ISSN 2347-3614

UNIQUE JOURNAL OF PHARMACEUTICAL AND BIOLOGICAL SCIENCES

Available online: www.ujconline.net

Research Article

SYNTHESIS AND ANTITUMOR ACYIVITY OF NEW 2-(PYRIDIN-4-YL) THIAZOLO [3, 2-B][1,2,4]TRIAZOL-6(5H)-ONE DERIVATIVES

Mruthyunjaya JH[1]* and Gopalakrishna B[2]

[1]Deparment of Pharmaceutical Chemistry, Bapuji Pharmacy College, Davangere, Karnataka (India) – 577004
[1]Vinayaka mission's University, Salem, Tamilnadu, (India) - 636308
[2]Deparment of Pharmaceutical Chemistry, R.R. College of Pharmacy, Bangaluru, Karnataka (India)- 560 090

Received 06-07-2013; Revised 12-08-2013; Accepted 11-09-2013

*Corresponding Author: **J.H. Mruthyunjaya**
Deparment of Pharmaceutical Chemistry, Bapuji Pharmacy College, Davangere, Karnataka. (India) – 577004, Email: jhmruthyunjaya@gmail.com

ABSTRACT

The synthesis of proposed biheterocyclic system was carried by using Isoniazide as starting material, which was converted in to 5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol **(INHTZ)** via 2-isonicotinoylhydrazine-1-carbothioamide by reacting with potassium thiocyanate and followed cyclisation with sodium hydroxide as catalyst upon refluxing in alcoholic medium. The 5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol **(INHTZ)** was converted into 2-(pyridin-4-yl) thiazolo [3, 2-b][1,2,4]triazol-6(5h)-one (TrTh 1-12) by one step multi-component reaction (via thioaceticacids followed by cyclisation in to 2-(pyridin-4-yl)thiazolo[3,2-b][1,2,4]triazol-6(5H)-ones which were not isolated) by refluxing for 8 hrs with chloro acetic acid in the presence of sodium acetate, acetic anhydride and glacial acetic acid in alcoholic medium. The synthesized compounds were characterized by physicochemical and analytical data and evaluated for *in-vitro* anticancer activity against the two cell lines, some of the derivatives showed excellent *in-vitro* cytotoxicity.

Keywords: Anticancer activity, cytotoxicity, MTT assays, Synthesis, Thiazoles, Triazoles.

INTRODUCTION

Substituted triazoles have received considerable attention during last two decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. A literature survey indicates that triazole derivatives possess different pharmacological and biological activities, of which the most potent is anti-microbial, analgesic, anti-inflammatory and anti-tubercular activities[1-5]. Compounds which contain two fused ring of thiazole and triazole, thiazolo triazoles, also show significant biological and pharmacological properties like anti-inflammatory, antitumor, analgesic, antipyretic & antimicrobial activities[6-12]. On the basis of the above mentioned reports the present work is concerned with the synthesis of fused thiazolo triazole derivatives with objective of discovering novel & potent anti-cancer agents that might be devoid of harmful side effects.

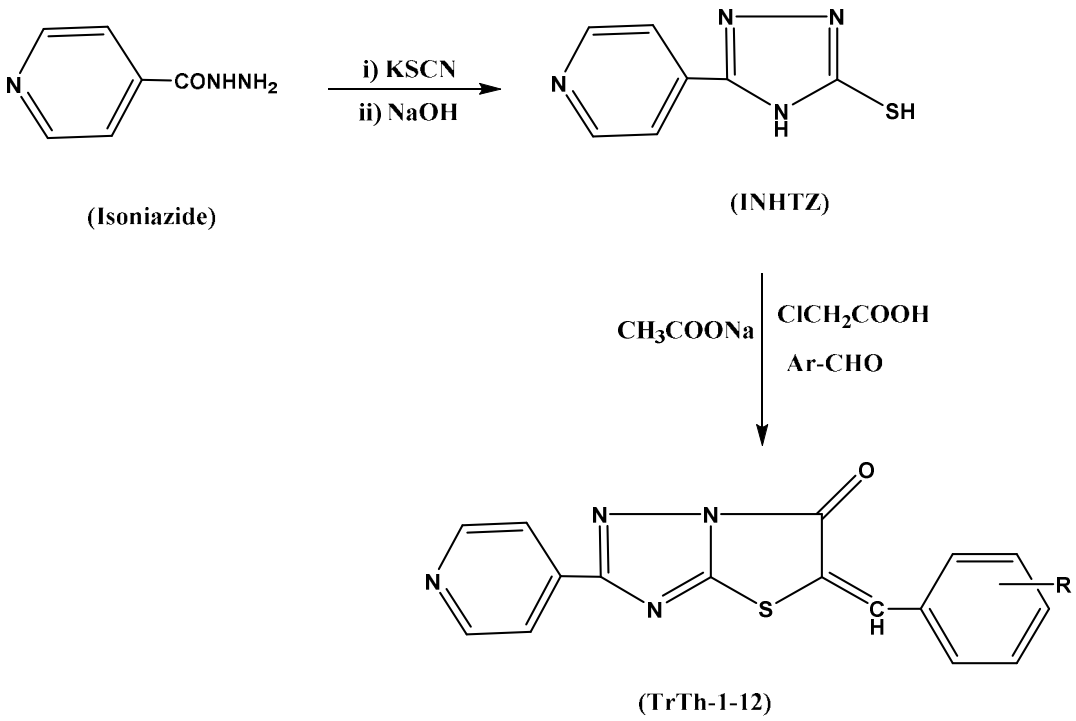
MATERIALS AND METHODS

The reagent grade chemicals were obtained from commercial sources and were purified by either recrystallization or distillation before use. Melting points were determined by using Toshniwal apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using chloroform: ethyl acetate (7:3) as solvent system and U.V lamp used as a visualizing agent. IR spectra were recorded KBr pellets on a Shimadzu 8000 series spectrophotometer. ^{1}H-NMR spectra on a Bruker/Varian Avance 400 MHz spectrophotometer using DMSO-d_6 as a solvent and TMS as internal standard (chemical shift values expressed in δ ppm). LC-MS spectra were recorded on a Shimadzu 2010A series spectrometer.

SYNTHETIC PROCEDURE: The general scheme for the synthesis of 2-(pyridin-4-yl)thiazolo[3,2-*b*][1,2,4]triazol-6(5*H*)-one derivatives has been represented in Figure 1.

Procedure for the preparation of 5-(pyridin-4-yl)-4H-1, 2, 4-triazole-3-thiol (INHTZ):

To a solution of isonicotinic acid hydrazide (1mmol) in absolute alcohol (50 ml) was added potassium thiocyanate (1mmol) and the reaction mixture was refluxed for 12 h. After the completion of reaction (monitored by TLC) the reaction mixture was cooled to room temperature and sodium hydroxide solution (2N, 10 ml) was added portion wise to the reaction mixture and further refluxed for 8 h. After the completion of reaction (monitored by TLC) the reaction mixture was cooled to room temperature and the solution was neutralized with hydrochloric acid. The precipitated solid was filtered, washed thoroughly with water, dried and recrystallised from alcohol, the yield was 80% and the melting point was 304-306°C.



R	1	2	3	4	5	6	7	8	9	10	11	12
	H	4-OH	4-Cl	3-Cl	2-Cl	4-F	2-F	4-OCH_3	2-NO_2	3-NO_2	4-NO_2	4-$N(CH_3)_2$

1: General Scheme for the Synthesis of 2-(pyridin-4-yl) thiazolo [3, 2-*b*][1,2,4]triazol-6(5*H*)-ones (TrTh 1-12)

General method for the preparation of 2-(pyridin-4-yl) thiazolo [3,2-b][1,2,4]triazol-6(5H) ones (TrTh 1-12):

To a solution of 5-(pyridin-4-yl)-4H-1 2 4-triazole-3-thiol **(INHTZ)** (1mmol) in absolute alcohol (50 ml) was added monochloroacetic acid (1.5 mmol), appropriate benzaldehyde (0.01 mol), acetic anhydride (1 ml), anhydrous sodium acetate (0.01 mol) and 2 ml glacial acetic acid and refluxed for 8 h. The reaction mixture was cooled, and poured onto crushed ice. The mixture was then allowed to reach room temperature, then filtered and washed with water to obtain a crude product. The resulting solid was collected and were recrystallized from ethanol. The physical characteristic data was given in table-1.

Compound- TrTh-1: ^{1}H NMR ($DMSO.d_6$) δ: 8.8-9.0 (2H, m, 2H of pyridine ring), 8.3-8.4 (1H, s, 1H of CH=C group), 7.5-8.2 (7H, m, 5H of Ar-H and 2H of pyridine ring): **^{13}C NMR** (400 MHz, $DMSO-d_6$): δ 190.0, 171.0, 150.0, 147.0,140.5, 130.9, 129.7, 124.0, 117.0, 116.0 and 115.2: **MS** (m/z): M^{+1}= 307.

Compound- TrTh-3: ^{1}H NMR ($DMSO.d_6$) δ: 8.7-8.8 (2H, m, 2H of pyridine ring), 8.3-8.4 (1H, s, 1H of CH=C group), 7.5-8.2 (6H, m, 4H of Ar-H and 2H of pyridine ring): **^{13}C NMR** (400 MHz, $DMSO-d_6$): δ 192.0, 171.0,170.0, 155.0, 148.0, 131.0, 125.0, 120.0, 117.0, 115.0, and 105.2: **MS** (m/z): M^{+1}= 341.

Compound- TrTh-6: ^{1}H NMR ($DMSO.d_6$) δ: 8.6-8.8 (2H, m, 2H of pyridine ring), 8.1-8.2 (1H, s, 1H of CH=C group), 7.1-8.0 (6H, m, 4H of Ar-H and 2H of pyridine ring): **^{13}C NMR** (400 MHz, $DMSO-d_6$): δ 188.0, 176.0, 170.0, 147.0,142.0, 132.0, 130.0, 128.0, 126.0, 115.0 114.0, and 104.0:**MS** (m/z): M^{+1}= 324.

Compound- TrTh-8: ^{1}H NMR ($DMSO.d_6$) δ: 8.8-9.0 (2H, m, 2H of pyridine ring), 8.3-8.4 (3H, m, 1H of CH=C group and 2H of Ar-H), 7.1-7.8 (4H, m, 2H of Ar-H and 2H of pyridine ring), 3.9-4.0 (3H, s, 3H of OCH_3): **^{13}C NMR** (400 MHz, $DMSO-d_6$): δ 188.0, 178.0, 164.0,162.0, 154.0, 150.0, 138.0, 135.0, 124.0, 120.0, 110.0, 105.0 and 58.0 : **MS** (m/z): M^{+1}= 336.

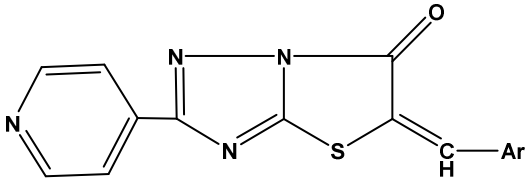
Compound- TrTh-12: ^{1}H NMR ($DMSO.d_6$) δ: 8.8-9.0 (2H, m, 2H of pyridine ring), 8.1-8.3 (3H, m, 1H of CH=C group and 2H of Ar-H), 6.8-7.7 (4H, m, 2H of Ar-H and 2H of pyridine ring), 3.1-3.3 (3H, s, 6H of 2xN-CH_3): **^{13}C NMR** (400 MHz, $DMSO-d_6$): δ 185.0, 172.0, 170.0, 154.0, 150.0, 139.0, 138.0, 134.0, 130.0, 117.0, 110.0, and 58.0: **MS** (m/z): M^{+1}= 349.

***IN VITRO* CYTOTOXICITY ASSAY**

The cytotoxicity of the newly synthesized compounds was evaluated by MTT assay according to the Mossman's method [13] against Human embryonic kidney 293 cell line (*HEK293*), colon carcinoma HT-29 cell lines. The MTT assay is based on the reduction of the soluble 3-(4, 5-methyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) into a blue-purple formazan products, mainly by mitochondrial reductase activity inside living cells. The cells used in cytotoxicity assay were cultured in MEM media supplemented with 10 % fetal bovine

serum. Cells suspended in the medium (104 cells/well in 100 µl) were placed in 96-well culture plates and incubated at 37 ^{o}C in a 5 % CO_2 incubator. After 12 h, the test sample (10, 20 and 30 µG) was added to the cells (104cells/well in 100 µl) in 96-well plate and cultured at 37oC for 3 days. The cultured cell were mixed with 100 µL of MTT solution and incubated for 4 h at 37 ^{o}C. The supernatant was carefully removed from each well and 100 µL of DMSO were added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by an ELISA micro plate reader using a test wavelength of 490 nm. The results were expressed as the IC_{50}, which inducing a 50% inhibition of cell growth of treated cells when compared to the growth of control cells. The drug concentration that causes 50% cell growth inhibition after 72 h of continuous exposure to the test compounds (IC_{50}) was determined by plotting the graph of concentration of the drug against the percent cytotoxicity.

TABLE-1: Physical Characteristic data of 2-(pyridin-4-yl) thiazolo [3,2-*b*][1,2,4] triazol-6(5*H*)-ones (TrTh 1-12)



(TrTh-1-12)

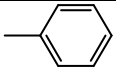
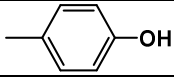
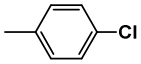
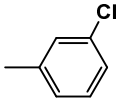
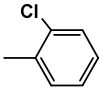
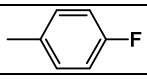
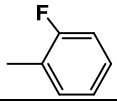
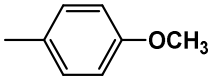
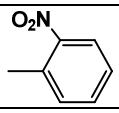
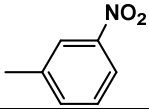
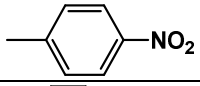
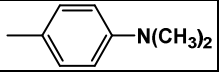
Sl. No	Compound Code	Ar	Mol. Formula	Mol.Wt. (gms)	% Yield	Melting Point (^{o}C)
1	TrTh-1		$C_{16}H_{10}N_4OS$	306	68	220
2	TrTh-2		$C_{16}H_{10}N_4O_2S$	322	50	295
3	TrTh-3		$C_{16}H_9N_4OSCl$	340	71	263
4	TrTh-4		$C_{16}H_9N_4OSCl$	340	56	225
5	TrTh-5		$C_{16}H_9N_4OSCl$	340	65	282
6	TrTh-6		$C_{16}H_9FN_4OS$	324	70	249
7	TrTh-7		$C_{16}H_9FN_4OS$	324	73	275
8	TrTh-8		$C_{17}H_{12}N_4O_2S$	336	72	249
9	TrTh-9		$C_{16}H_9N_5O_3S$	351	64	260
10	TrTh-10		$C_{16}H_9N_5O_3S$	351	55	265
11	TrTh-11		$C_{16}H_9N_5O_3S$	351	62	248
12	TrTh-12		$C_{18}H_{15}N_5OS$	349	65	229

Table 2: Cytotoxicity data of synthesized compounds (TrTh 1-12) against HT-29 cell lines.

Sl. No.	Compound	Concentration (µG)	% of inhibition	IC_{50}
1	**TrTh-1**	10	60.75	8.25 µg
2		20	71.38	
3		30	75.22	
4	**TrTh-2**	10	27.29	29.5 µg
5		20	34.11	
6		30	51.06	
7	**TrTh-3**	10	77.59	6.20 µg
8		20	85.43	
9		30	86.32	
10	**TrTh-4**	10	11.43	-----
11		20	14.83	
12		30	31.54	
13	**TrTh-5**	10	31.80	28.10 µg
14		20	32.71	
15		30	54.24	
16	**TrTh-6**	10	52.54	8.40 µg
17		20	65.13	
18		30	71.56	
19	**TrTh-7**	10	No lysis`	-----
20		20	No lysis`	
21		30	13.35	
22	**TrTh-8**	10	88.42	5.74 µg
23		20	91.38	
24		30	91.71	
25	**TrTh-9**	10	12.14	-----
26		20	32.35	
27		30	41.85	
28	**TrTh-10**	10	15.24	-----
29		20	26.85	
30		30	44.35	
31	**TrTh-11**	10	1.93	-----
32		20	5.24	
33		30	18.64	
34	**TrTh-12**	10	40.74	15.9 µg
35		20	56.37	
36		30	62.95	
37	**Control**	-	No lysis`	-

RESULTS AND DISCUSSION

The synthetic strategies adopted for the preparation of intermediate and targeted compounds are depicted in scheme 1. The intermediate 5-(pyridin-4-yl)-4H-1, 2, 4-triazole-3-thiol (INHTZ) was synthesized by treating isonicotinic acid hydrazide with potassium thiocyanate in absolute alcohol under reflux for 12 h and followed by cyclisation with sodium hydroxide. The 5-(pyridin-4-yl)-4H-1, 2, 4-triazole-3-thiol (INHTZ) was further converted smoothly in final compounds by treating with monochloroacetic acid, appropriate benzaldehyde, , anhydrous sodium acetate (0.01 mol) acetic anhydride and glacial acetic acid under reflux for 8 h.

In vitro MTT Cytotoxic activity of synthesized compounds was evaluated for their in vitro cytotoxic activity by the standard MTT (3-(4, 5-methyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide) assay method against a panel of two human tumor cell lines namely; embryonic kidney 293 cell line (*HEK293*), colon carcinoma HT-29 cell lines. The results of in vitro cytotoxic activity were presented in table 2 and 3 as IC_{50} (µg) value, which is the inhibitory concentration of the compounds, which cause inhibition of 50% the cells in 24h. The obtained data revealed that, the two tested human tumor cell lines exhibited variable degree of sensitivity profiles towards the tested compounds. The in vitro cytotoxic activity data against HT-29 cell line reveals that the compounds TrTh-1, TrTh-3, TrTh-6 and TrTh-8 exhibited outstanding potential as evidenced from their IC_{50} values 8.25, 6.20, 8.40 and 5.74 µg respectively. Where as the compounds TrTh-2, TrTh-5 and TrTh-12 displayed pronounced sensitivity with IC_{50} values 28.1, 29.5 and 15.9 µg respectively. While rest of the

compounds showed insignificant activity. Among the tested compounds, the *HEK293* cell lines showed pronounced sensitivity against compounds, TrTh-3, TrTh-6 and TrTh-8 with IC_{50} values of 6.40, 9.60 and 5.87 µg respectively. Moreover a remarkable cytotoxic potential was displayed by compounds TrTh-1 and TrTh-12 against the same cell line (14.9 and 18.4 µg) and rest of the compounds showed insignificant activity. In particular, compounds TrTh-1, TrTh-3, TrTh-6, TrTh-8 and TrTh-12 proved to be the most active members in this study with a broad spectrum of activity against the tested cell lines. A close examination of the structure of the active compounds showed that they contains electron donating groups like OH, OCH_3, $N(CH_3)_2$ etc. The differences in the IC_{50} values may be attributed to factors such as substitution on the phenyl ring attached to thiazole nucleus, genetic and biochemical background of the cell lines.

Table 3: Cytotoxicity data of synthesized compounds (TrTh 1-12) against HEK-293 cell lines.

Sl. No.	Compound	Concentration (µg)	% of inhibition	IC_{50}
1	**TrTh-1**	10	38.39	14.9 µg
2		20	62.38	
3		30	62.87	
4	**TrTh-2**	10	10.86	----
5		20	32.90	
6		30	46.75	
7	**TrTh-3**	10	79.04	6.40 µg
8		20	79.39	
9		30	80.22	
10	**TrTh-4**	10	2.69	----
11		20	29.58	
12		30	37.98	
13	**TrTh-5**	10	No lysis`	----
14		20	No lysis`	
15		30	12.35	
16	**TrTh-6**	10	54.14	9.60 µg
17		20	67.12	
18		30	78.75	
19	**TrTh-7**	10	No lysis`	----
20		20	No lysis`	
21		30	No lysis`	
22	**TrTh-8**	10	84.14	5.87 µg
23		20	87.12	
24		30	88.75	
25	**TrTh-9**	10	No lysis`	----
26		20	15.68`	
27		30	31.55`	
28	**TrTh-10**	10	3.52	----
29		20	12.62`	
30		30	20.30	
31	**TrTh-11**	10	5.68`	----
32		20	21.55`	
33		30	34.12	
34	**TrTh-12**	10	32.41	18.4 µg
35		20	53.17	
36		30	54.89	
37	**Control**	----	No lysis	----

CONCLUSION

Novel class of Triazolo Triazines were synthesized and characterized for their structure activity relationship. Cytotoxicity studies of these compounds indicated that the compounds TrTh-1, TrTh-2, TrTh-3, TrTh-5, TrTh-6, TrTh-8 and TrTh-12 were found to be the most active anticancer compounds. Compounds TrTh-1, TrTh-3, TrTh-6, TrTh-8 and TrTh-12 were found to have broad spectrum of activity against the tested cell lines.

ACKNOWLEDGEMENTS

The authors are thankful to the principal and management of Bapuji College of Pharmacy, Davangere for providing the

necessary facilities to carry out the research work. The authors are highly indebted to the SAIF Punjab University, Chandigarh for providing NMR Spectra. The authors express sincere thanks to Dr. Kishore G. Bhat, Maratha Mandal Dental College Belgaum for their help in *in-vitro* cytotoxicity studies.

REFERENCES

1. Demirayaka S, Benklia K, Guven K. Eur. J. Med. Chem., 2000; 35: 1037-40.
2. Klimesovs V, Zahajska L, Waisser K, Kaustova J, Mollmann U., IL Farmaco., 2004; 59: 279-88.
3. Costa MS, Boechat N, Rangel EA, Silva FDCD, Souza AMTD, Rodrigues CR et al. Bioorg. Med. Chem., 2006; 14: 8644-53.
4. Ezabadi IR, Camoutsis C, Zoumpoulakis P, Geronikaki A, Sokovic M, Glamocilijad et al. J. Bioorg. Med. Chem., 2008; 16: 1150-61.
5. Bayrak H, Demirbas A, Karaoglu SA, Demirbas N. Eur. J. Med. Chem., 2009; 44: 1057-66.
6. Roy P, Leblanc Y, Ball RG, Brideau C, Chan CC, Chauret N, et al. Bioorg. Med. Chem. Lett., 1997; 7: 57.
7. Dodian E, Tozkoparan B, Kayanak FB, Eriksson L, Kupeli E, Yeilada E, et al. Arzneim. Forsch., 2007; 57: 196.
8. Tozkoparan B, Gokhan N, Aktay G, Yeailada E, Ertan M. Eur J. Med. Chem., 2000; 35: 743.
9. Lesyk R, Vladzimirska O, Holota S, Zaprutko L, Gzella A. Eur. J. Med. Chem., 2007; 42: 641.
10. Pignatello R, Mazzone S, Panico AM, Mazzone G, Pennisi G, Castana R, et al. Eur. J. Med. Chem., 1991; 26: 929.
11. Erol DD, Calia U, Demirdamar R, Yuluo N, Ertan M. J. Pharm. Sci., 1995; 84: 462.
12. El-sherif HAH, Mahmoud AM, Sarhan AAO, Hozien ZA, Habib OMA. J. Sulfur. Chem., 2006; 27: 65.
13. Mosmann T. J. Immunol. Meth., 1983; 65: 55.

Source of support: Nil, Conflict of interest: None Declared