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Synthesis, characterization and evaluation of new thiazole derivatives as anthelmintic agents

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A series of 2-amino substituted 4-phenyl thiazole derivatives has been synthesized by the conventional method. The thiazole derivatives have been synthesized by three steps. The obtained five derivatives have been purified by recrystallization process by using methanol as solvent and column chromatography [IVd Compound] and have been characterized by melting point, TLC, FTIR, ^{1}H NMR and mass spectral data. All the five derivatives have been evaluated using *in silico* studies by using different softwares (Lipinski's Rule of 5, OSIRIS molecular property explorer, Molsoft molecular property explorer, PASS and docking studies). These compounds have then been evaluated for anthelmintic activity against Indian adult earth worms (*Pheretima postuma*). All the compounds show significant anthelmintic activity. The compound IVc and IVe are shown to be potent compounds when compared with the standard drug (Mebendazole). Molecular docking studies have guided and prove the biological activity of the sythesised compounds against beta tubulin protein (1OJ0).

Keywords: Anthelmintic activity, *Pheretima postuma*, molecular docking, thiazole derivatives, β-tubulin protein

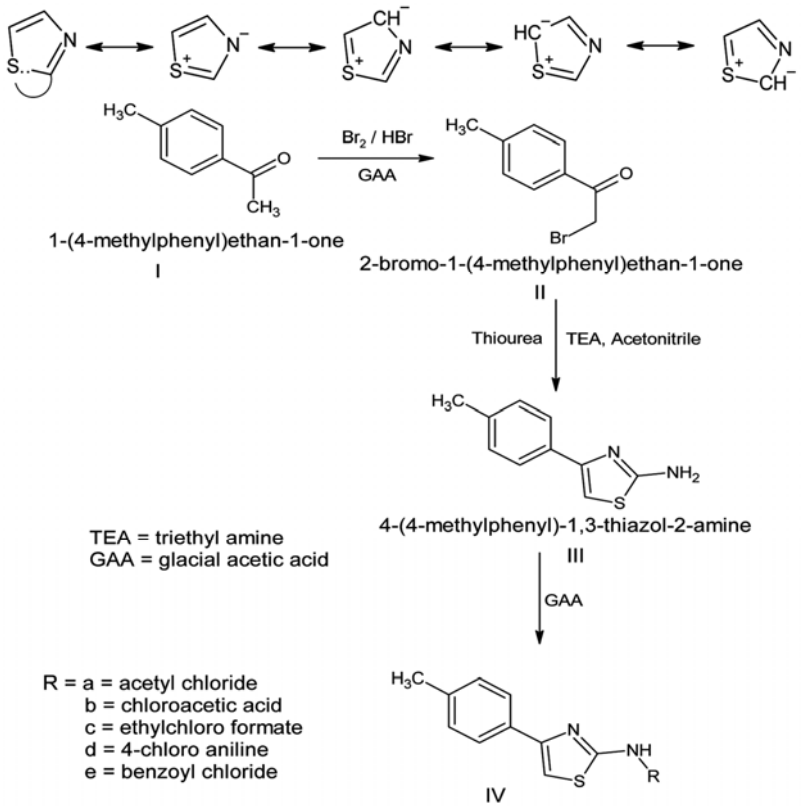
Helminthic infections are one of the World's long standing health problems in humans and domestic animals. We can recognize many of the characteristic clinical features of helminthes infections from the ancient writings of Hippocrates, Egyptian medical papyri, and the Bible. In recent past, several reports of failures in the treatment of human helminthes have been published and suspected for anthelmintic resistance (AR). AR is the most important disease problem faced by sheep-farming industry in Australia, South Africa. Even multiple-drug resistance is not uncommon in helminthes of veterinary importance. Helminthes are resistant to all available broad spectrum anthelmintics[1-5]. Considering the fact of AR, its potential threat and potential anthelmintic activity of thiazole derivatives, it was planned to synthesize new thiazole derivatives as anthelmintic drugs.

Thiazole is a five-membered heterocyclic ring with nitrogen and sulfur atom. Thiazole and related compounds are called 1,3-azoles (nitrogen and one other heteroatom in a five- membered ring). They are isomeric with the 1,2-azoles, containing nitrogen and sulfur atoms called isothiazole. Thiazole itself is a clear to pale yellow liquid with a boiling point of 116-118°C. Its specific gravity is 1.2 and it is sparingly soluble in water. It is soluble in alcohol and ether[6]. Thiazole is an aromatic ring on the basis of delocalization of a lone pair of electrons from the sulfur atom. The resonance forms of thiazole are shown in Scheme I. The thiazoles synthesized by using different techniques are from haloketones using halogen and thiourea[7], using NBS and thiourea[8], using oxidizing agent[9], using formamide disulfide dihydrobromide[10], from α-haloketones[11] (Scheme I).

Experimental Section

Chemicals used for the synthetic work were 4-methyl acetophenone, Bromine (Br_2), hydrobromic acid (HBr), glacial acetic acid, thiourea, thionyl chloride ($SOCl_2$), acetonitrile, acetyl chloride, chloro acetic acid, ethyl chloro formate, 4-chloro aniline, benzoyl chloride.

All the reactions were performed in the dried Borosil glass beakers, round bottomed flasks, conical flasks. Precoated silica gel plates (Merck) were used for TLC to monitor progress of the reaction. Compounds melting points were determined by capillary method and are uncorrected. JASCO UV chamber was used for detection of spots in TLC. IR spectra were recorded on Bruker FTIR spectrometer. ^{1}H NMR spectra were recorded on Bruker-400MHz spectrometer using DMSO-d_6 as solvent. The chemical shift data were expressed as values relative to TMS in δ (ppm).



Scheme I

In silico screening

Lipinski's rule of 5 filtration

The files were inserted in *.pdb, *.mol, *.mol2, *.xyz, *.sdf, or .smile formats. Care was taken to avoid whitespace(s) in the input file name. The window opened and the files were uploaded in the above mentioned formats. *p*H was adjusted from 0-14 as required. Upon submission, results were obtained.

OSIRIS property explorer (version 2)

OSIRIS Molecular Property Explorer version 2 (which requires JAVA platform to run) was used in the present study. The structure of designed molecule when drawn in or when pasted in smiles format shows the results at right side with colour coding. A green colour indicates non-toxic and red indicates toxicity (Figure 1).

Prediction of activity spectra for substances (PASS)

Molecules which have been filtered through Lipinski rule were subjected to online PASS software to predict their biological activities. The Pa and Pi values from 0.000 to 1.000. To define the threshold for selecting type of activity to be predicted.

Molsoft property explorer (version v.3.7-2)

Molsoft property explorer version v.3.7-2 was used in the present study. The structure when drawn directly on the window or when inserted in mol, Inch, smiles formats will calculate properties like MlogP, MlodS.

Docking (version 4.0)

AutoDock is a molecule modeling simulation software. It is especially effective for protein ligand docking. It has two versions Auto Dock 4.0, vina. Vina is a advanced version.

Synthesis of 2-bromo-1-(4-methylphenyl)-ethan-1-one, II

A round bottom flask was charged with substituted acetophenone (0.01 mol), glacial acetic acid (20 mL) and aqueous hydrobromic acid (HBr). The flask was immersed in an ice salt mixture (0-5°C) and bromine (0.01 mol) was added from the droping funnel at such a rate that the temperature of the reaction mixture does not exceeded 5°C. After completion of addition, it was stirred at RT for 2-3 hours. The suspension was poured onto crushed ice. The colourless precipitate

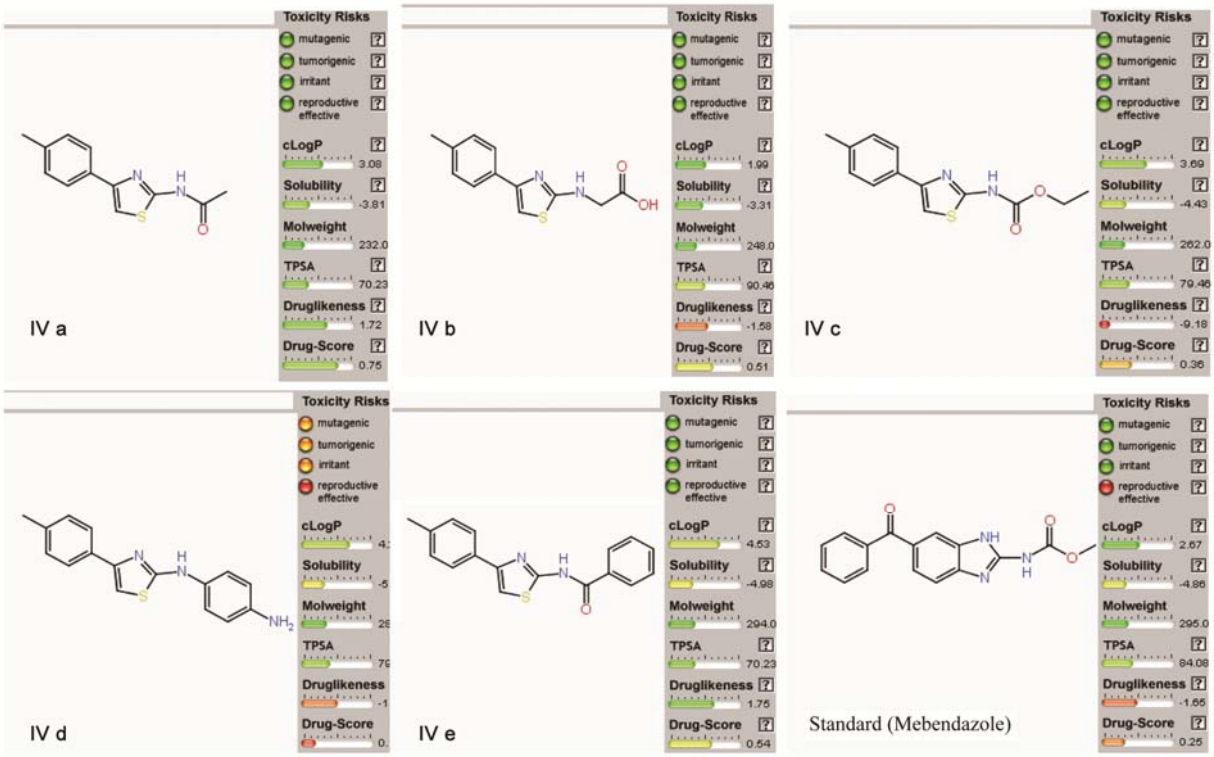


Figure 1 — OSIRIS molecular property explorer of compounds IVa-IVe and standard

was filtered, repeatedly washed with water and dried at RT[12].

Synthesis of 4-(4-methylphenyl)-1,3-thiazol-2-amine, III

A mixture of substituted pheacyl bromide (0.01 mol) of thiourea (0.01 mol) and of triethyl amine (0.01 mol) was refluxed in 20 mL of acetonitrile for 8-10 hours. The reaction mixture was then cooled, poured onto ice. The precipitate was filtered, dried and recrystallised from ethanol:water mixture (70:30)[12].

Synthesis of N-[4-(4-methylphenyl)-1,3-thiazol-2-yl] acetamide, IVa

A mixture of **2a** (0.01 mol) with acetyl chloride (0.01 mol) in glacial acetic acid (30 mL) was refluxed under stirring 5 hours, after that the mixture cooled at RT, then poured on crushed ice. The precipitate was collected by filtration and recrystallization from ethanol and gives orange to brown crystals.

Synthesis of {[4-(4-methylphenyl)-1,3-thiazol-2-yl] amido} acetic acid, IVb

A mixture of **2a** (0.01 mol) with chloro acetic acid (0.01 mol) in glacial acetic acid (30 mL) was refluxed under stirring 5 hours, after that the mixture cooled at RT, then poured on crushed ice. The precipitate was collected by filtration and recrystallization from ethanol and gives orange to brown crystals.

Synthesis of [4-(4-methylphenyl)-1,3-thiazol-2-yl] carbamate, IVc

A mixture of **2a** (0.01 mol) with ethyl chloro formate (0.01 mol) in glacial acetic acid (30 mL) was refluxed under stirring 5 hours, after that the mixture cooled at RT, then poured on crushed ice. The precipitate was collected by filtration and recrystallization from ethanol and gives orange to brown crystals.

Synthesis of N-[4-(4-methylphenyl)-1,3-thiazol-2-yl] benzene-1,4-diamine, IVd

A mixture of **2a** (0.01 mol) with 4-chloro aniline (0.01 mol) in glacial acetic acid (30 mL) was refluxed under stirring 5 hours, after that the mixture cooled at RT, then poured on crushed ice. The precipitate was collected by filtration and recrystallization from ethanol and gives orange to brown crystals.

Synthesis of N-[4-(4-methylphenyl)-1,3-thiazol-2-yl] benzamide, IVe

A mixture of **2a** (0.01 mol) with benzoyl chloride (0.01 mol) in glacial acetic acid (30 mL) was refluxed

under stirring 5 hours, after that the mixture cooled at RT, then poured on crushed ice. The precipitate was collected by filtration and recrystallization from ethanol and gives orange to brown crystals[13].

Evaluation of Anthelmintic activity

Adult earth worms *Pheretima postuma* were collected (due to anatomical and physiological resemblance with the intestinal round worm parasites of human being[14]) from moist soil obtained from agricultural fields. Five test groups were taken each containing two earth worms of approximately equal size 8±1 cm. Mebendazole was taken as standard drug and different concentrations (20 mg/mL, 35mg/mL, 50mg/mL) were prepared in normal saline containing 15% tween 80. The synthesized compounds of different concentrations were prepared by dissolving in minimum quantity of tween 80 and making up to the final volume with normal saline to obtain 20,35,50 mg/mL concentrations. One of the group is taken as control group, which was treated with normal saline containing 1% tween 80. Paralysis onset time and death time of individual worms were noted. Paralysis was said to occuer when the worms donot revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of colour of worms[15].

Results and Discussion

***In silico* screening and Lipinski rule of 5 filtration**

Prediction of activity spectra for substances (PASS)

From Table I, it is seen that the synthesized thiazole derivatives obey Lipinski rule of 5. From Table II it is seen that all the synthesized compounds obey PASS values Pa > 0.3. and the standard (Mebendazole) drug has the Pa value is 0.821. The targeted synthesized molecules IVc and IVe has the more active when compared to the other target molecules.

By the results of OSIRIS Molecular Property explorer the standard drug shows the toxicity (reproductive effective) and the synthesized molecules has the no toxicity.

Molsoft property explorer

The results of Molsoft Molecular property explorer are shown in Table III.

Docking Analysis

Name of the protein: 1OJ0

Docking is used to find the exact binding conformation and orientation of the ligand molecule into the active site of the protein. The synthesized five thiazole compounds and standard (Mebendazole) were docked against beta tubulin using Auto-Dock Tool 4.0, an automated docking tool. The docking results of all synthesized compounds and standard are shown in Table IV.

The docking process involves four main steps,
(i) Protein preparation
(ii) Ligand preparation
(iii) Grid preparation and
(iv) Docking.

The Lamarckian genetic algorithm has been used as the search algorithm to search for the best conformers. The initial population size was set randomly as 150 individuals and ten generations was set for each genetic algorithm run and the maximum number of energy evaluations was set to 2,500,000. The grid box size was set as to include all the active site residues present in rigid macromolecules. The

Table II — Results of PASS prediction

Compd	Anthelmintic activity	
	Pa	Pi
IVa	0.439	0.030
IVb	0.487	0.019
IVc	0.773	0.003
IVd	0.407	0.040
IVe	0.537	0.011
Standard (Mebendazole)	0.821	0.002

Table I — Results of Lipinski's Filtration

Compd	Mol. Wt.	Hydrogen bond donors	Hydrogen bond acceptors	Log p	Molar refractivity
IVa	220	0	3	0.2391	55.6185
IVb	236	0	4	−0.04677	58.2515
IVc	248	0	4	1.5429	63.12
IVd	287	0	2	1.67	70.5
IVe	280	0	3	0.5868	71.3939
Standard (Mebendazole)	282	0	5	0.4483	67.692

Table III — Results of Molsoft Molecular property explorer

Compd	Mol. formula	Mol. Wt.	HBA	HBD	Mlogp	Mlogs	Mol. PSA	Mol. voume
IVa	$C_{12}H_{12}N_2OS$	232.07	3	1	3.12	−4.17	33.28	221.02
IVb	$C_{12}H_{12}N_2O_2S$	248.06	4	2	2.78	−4.22	48.90	218.44
IVc	$C_{13}H_{14}N_2O_2S$	262.08	4	1	3.82	−4.86	41.46	246.44
IVd	$C_{16}H_{15}N_3S$	281.10	2	3	4.73	−5.50	39.73	250.28
IVe	$C_{17}H_{14}N_2OS$	294.08	3	1	4.56	−5.58	33.10	275.74

Table IV — Docking results of all synthesized compounds and standard

Compd	Key Residues	Distance (Å)	No. of hydrogens	Docking Score (Kcal/Mol)
IVa	ILE	2.847	1	−8.58
IVb	–	–	0	−7.09
IVc	ILE	2.806	1	−8.98
IVd	–	–	0	−8.98
IVe	GLN PHE	3.093 2.506	2	−8.76
Standard (Mebendazole)	SER GLU	3.182 2.339	2	−9.80

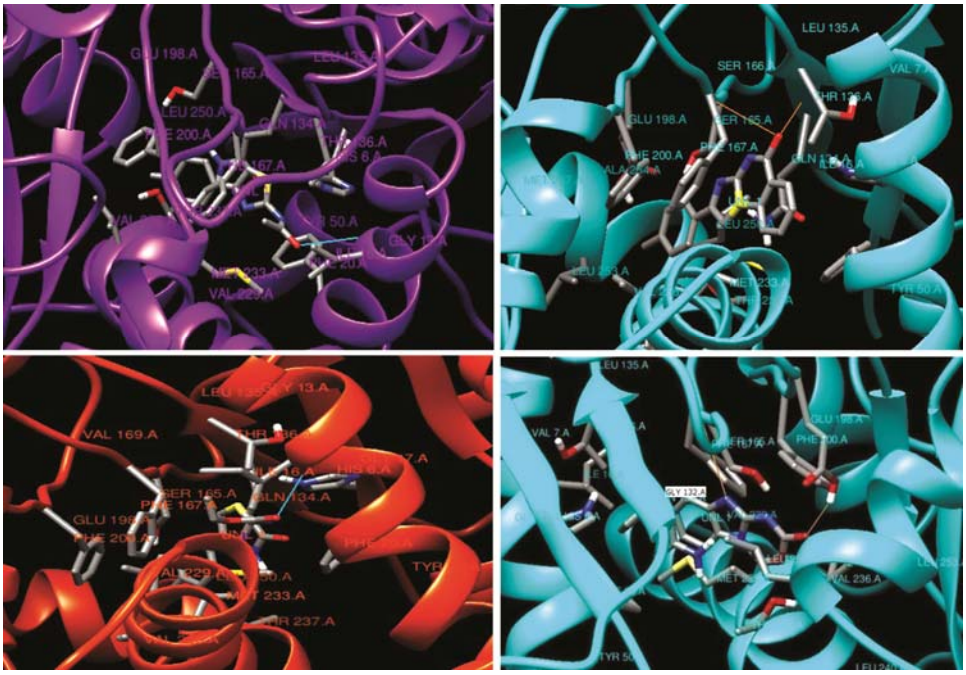


Figure 2 — Docking result of IVa, IVc, IVe and standard

grid box was centered at 8.671 Å × -8.036 Å × 0.67 Å and the dimensions of the grid box have been set as 40, 40, 40 (X,Y,Z co-ordinates) so as to include all the active site residues.

Docking studies showed that all ligands chosen for analysis possessed a least binding affinity with the target protein β-tubulin. The protein ligand interactions were studied in terms of minimum binding energy (Kcal/mol) and the number of hydrogen bonds formed with active site residues. The docking interactions of the six ligands and the protein β-tubulin were visualised using Chimera 1.13.1 viewer and shown in Figure 2. The final docked confirmation obtained for the different ligands based on the binding energy, number of hydrogen bonds formed, bond distance and the interacting residues were shown in Table II. IVc and Mebendazole show a least binding energy with the docking score of -−8.98 Kcal/mol (forms one hydrogen

bonds with ILE) and −9.80 Kcal/mol (forms two hydrogen bonds with GLU and SER), respectively, when docked against β-tubulin.

The length of the hydrogen bonds formed with interacting residues for all the ligands, which shows that the bonding was good. Most of the key residues shown in the Table II are the active site residues of the target protein predicted by PDBsum. Based on the docking score all the ligands have docking interactions with the protein β-tubulin. The compounds with anthelmintics activity was chosen as ligands in this study have different mode of action. This *in silico* molecular docking study shows that the IVa, IVc, IVe inhibits the drug target β-tubulin.

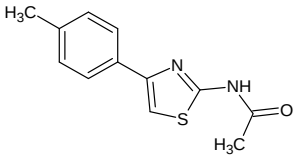
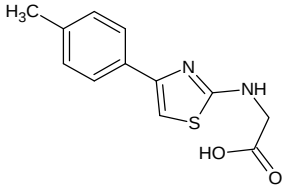
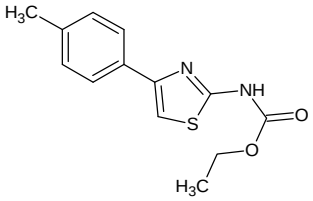
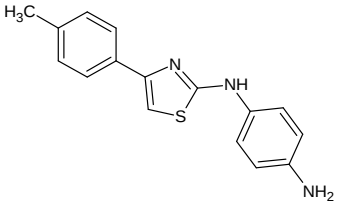
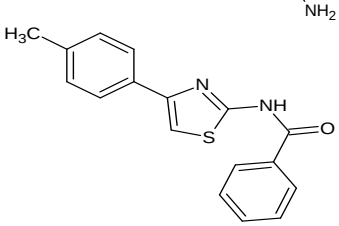
All the newer thiazole derivatives were synthesized by different chemical reagents. In that two derivatives are more yields. That are IVc (Ethylchloro formate derivative) and IVd (4-Chloroaniline derivative). Remaining three derivatives are less yields. All the synthesized compounds were varied the melting point and TLC profiling when compared with the second step of the synthesized molecule. All the synthesized molecules were recrystallized from the methnol and the one particular compound is purified by using the column chromatography and that purified compound was characterized by using the different spectroscopic techniques like FTIR, 1H NMR and mass spectroscopy. Physical data for synthesized compounds are shown in Table V. Representative mass fragmentation pattern of compound **IVd** is shown in Scheme II.

Spectral data for the synthesized compounds

N-[4-(4-Methylphenyl)-1,3-thiazol-2-yl] acetamide, IVa

FTIR (KBr): 3462 (N-H, str), 2884 (C-H, str), 2672 (C=C, str), 1654 (C=O, Str), 1462 (C=N, str), 1168

Table V — Physical data for synthesized compounds

Compd	Structure	Mol. Formula	Mol. Wt.	m.p. (°C)	R_f value
IVa		$C_{12}H_{12}N_2OS$	232.30	192-198	0.68
IVb		$C_{12}H_{12}N_2O_2S$	248.30	252-256	0.55
IVc		$C_{13}H_{14}N_2O_2S$	262.32	238-242	0.51
IVd		$C_{16}H_{15}N_3S$	281.37	168-174	0.59
IVe		$C_{17}H_{14}N_2OS$	294.37	269-271	0.82

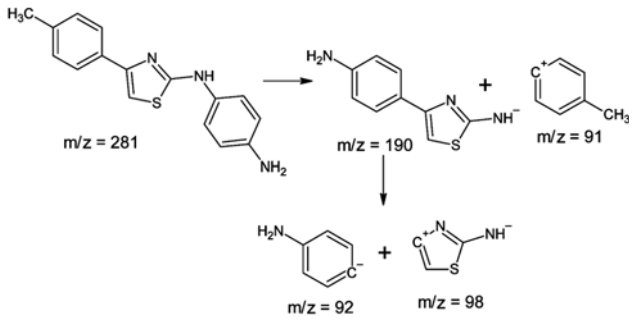
(C-N, str), 722 cm^{-1} (C-H, str); ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 7.2 (d, 2H, Ar-H), 7.8 (d, 2H, Ar-H), 7.9 (s, 1H, Ar-H), 12.5 (s, 1H, NH).

{[4-(4-Methylphenyl)-1,3-thiazol-2-yl] amido} acetic acid, IVb

FTIR (KBr): 3462 (N-H, str), 3280 (O-H, bend), 2884 (C-H, str), 2672 (C=C, str), 1654 (C=O, Str), 1462 (C=N, str), 1368 (C-O, str), 1168 (C-N, str), 722 cm^{-1} (C-H, str); ^{1}H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 4.1 (s, 2H, CH_2), 5.9 (s, 1H, NH), 7.2 (d, 2H, Ar-H), 7.8 (d, 2H, Ar-H), 7.9 (s, 1H, Ar-H), 13.5 (s, 1H, OH).

[4-(4-Methylphenyl)-1,3-thiazol-2-yl] carbamate, IVc

FTIR (KBr): 3462 (N-H, str), 2884 (C-H, str), 2672 (C=C, str), 1654 (C=O, Str), 1462 (C=N, str), 1368 (C-O, str), 1168 (C-N, str), 722 cm^{-1} (C-H, str); ^{1}H NMR (DMSO-d_6): δ 1.2 (t, 3H, CH_3), 2.3 (s, 3H, CH_3), 4.1 (q, 2H, CH_2), 7.2 (d, 2H, Ar-H), 7.8 (d, 2H, Ar-H), 7.6 (s, 1H, Ar-H), 11.8 (s, 1H, NH).



Scheme II — Mass fragmentation of compound **IVd**

N-[4-(4-Methylphenyl)-1,3-thiazol-2-yl] benzene-1,4-diamine, IVd

FTIR (KBr): 3462 (N-H, str), 2884 (C-H, str), 2672 (C=C, str), 1462 (C=N, str), 1168 (C-N, str), 722 cm^{-1} (C-H, str); ^{1}H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 4.1 (s, 1H, NH), 6.2 (s, 2H, NH_2), 6.4 (s, 4H, Ar-H), 7.3 (d, 3H, Ar-H), 7.45 (d, 2H, Ar-H); EI-MS: *m/z* 282 (M^++1).

N-[4-(4-Methylphenyl)-1,3-thiazol-2-yl] benzamide, IVe

FTIR (KBr): 3462 (N-H, str), 2884 (C-H, str), 2672 (C=C, str), 1654 (C=O, Str), 1462 (C=N, str), 1168 (C-N, str), 722 cm^{-1} (C-H, str); ^{1}H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 7.2 (d, 2H, Ar-H), 7.5 (t, 2H, Ar-H), 7.6 (t, 2H, Ar-H), 7.8 (d, 2H, Ar-H), 7.9 (s, 1H, Ar-H), 12.5 (s, 1H, NH).

Evaluation of Anthelmintic activity

The thiazole derivatives were screened for their *in vitro* anthelmintic activity by using standard protocol against Indian adult earth worms (*Pheretima postuma*) and compared with the standard.

The Indian adult earth worms (*Pheretima postuma*) resembles both anatomically, biologically and physiologically to the intestinal round worm parasites of human beings. The results for antihelmintic activity of the synthesized compounds is shown in Table VI.

Table VI — Results of anthelmintic activity

S. No.	Compd	Concentration (mg/mL)	Paralysis time (min)	Death time (min)
1	Normal saline (control)	–	–	–
		20	35	38
2	Standard	35	28	33
		50	15	21
		20	44	51
3	IVa	35	33	39
		50	22	27
		20	47	55
4	IVb	35	40	47
		50	21	28
		20	30	32
5	IVc	35	21	29
		50	13	18
		20	38	45
6	IVd	35	30	38
		50	17	24
		20	33	36
7	IVe	35	24	31
		50	14	20

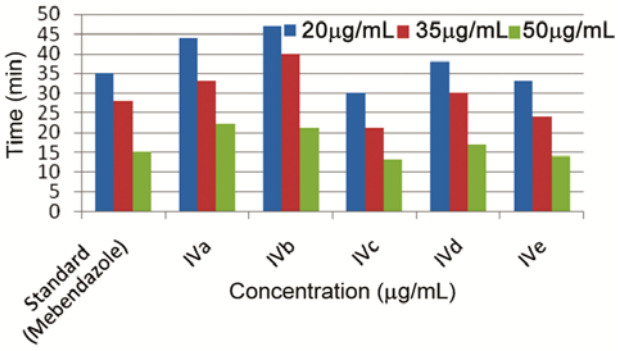


Figure 3 — Comparison of paralyzing time of worms

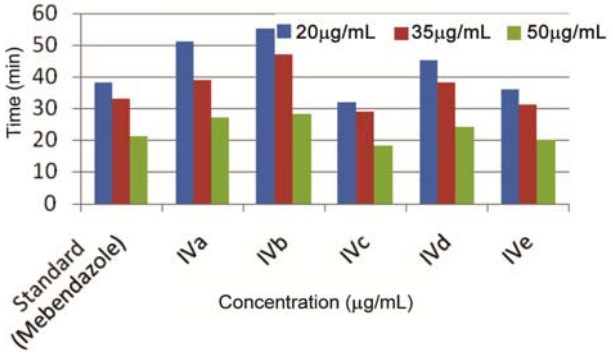


Figure 4 — Comparison of death time of worms

All the synthesized compounds show significant action on the worms *Pheretima postuma* (Figure 3 and Figure 4).

In that of two compounds (IVc, IVe) were found to be equivalent or more potent than the standard drug (mebendazole).

Conclusion

Thiazole derivatives were synthesized as per Scheme I. The thiazole derivatives (IVa - IVb) were studied for their molecular properties by various softwares. Amongst them drug-likeness, non-toxic molecules (compounds IVa – IVe) were subjected to docking studies and synthesized as per Scheme I. The new thiazole derivatives exhibited greater binding affinity than the standard drug (Mebendazole) against the target enzyme tubulin (1OJ0) *in silico*. Among all the compounds tested IVc and IVe exhibited more potent Anthelmintic activity towards *Pheretima postuma* at the concentrations of 20, 35, 50 mg/mL respectively. The compounds IVc and IVe were more potent when compared to standard drug, Mebendazole.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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