Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2014; 3(6): 432-437 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Evaluation of Some Novel Synthesised 2-Amino-1,3,4-Thiadiazole Derivatives

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Abstract: A series of 1, 3, 4-thiadiazole derivatives had been synthesized from different Polyhydroxylated aldehydes and ketones. The structures were characterized by IR, ^{1}H NMR and ^{13}C NMR spectral data. The compounds were evaluated for antibacterial activity against gram-positive and gram-negative bacteria. Insilco evaluation was carried, aiming to present potential selective activities as enzyme inhibitors. These activities were suggested by the score values using Molinspiration Cheminformatics program.
Keywords: Enzyme inhibitors, 1, 3, 4-thiadiazole, Molinspiration Cheminformatics program.

INTRODUCTION

Thiadiazoles constitute a class of heteroaromatic compound containing two heteroatoms (sulphur and nitrogen). This structural moiety (Fig1) is found in natural products and has been used as an essential skeleton in pharmaceutics and many medicinal compounds. Efficient and versatile synthetic methods for producing 1, 3, 4-Thiadiazole derivatives have been actively investigated.

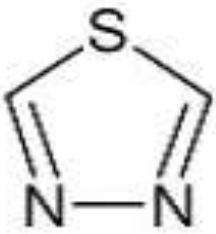


Fig-1: Structure of 1, 3, 4-Thiadiazole

Even if efficient synthetic methods for producing 1, 3, 4-Thiadiazole derivatives have been continuously investigated[1]. Many synthetic methods require an organized thiosemicarbazide derivative with functionalized N-alkynyl or aryl groups. Therefore, the investigation of an alternative method having various functional group variations on 1, 3, 4-Thiadiazole nucleus is highly desirable for biological activity studies[2].

Curiously, according to our knowledge, it is not described in literature efficient 1, 3, 4- thiadiazole derivative synthesis by green chemistry. In a recent work, a standard method for the preparation of 1, 3, 4-thiadiazoles is dehydrative cyclization of acyl thiosemicarbazide[3]. Different acidic reagents have been used for dehydration e.g. sulphuric acid, phosphoric acid, acetic anhydride and phosphorus halides. In connection with our interest in the new bioactive compound synthesis our group decided to investigate the green chemistry use on synthetic methodologies development to obtain new 1, 3, 4-Thiadiazole derivatives. The structures synthesized in this work are shown in (Table-1)[4]. The Molinspiration Cheminformatics program presents specific activity scores for each of these six receptor classes (GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor). Using this program, it is also possible to calculate the Lipinski's rule of five proprieties[5] Molinspiration Cheminformatics virtual screening methodology can efficiently separate drug-likeness from inactive structures[6]

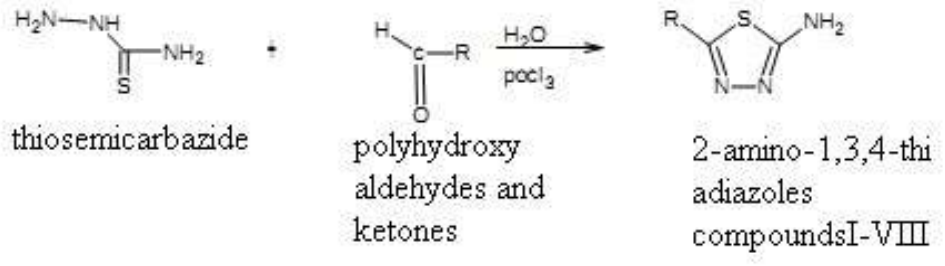
MATERIALS AND METHODS

The chemicals used for the synthesis were supplied by LOBA chemicals and used as received. Purity of the compounds was checked on thin layer chromatography (TLC) plates (Silica Gel G) using the solvent systems ethyl acetate: hexane (1:1). The spots were identified in iodine chamber. Melting points were determined on GallenKamp (MFB-600) melting point apparatus an were uncorrected. The IR spectra of the compounds were recorded on a shimadzu FT-IR-8300 Spectrometer as KBr disk. The ^{1}H-NMR and ^{13}C-NMR spectra (solvent DMSO-d6) were recorded on Bruker 400δMHz spectrophotometer using TMS as internal standard.

Experimental work:

General procedure for the synthesis of Thiadiazole:

Thiosemicarbazide (0.01moles), Polyhydroxylated aldehydes or ketones (0.01moles) were dissolved in minimum amount of water. To the mixture phosphorous oxychloride was added drop by drop. The temperature of the mixture rises spontaneously. The beaker was shaken for 15 min, and then cooled. A white crystalline product separates out. The product was filtered, washed with water, dried and recrystallized from water. The brief illustration of the scheme was represented in scheme-1.the general properties were mentioned in Table-1.



Scheme1

Table-1: General Properties

S .NO	Preferred IUPAC Name	M.W	Melting Point	ElementalComposition
I	1-(5-amino1,3,4Thiadiazol--2yl)pentane1,2,3,4,5-pentol	251.26	140-180^{o}C	C (33%), H (5.2%), N (16.0%) O (31.84%), S (12.76%)
II	1(5-amino1,3,4thiadiazol2-yl) pentane-1,2,3,4,5pentol	251.26	158-198^{0}C	C(33.46%),H(5.22%),N(16.7%) O (31.84%), S (12.76%)
III	1(1,3,4thiadiazol2yl)pentane1,2,3,4,5-pentol	251.26	140-150^{0}C	C(35.59%),H(5.12%)N(11.86%) O (33.86%),S (13.57%)
IV	1(5a min o1,3,4thiadiazol2yl) butane1,2,3,4-tetrol	221.23	150-170^{0}C	C(32.57%),H(5.01%),N(18.99%) O (28.93%),S (14.49%)
V	1(5a min o1,3,4 thiadiazol2yl)butane1,2,3,4-tetrol	221.23	130-140^{o}C	C(32.57%),H(5.01%),N(18.99%) O (28.93%),S (14.49%)
VI	1(5amino-1,3,4thiadiazol2-yl)3 {[3,4,5trihydroxy6(hydroxymethyl) oxan2yl]oxy}pentane1,2,4,5-tetrol	413.405	140^{o}C - degraded	C (37.77%), H (5.61%) N(10.16%),O (38.7%) S (7.76%)
VII	5(5a min o-1,3,4thiadiazol2-yl)5 {[3,4dihydroxy2,5 bis(hydroxymethyl)oxolan2yl]oxy} pentane1,2,3, 4 tetrol	413.405	140170^{o} C	C(37.7%), H (5.61%)N (10.16%) O (38.7%),S (7.76%)
VIII	5(5a min o-1,3,4-thiadiazol-2-yl)5 {[4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxy}pentane-1,2,3,4-tetrol	413.405	150-185^{o}C	C(37.77%),H (5.61%)N (10.16%) O (38.7%),S (7.76%)

1- (5a mino1, 3, 4-thia diazol2yl) pent ane1, 2, 3, 4, 5-pentol (I):
IR Data: (KBr, V_{max}, Cm^{-1}) 3243, 3184 (N H2), 2936 (OH and H - bonding), 2360 (CH groups), 1507 (C=N), 1362 (CS bonds) ^{1}H MR(500MHZ,$CDCl_3$):4.84(CH(d)), 3.91(CH_2(dd)), 3.79(CH(m)), 3.30(OH(s)), 2.58(NH2(s)), 2.41(OH(s)); ^{13}C NMR (125MHZ,CDCl3): 157.84 , 154.63, 73.72,72.16,71.61,63.41.(basic ring carbons).

1- (5amino1, 3, 4 - thiadiazol2yl) pentane 1, 2, 3, 4, 5-pentol (II):
IR Data: (KBr, V_{max}, Cm^{-1}) 3250, 3145 (N H2), 2880 (OH and H-bonding), 2361 (CH groups), 1514 (C=N), 1361 (CS bonds). ^{1}H NMR(500MHZ,$CDCl_3$):4.84 (CH(d)), 3.91(CH2(dd)), 3.79(CH(m)), 3.30(OH(s)), 2.58(NH2(s)), 2.41(OH(s)); ^{13}C NMR (125MHZ,$CDCl_3$): 157.84 , 154.63, 73.72,72.16,71.61,63.41.(basic ring carbons).

1- (1, 3, 4-thiadiazol -2-yl) pent ane1, 2, 3, 4, 5-pentol (III):
IR Data: (KBr, V_{max}, Cm^{-1}) 3246, 3154 (N H2),2862 (OH and H- bonding),2359 (CH groups),1508 (C=N),1364 (CS bonds). ^{1}H NMR (500MHZ, $CDCl_3$): 6.22(NH_2(s)), 5.32(NH(s)), 5.12(OH(s)), 4.51(CH2 (d)) 3.92(CH (dd)), 2.36(OH(s)); ^{13}C NMR (125MHZ, CDCl3): 151.91, 82.38, 76.22, 72.87, 68.08, 63.91 (basic ring carbons).

1- (5a mino1, 3, 4 -t hiadiazol2yl) but ane1, 2, 3, 4-tetrol (IV):
IR Data: (KBr, V_{max}, Cm^{-1}) 3245, 3142 (N H2), 2905 (OH and H- bond in g), 2360 (CH groups), 1507 (C=N), 1360 (CS bonds). ^{1}H NMR(500MHZ,$CDCl_3$): 5.93(s)(OH), 5.33(s) (OH), 4.47(m)(CH), 4.23(m)(CH),3.85(m), (C=C),2.58(s)(NH2),2.15(s)(OH),; ^{13}C NMR (125MHZ,$CDCl_3$): 157.84, 155.29, 105.48, 82.63,77.61,72.93,74.7,68.65,63.15.(basic ring carbons).

1- (5a mino1, 3, 4 -thiadiazol2yl) but ane1, 2, 3, 4-tetrol (V):
IR Data: (KBr, V_{max}, $Cm^{-1)}$3244, 3141 (N H2), 2917 (OH and H- bond in g), 2360 (CH groups), 1498 (C=N), 1357 (CS bonds). ^{1}H NMR(500MHZ,$CDCl_3$): 5.93(s)(OH), 5.33(s) (OH), 4.47(m)(CH), 4.23(m)(CH),3.85(m), (C=C),2.58(s)(NH2),2.15(s)(OH),; ^{13}C NMR (125MHZ,$CDCl_3$): 157.84 , 155.29, 105.48, 82.63,77.61, 72.93,74.7, 68.65, 63.15.(basic ring carbons).

1- (5a mino1, 3, 4-thia diazol2yl) -3-{[3,4,5trihy droxy6 (hydroxy methyl) oxan2yl] oxy} pent ane-1,2,4,5tetrol(VI):
IR Data: (KBr, V_{max}, Cm^{-1})3243, 3138 (N H2), 2884 (OH and H - bonding), 2360 (CH groups), 1513 (C=N), 1359 (CS bonds). ^{1}H NMR(500MHZ,$CDCl_3$):5.33(OH(s), 4.945(OH(s)),4.47(CH2(d)), 3.69(CH(m)), 2.58(NH2(s)); ^{13}C NMR (125MHZ,CDCl3): 157.84,154.63, 106.40, 82.82,77.42, 73.96, 73.55, 62.47, 63.95.(basic ring carbons).

5- (5a mino1, 3, 4-thia diazol2yl) - 5-{[3, 4-di hydroxy 2, 5 -bis (hydroxy methyl) oxolan2yl] oxy} pent ane1, 2, 3, 4- tetrol (VII)
IR Data: (KBr, V_{max}, Cm^{-1})3245, 3134 (N H2), 2853 (OH and H - bonding), 2360 (CH groups), 1497 (C=N), 1359 (CS bonds). ^{1}H NMR(500MHZ,$CDCl_3$): 5.93(s)(OH), 5.33(s) (OH), 4.47(m)(CH), 4.23(m)(CH),3.85(m), (C=C),2.58(s)(NH_2), 2.15(s) (OH),;^{13}C NMR (125MHZ,$CDCl_3$): 157.84, 155.29, 105.48, 82.63,77.61, 72.93, 74.7,68.65,63.15.(basic ring carbons).

5-(5a mino1,3,4t hiadiazol2yl)5{[4,5,6t r ihydroxy2-(hydroxy methyl)oxan3yl] oxy pent ane1,2,3,4tetrol (VIII):
IR data : (KBr,V_{max}, Cm^{-1})3246, 3146 (N H2), 2991 (OH and H - bonding), 2360 (CH groups), 1506(C=N),1359(CSbonds).^{1}HNMR(500MHZ,$CDCl_3$) :5.93(s)(OH),5.33(s)(OH),4.47(m)(CH),4.23(m)(CH),3 .85(m),(C=C),2.58(s)(NH_2),2.15(s)(OH), ^{13}C NMR (125MHZ,$CDCl_3$):157.84, 155.29, 105.48, 82.63, 77.61,72.93, 74.7,68.65, 63.15. (basic ring carbons).

INSILICO ACTIVITY:

Molinspiration, web based software [7-8] was used to obtain parameter such as drug likeness and bioactive scores. Drug likeness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs following Lipinski rule of five [9-10]. Calculated drug likeness score of each compounds and compared with the specific activity of each compound, and the results were compared with standard drug. For organic molecules the probability is if the bioactivity score is (>0), then it is active, if (-5.0-0.0) then moderately active, if (< -5.0) then inactive [11-15]. The drug likeness score and the calculated value of various parameters of the isolated compounds (I-VIII) are in Table 2. The Bioactivity scores of the isolated compounds (I-VIII) were compared with standard drug on the basis of GPCR ligand, ion channel modulator, nuclear receptor legend, kinase inhibitor, protease inhibitor, enzyme inhibitor in Table-3.

Table-2: Molecular Properties

S .NO	Log p	TPS A	N atoms	M. wt	NON	NOHNH	N violation	Nrotb	vol
I	-3.29	152.94	16	251.26	8	7	1	5	201.97
II	-3.29	152.94	16	251.26	8	7	1	5	201.97
III	-3.15	151.55	16	251.26	8	8	1	5	207.55
IV	-2.85	132.71	14	221.23	7	6	1	4	177.08
V	-2.85	132.71	14	221.23	7	6	1	4	177.08
VI	-4.39	132.09	27	413.405	13	10	2	8	334.05
VII	-4.229	232.099	27	413.405	13	10	2	9	333.705
VIII	-4.7.32	232.099	27	413.405	13	10	2	8	333. 05

Table 3: Bioactive Scores

S.NO	GPCR ligand	Ion channel Modulator	Kina se inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
I	-0.75	-0.61	-0.57	-1.10	-0.54	0.04
II	-0.75	-0.61	-0.57	-1.10	-0.54	-0.04
III	-0.51	-0.19	-0.70	-0.94	-0.34	0.14
IV	-1.01	-0.80	-0.83	-1.43	-0.81	-0.23
V	-1.01	-0.80	-0.83	-1.43	-0.81	-0.23
VI	-0.16	-0.29	-0.12	-0.49	0.00	0.25
VII	-0.06	-0.39	0.03	-0.57	-0.16	0.34
VIII	-0.13	-0.35	-0.13	-0.52	-0.07	0.31
STD	-0.14	-1.53	-0.69	-0.82	0.7	0.13

ANTIBACTERIAL ACTIVITY

Cup plate method [16-18] using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of compound I to VIII against Escherichia coli ,Staphylococcus aureus, Pseudomonas aeruginos and Bacillus subtilis. The agar media was purchased from HI-media laboratories limited, Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure .Each test compound (5mg) was dissolved in 5ml of dimethyl formamide. Benzyl Penicillin was employed as reference standard (1000µg/ml) to compare the results. All the compounds were tested at a concentration of 0.15ml (150µg) level and DMF as control did not show any inhibition. The medium was inoculated at one percent level using 18hrs old cultures of the test organism mentioned above aseptically into sterile Petri dishes and allowed to set at room temperature for about 30 minutes. The test and standard solutions were added into cups, left for 90 minutes in a refrigerator for diffusion. After incubation for 24 hours at 37 ^{0} c, the plates were examined for inhibition zones. The results were represented in Table 4

Table: 4 Antibacterial Activity

Zone of Inhibition in mm Per mg of compound				
COMPOUND	B.subtilis	E. coli	P. aeruginos	S. aureus
1	17	16	15	12
2	16	16	16	13
3	16	17	18	13
4	16	17	14	12
5	15	18	10	15
6	15	14	12	13
7	16	17	12	14
8	19	19	15	15
Std	20	20	18	19

RESULTS AND DISCUSSIONS

Insilico Activity

All the compounds (I-VIII) fulfil Lipinski's rule and show good drug likeness score (Table 2). Milog P < 5. TPSA< 160 Å2, n violations = 0 it means compound easily bind to receptor, molecular mass <500, nrotb < 5, No. hydrogen bond donors ≤ 5 (The sum of OHs and NHs), No. hydrogen bond acceptor ≤ 10 (The sum of Os and Ns). Compound I - VIII were taken further calculation of bioactivity score from Table 3. Compounds III, VI, VII andVIIIshowedgoodbioactivityscore.CompoundVI I[5(5amino1,3,4thiadiazol2yl)5{[3,4dihydroxy2,5bis(hy droxymethyl)oxolan2yl]oxy}pentane1,2,3,4tetrol]show ed good drug likeness score and bioactivity score, on comparison with other compounds.

Antibacterial Activity:

The synthesized compounds were also screened for their antibacterial activity against Escherichia coli (ATTC-25922), Staphylococcus aureus (ATTC-25923), Pseudomonas aeruginosa (ATCC-27853), and Bacillus subtilis (recultured) bacterial strains by cup plate method. Benzyl penicillin was used as standard antibiotic for antibacterial activity. The results infer that the compounds exhibited moderate antibacterial activity. of all the eight compounds synthesized, compoundsVII and compound VIII showed significant antimicrobial activity. Among gram positive and gram negative bacteria, the compounds are more sensitive towards gram positive microorganisms. Compound III showed highest activity against pseudomonas aeuroginosa.

CONCLUSION

The work represents a simpler and efficient synthetic method for thiadiazoles from polyhydroxlated carbonyl compounds by using water as a solvent considered as green solvent .the results from insilico and invitro antibacterial evaluation infers that deritivatives synthesised from Polyhydroxylated ketones , presence of electron donating substituents on the ring and increased substitution of the ring leads to significant increase in the activities.this work may further serve for the development of thiadiazole moiety as a lead molecule.

ACKNOWLEDGEMENTS

The authors are thankful to the Siddhartha Academy for General and Technical Education for providing necessary facilities to carry out this research work.

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