H,NH), 8.0-8.45 (4H, Ar-H), 5.70 (s, 2H, CH_2 attached to tetrazole), 4.92 (s, 1H, OH), 3.45 (t, 2H, CH_2 of CH_2OH), 3.25 (t, 2H, CH_2 of NH-CH_2); ^{13}C NMR (DMSO-d_6): δ 42.27, 55.23, 59.93, 126.19, 128.53, 129.88, 135.70, 163.67, 164.58; Mass: *m/z*: 281(M^+).

2-[5-(2-chlorophenyl)-1*H*-tetrazol-1-yl]-*N*-(2-hydroxyethyl) acetamide (5b)

Cream colored amorphous powder, m.p. 293-245 ^{o}C, yield, 62 %. IR [KBr] cm^{-1}: 3429 (OH), 1600 (C=N), 1663 (C=O of amide); 743 (C-Cl); ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.50 (s, 1H, NH of amide), 7.9-8.20 (4H, Ar-H), 5.30 (s, H, CH_2 attached to tetrazole), 4.60 (s, 1H,

OH), 3.31 (t, 2H, CH_2 of CH_2OH), 3.19 (t, 2H, NH-CH_2); ^{13}C NMR (DMSO-d_6):δ 42.37, 55.25, 59.99, 126.09, 128.23, 129.65, 135.59, 163.29, 164.18; Mass: *m/z*: 281(M^+).

2-[5-(3, 4-dimethoxyphenyl)-1*H*-tetrazol-1-yl]-*N*-(2-hydroxyethyl) acetamide (5c)

Light yellow solid, m.p. 237 ^{o}C, yield, 72 %. IR [KBr] cm^{-1}: 3286 (OH), 1654 (C=O of amide), 1060 (C-O-C), 1608 (C=N); ^{1}HNMR (400 MHz, DMSO-d_6):δ 8.50 (s, 1H, NH of amide), 7.25-7.75 (3H, Ar-H), 5.50 (s, 2H, CH_2 attached to tetrazole), 4.75 (s, 1H, OH), 3.75 (s, 6H, OCH_3), 3.40 (t, 2H, CH_2 of CH_2OH), 3.20 (t, 2H, NH-CH_2); ^{13}C NMR (DMSO-d_6):δ 42.25, 55.03, 55.94, 56.00, 59.92, 109.59, 112.45, 119.72, 149.50, 151.06, 164.59, 164.70; Mass: *m/z*: 307(M^+).

***N*-(2-hydroxyethyl)-2-[5-(4-nitrophenyl)-1*H*-tetrazol-1-yl] acetamide (5d)**

Light yellow solid; m.p. 182 ^{o}C, yield, 69%. IR [KBr] cm^{-1}: 3402 (OH), 1674 (C=O of amide) cm^{-1} 1537, 1342 (NO_2); ^{1}HNMR (400 MHz, DMSO-d_6):δ 8.65 (s, 1H, NH of amide), 8.35-8.40 (4H, Ar-H), 5.50 (s, 2H, CH_2 attached to tetrazole), 4.80 (s, 1H, OH), 3.40 (t, 2H, CH_2 of CH_2OH), 3.20 (t, 2H, NH-CH_2); ^{13}C NMR (DMSO-d_6):δ 40.55, 42.27, 55.23, 59.93, 126.19, 128.53, 129.88, 135.70, 163.67, 164.58; Mass: *m/z*: 290.60 (M-1).

***N*-(2-hydroxyethyl)-2-[5-(3-nitrophenyl)-1*H*-tetrazol-1-yl] acetamide (5e)**

Light yellow solid; m.p. 171 ^{0}C, yield, 65%. IR [KBr] cm^{-1}: 3421 (OH), 1650 (C=O of amide); ^{1}HNMR (400 MHz, DMSO-d_6):δ 8.55 (s, 1H-NH of amide), 8.20-8.39 (4H, Ar-H), 5.35 (s, 2H, CH_2 attached to tetrazole), 4.65 (s, 1H, OH), 3.29 (t, 2H, CH_2 of CH_2OH), 3.16 (t, 2H, NH-CH_2); ^{13}C NMR (DMSO-d_6): δ 42.273, 55.234, 59.931, 126.19, 128.53, 129.88, 135.70, 163.67, 164.58; Mass: *m/z*: 290.60 (M-1).

***N*-(2-hydroxyethyl)-2-[5-(4-methoxyphenyl)-1*H*-tetrazol-1-yl] acetamide (5f)**

Cream colored amorphous powder, m.p. 185 ^{o}C, yield, 69 %. IR [KBr] cm^{-1}: 3286 (OH), 1654 (C=O of amide), 1608 (C=N), 1058 (C-O-C); ^{1}HNMR (400 MHz, DMSO-d_6):δ 8.50 (s, 1H, NH of amide), 8.0-8.45 (4H, Ar-H), 5.50(s, 2H, CH_2 attached to tetrazole), 4.75 (s, 1H, OH), 3.75 (s, 3H, OCH_3), 3.40 (t, 2H, CH_2 of CH_2OH), 3.20 (t, 2H, NH-CH_2); ^{13}C NMR (DMSO-d_6):δ 42.25, 55.03, 55.94, 56.00, 59.92, 112.45, 119.72, 149.50, 151.06, 164.59, 164.70; Mass: *m/z*: 277 (M^+).

***N*-(2-hydroxyethyl)-2-[5-(4-hydroxyphenyl)-1*H*-tetrazol-1-yl] acetamide (5g)**

White solid, m.p. 197 ^{o}C, yield, 67 %. IR [KBr] cm^{-1}: 3433 (OH), 1654 (C=O of amide), 1608 (C=N); ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.33 (s, 1H, NH of amide), 8.17 (s, 1H, OH), 7.8-8.20 (4H, Ar-H), 5.32(s, 2H, tetrazolyl-CH_2), 4.59(s, 1H, OH), 3.21 (t, 2H, CH_2 of CH_2OH), 3.00 (t, 2H, NH-CH_2); ^{13}C NMR (DMSO-d_6): δ 42.273, 55.234, 59.931, 126.19, 128.53, 129.88, 135.70, 163.67, 164.58; Mass: *m/z*: 263 (M^+).

***N*-(2-hydroxyethyl)-2-[5-(4-hydroxy-3-methoxyphenyl)-1*H*-tetrazol-1-yl] acetamide (5h)**

White solid, m.p. 221 ^{0}C, yield, 70%. IR [KBr] cm^{-1}: 3286 (OH), 1670 (C=O of amide), 1608 (C=N) 1060 (C-O-C); ^{1}HNMR (400 MHz, DMSO-d_6):δ 8.50 (s, 1H, NH of amide), 8.30 (1H, OH), 7.25-7.75 (3H,Ar-H), 5.50 (s, 2H, CH_2 attached to tetrazole), 4.75 (s, 1H, OH), 3.75 (s, 3H, OCH_3), 3.40 (t, 2H, CH_2 of CH_2OH), 3.20 (t, 2H, NH-CH_2); ^{13}C NMR (DMSO-d_6):δ 42.25, 55.03, 55.94, 59.92, 109.59, 112.45, 119.72, 149.50, 151.06, 164.59, 164.70; Mass: *m/z*: 293 (M^+).

RESULTS AND DISCUSSION

Chemistry

The synthetic methodology followed to obtain the target compounds is outlined in the figure 2. In the first step, arylaldoximes **(1a-h)** were prepared by reacting aromatic aldehydes with hydroxylamine hydrochloride in methanol under reflux for 6-12 h. Further, the heating of oximes with acetic anhydride at 140-150 ^{o}C resulted in the formation of different arylnitriles **(2a-h)**. 1,3-dipolar cycloaddition of arylnitriles with sodium azide in presence of triethyl ammonium chloride in DMF gave substituted 5-phenyl tetrazoles in good yields **(3a-h)**. Compounds **(3a-h)** were reacted with ethyl chloroactetate in dry acetone to give tetrazolyl esters **(4a-h)** which on reaction with ethanolamine in methanol under reflux for 4-5 h gave *N*-(2-hydroxyethyl)-2-(5-phenyl-1*H*-tetrazol-1-yl) acetamides **(5a-h)** in reasonable yields.

The synthesized compounds were confirmed on the basis of spectral data. The ^{1}H NMR spectrum of compound 5a showed a singlet at δ 4.9 due to the presence of OH proton which was further confirmed by deuterium exchange and two triplets at δ 3.45 and δ 3.25 indicates two sets of methylene protons attached to OH and NH. A singlet at δ 8.9 due to NH proton and was further confirmed by deuterium exchange and singlet at δ 5.7 can be assignable to two methylene protons attached to tetrazole nucleus. The four aromatic protons appeared in the range of δ 8.0 -8.45, thus confirmed the structure of the compound 5a. Moreover, the mass spectrum of compound revealed a molecular ion peak at *m/z* 281(M^+). The structure was

further supported by ^{13}CNMR spectrum. In IR spectrum, the disappearance of ester absorption peak and the appearance of amide stretching at 1670 cm^{-1} further confirmed the formation of the compound. Similarly the structures of other compounds were confirmed on the basis of IR, proton NMR, ^{13}C NMR and mass spectral data.

Anti bacterial screening

All the synthesized compounds were screened for their antibacterial activity against two gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and two gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) with ciprofloxacin as reference standard. The study was carried out by cup-plate method [17, 18] to determine the zone of inhibition (mm) against four strains of bacteria. Antibacterial activity was carried out at a concentration of 100 µg/50 µL. Investigation of antibacterial data revealed that compounds 5b, 5e, and 5h have good activity against all the bacterial strains as shown in table 1. The MIC of these compounds and ciprofloxacin were determined by using the standard protocol of NCCLS Broth Micro dilution MIC method [19] and the results are tabulated in Table 2. MIC values were determined for compounds 5b, 5e and 5h by preparing two fold dilutions to give concentrations 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0.39 µg/mL. Compounds showed activity against both gram positive and gram negative strains with MIC values ranging between 1.56 and 12.5 µg/mL.

Table 1. Anti bacterial data of compounds 5a-5h.

Compound	Concentration (µg/50µL)	Zone of inhibition (mm)			
		Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
Solvent(control)	----	---	---	---	---
Ciprofloxacin	**25**	**20**	**28**	**30**	**20**
5a	100	6	8	14	9
5b	100	**17**	**19**	**22**	**20**
5c	100	5	4	10	6
5d	100	8	7	15	8
5e	100	**15**	**19**	**18**	**21**
5f	100	10	9	10	12
5g	100	9	11	10	13
5h	100	**16**	**21**	**23**	**15**

Table 2. Antibacterial activity of compounds5b, 5e and 5h by broth Micro dilution MIC method.

Concentration (µg/mL)	Antibacterial activity against standard strains		
	5b	**5e**	**5h**

	1	2	3	4	1	2	3	4	1	2	3	4
0.39	+	+	+	+	+	+	+	+	+	+	+	+
0.78	+	+	+	+	+	+	+	+	+	+	+	+
1.56	−	−	−	−	+	−	−	−	−	−	+	−
3.125	−	−	−	+	−	−	−	−	−	+	−	−
6.25	−	−	−	−	−	−	−	−	−	−	−	−
12.5	−	−	−	−	−	−	−	−	−	−	−	−
25	−	−	−	−	−	−	−	−	−	−	−	−
50	−	−	−	−	−	−	−	−	−	−	−	−
100	−	−	−	−	−	−	−	−	−	−	−	−

1, *Bacillus subtilis*; 2, *Staphylococcus aureus*; 3, *Escherichia coli*; 4, *Pseudomonas aeruginosa*; (+) resistant; (−) Susceptible. Ciprofloxacin was taken as a standard drug and its MIC was 1.56 µg/mL against all the four strains.

Antiprotozoal screening

All the synthesized compounds were screened *in vitro* against *Entamoeba histolytica* by micro dilution method[20]. All the experiments were carried out in triplicates at each concentration level. The results are summarized in table 3. The antiamoebic activity of the synthesized compounds was compared with widely used antiamoebic medication, metronidazole with 50% inhibitory concentration (IC_{50}) of 1.70 µM.

The antiamoebic activity of the test compounds was found to be substituent dependent. As depicted in table 3, compounds exhibited an interesting inhibition pattern on *Entamoeba histolytica*. The tetrazole derivatives showed IC_{50} values in the range of 1.02-5.32 µM. It was observed that the compounds bearing chloro, nitro substitutions at para position and methoxy group at ortho and para positions showed excellent activity. The presence of chloro and nitro group at meta and ortho positions respectively showed moderate activity. These findings indicate that the presence of electron withdrawing groups like chloro and nitro group at para position of the phenyl ring, generally increase the antiamoebic activity of the compounds under study than the compounds bearing electron releasing groups.

Table 3. Antiamoebic activity of compounds 5a – 5h against *Entamoeba histolytica*.

Compound	R	Antiamoebic activity	
		IC_{50} (µM)	S.D (±)
5a	4-Cl	**1.05**	0.17
5b	2-Cl	3.02	0.12
5c	3,4-OCH_3	**1.08**	0.10

5d	4-NO_2	**1.02**	0.14
5e	3-NO_2	2.63	0.13
5f	4-OCH_3	1.16	0.16
5g	4-OH	5.32	0.09
5h	4-OH,3- OCH_3	3.15	0.14
Standard	Metronidazole	1.70	0.10

CONCLUSION

In this investigation, a new series of tetrazolyl acetamides (**5a - 5h**) have been synthesized by substituting imidazole moiety of etanidazole with tetrazole and the resulting compounds were characterized on the basis of spectral data. All the compounds were evaluated for *in vitro* anti bacterial and antiprotozoal activities. Some of them have exhibited potent amoebicidal activity against *Entamoeba histolytica* and anti bacterial activity against both gram positive and gram negative organisms. This study suggests that tetrazolyl acetamides may serve as promising scaffolds for design of new anti protozoal and anti bacterial agents.

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REFERENCES

1. Koldobskii GI, Kharbash RB. 2-Substituted and 2, 5-disubstituted tetrazoles. *Russian J. Org. Chem*, 2003; 39(4): 453-470.
2. Israili ZH. Clinical pharmacokinetics of angiotensin II (AT1) receptor blockers in hypertension. *J. Hum. Hypertension*, 2000; 14: S73-S86.
3. Master HE, Khan SI, Poojari KA. Design and synthesis of low molecular weight compounds with complement inhibition activity. *Bioorg. Med. Chem*, 2005; 13: 4891-4899.
4. Herr RJ. 5-Substituted-1H-tetrazoles as carboxylic acid isosteres: medicinal chemistry and synthetic methods. *Bioorg. Med. Chem*, 2002; 10: 3379-3393.
5. Rajasekaran A, Thampi PP. Synthesis and antinociceptive activity of some substituted-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}alkanones. *Eur. J. Med. Chem*, 2005; 40: 1359- 1364.

6. Makara GM, Keseru GM. On the conformation of tiazofurin analogues. *J. Med. Chem*, 1997; 40: 4154-4159.
7. Holy A, Phosphonylmethyl analogs of nucleotides and their derivatives chemistry and biology. *Nucleosides Nucleotides*, 1987; 6: 147.
8. Remy RJ, Secrist JA. Acyclic nucleosides other than acyclovir as potential antiviral agents. *Nucleosides Nucleotides*, 1985; 4: 411.
9. Buckler RT, Hayao S, Lorenzetti OJ, Sancilia LF. Synthesis and anti-inflammatory activity of some aryltetrazolylalkanoic acids. *J. Med. Chem*, 1970; 13: 725-729.
10. Baker Jawabrah Al-Hourani, Sai Kiran Sharma, Mavanur Suresh. Frank Wuest Novel 5-substituted 1*H*-tetrazoles as cyclooxygenase-2 (COX-2) inhibitors. *Bioorg. Med. Chem. Lett*, 2012; 22: 2235-2238.
11. Adnan A. Bekhit a, Ola A. El-Sayed, Elsayed Aboulmagd, Ji Young Park. Tetrazolo [1,5-*a*] quinoline as a potential promising new scaffold for the synthesis of novel anti-inflammatory and antibacterial agents. *Eur J. Med. Chem*, 2004; 39: 249-255.
12. Katritzky AR, Jain R, Petrukhin R, Denisenko S, Schelenz T. QSAR correlations of the algistatic activity of 5-amino-1-aryl-1H-tetrazoles. *SAR QSAR Environ. Res*, 2001; 12: 259-266.
13. Gundugola AS, Chandra KL, Perchellet EM, Waters AM, Rayat S. Synthesis and antiproliferative evaluation of 5-*oxo* and 5-thio derivatives of 1,4-diaryl tetrazoles. *Bioorg Med. Chem. Lett*, 2010; 20: 3920-3924
14. Thomas MB, Daniel MA, Andreas B, Peter JB. A-ring dihalogenation increases the cellular activity of combretastatin - templated tetrazoles. *Med. Chem. Lett*, 2012; 3: 177-181.
15. Mohmmad YW, Abdul RB, Amir A, Dae HL, Inho C, Fareeda A. Synthesis and *in vitro* evaluation of novel tetrazole embedded 1,3,5-trisubstituted pyrazoline derivatives as Entamoeba histolytica growth inhibitors. *Eur. J. Med. Chem*, 2012; 54: 845-854.
16. Swetha KS, Parameshwar R, Reddy BM, Babu VH. Synthesis of novel pyrazolyl tetrazoles as selective COX-2 inhibitors. *Med. Chem. Res*, 2013; 22(10): 4886-4892.
17. Gunasekaran P. Laboratory manual in microbiology, 1st edition, New age international, New Delhi, 1995; 39-42.
18. Indian Pharmacopoeia. Ministry of health and family welfare, New Delhi, 1996; 2: A-105.

19. National committee for clinical laboratory standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A4. Wayne, Pa, USA: National Committee for Clinical Laboratory Standards; 2000.
20. Wright CW, O'Neill MJ, Phillipson JD, Warburst DC. Use of microdilution to assess in vitro antiamoebic activities of Brucea javanica fruits, Simarouba amara stem and a number of quassinoids. *Antimicrob. Agents. Chemother*, 1988; 32: 1725-1729.