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Original Article

DEVELOPMENT OF NOVEL 1, 3, 4-THIADIAZOLES AS ANTITUBERCULAR AGENTS-SYNTHESIS AND *IN VITRO* SCREENING

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ABSTRACT

Objective: Tuberculosis, known in short as TB, is considered to be a global crisis caused by *Myco bacterium tuberculosis*, and which continues to be a serious challenge to public health world wide especially in the developing countries. A serious problem related to tuberculosis is the development of drug resistant strains.

Methods: Present study describes the synthesis of eight novel derivatives of 1,3,4-thiadiazole derivatives and *in vitro* evaluation of their anti tubercular activity against *Mycobacterium tuberculosis* H37 Rv strain by alamar blue assay. Pyrazinamide and Streptomycin were the standards, used for the evaluation.

Results: Among the eight compounds synthesized one derivative was found to be active against the strain tested. All the other derivatives tested exhibited negligible activity against the strain.

Conclusion: It can be concluded that novel 1, 3, 4-thiadiazole derivatives can be developed as novel agents in the fight against TB.

Keywords: Tuberculosis, *Mycobacterium tuberculei,* 1,3,4-thiadiazole, Alamar blue, Minimum inhibitory concentration

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INTRODUCTION

Tuberculosis, the dreaded disease caused by *Myco bacterium tuberculosis*, continues to be a serious challenge to public health worldwide especially in the developing countries, where infections due to *Myco bacterium tuberculosis* has affected majority of the population in active or latent form [1]. A study by WHO revealed that around 3 million deaths are occurring every year due to the disease, tuberculosis worldwide and World Health Organization (WHO) has declared tuberculosis a global crisis [2]. In spite of this, the pace of research leading to the development of newer anti tubercular agents is considerably low [3]. The unusual chemical composition and structure of the cell wall of *M. Tuberculosis* acts as a hindrance for the development of newer antitubercular agents [4]. A serious threat in anti-tubercular therapy is the development of multidrug resistant (MDR) and extreme drug resistant (XDR) strains of *M. Tuberculei*. Thus in order to control the rapid spread of tuberculosis, there is an urgent need for developing newer antitubercular drugs with unique modes of action and improved properties such as enhanced activity against MDR and XDR strains, reduced toxicity and shortened duration of therapy [5].

Till date, heterocyclic compounds provided the required arsenal and ammunition for drug discovery groups in their efforts for the development of newer therapeutic agents [6]. 1,3,4-thiadiazole has acted as a promising scaffold in the design and discovery of novel pharmaco therapeutic agents [7]. Substituted 1,3,4-thiadiazoles have become highly successful compounds in the field of medicine. One of the best known drugs having a 1, 3, 4-thiadiazole moiety is acetazolamide, which is a carbonic anhydrase inhibitor launched in 1954. Its indications and usage are many, including hypertension, [8] epilepsy [9], mountain sickness [10] and congestive heart failure [11]. Further 1, 3, 5-thiadiazole derivatives have proven to be effective as antimicrobial [12], antimalarial [13], analgesic and anti-inflammatory [14], anticonvulsant [15], anticancer [16], ant diabetic, [17] antileishmanial agents [18]. Prompted by these findings and our quest for developing a novel, safe anti TB agent we here in report the synthesis of certain novel thiadiazoles and evaluation of their *in vitro* anti tubercular activity.

MATERIALS AND METHODS

N-Phenyl thiosemicarbazide was synthesized by refluxing hydrazine hydrate (0.03 mol) with phenylisothiocyanate (0.03 mol) in 20 ml of ethanol on a boiling water bath for 5-6 h. After completion of reaction, the reaction mixture was concentrated and kept overnight at room temperature. The needle shaped crystals of N-Phenyl thiosemicarbazide so obtained were filtered and recrystallized from ethanol water mixture (4:1 ratio). Yield: 90.24%W/W.

N-Phenyl thiosemicarbazide (0.01 mol, 1.67g), aromatic acid (0.01 mol) in DMF (25 ml)

Were taken and subjected to microwave irradiation for 8-12 min at 245-350W power in the presence of conc. H2SO4. In between, the completion of reaction was checked by TLC.

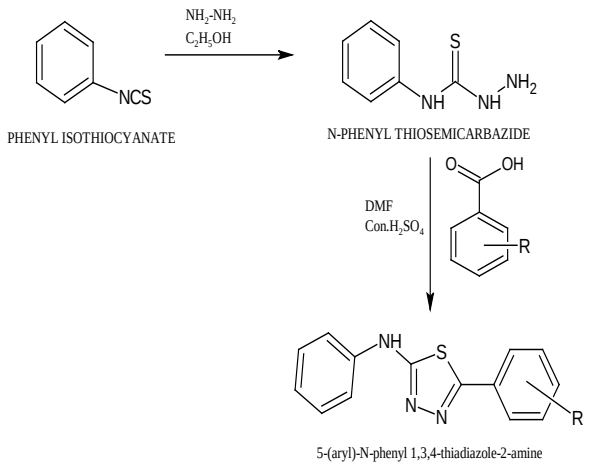

Fig. 1: Scheme of synthesis

After the reaction mixture was slowly poured into crushed ice and kept overnight. Separated solids was filtered, washed with water and dried. Crude product was then purified by recrystallization from ethanol-water mixture (4:1 ratio) to yield desired compounds (TD1–TD8) respectively. The scheme of synthesis is presented in fig. 1. The physical data and solubility profile of the synthesized derivatives are listed in table 1 and 2.

STEP 2.1: Synthesis of a)2-(5-anilino-1,3,4-thiadiazol-2-yl)-3,6-dinitro phenol(TD1)

N-Phenyl thiosemicarbazide (0.01 mol, 1.67g), 3,5-dinitro benzoic acid (0.01 mol) in DMF (25 ml) were taken and subjected to the steps explained under materials and methods to yield yellow crystals of compound TD1. M. P. 136 °C-140 °C Yield: 60%W/W.

IR: (KBR cm-1):3050.27(aromatic C-H stretching), 1580.27 (N-H bend), 1456.97, 1425.40 (aromatic C=C ring stretching), 1356.24 (secondary aromatic C-N stretching), 1160.00(N-N=C), 1014.56 (N-N stretching), 758 (C6H5 str), 1637, 1670 (aliphatic C=C), 2500-3000 (aliphatic C-C).

^{1}HNMR: (400 MHz, DMSO-d6) 8.850-Singlet,-NH (1H) 7.58-7.54, Triplet,-HC=CH (2H) 7.652-7.400,

^{13}CNMR (100 MHz, DMSO-d6)**:** 168.48, 142.27 (C-thiadiazole ring), 123.34 (aromatic ring)

MS: 275.4359(M+)

Elemental analysis: calcd**:** C(46.80%) H(2.52%) N(19.49%)O (22.26%) S(8.92%)

Found: C (46.73%) H (2.56%), N (19.23 %), O (22.35 %), S (8.74%)

b) 2-(5-anilino-1, 3, 4-thiadiazol-2-yl)-2-ethinyl benzene (TD2)

N-Phenyl thiosemicarbazide (0.01 mol, 1.67g), and cinnamic acid (0.01 mol) in DMF (25 ml) were taken and subjected to the steps explained under materials and methods to yield pale yellow crystals of compound TD2. M. P-153 °C-158 °C,Yield: 85%W/W.

IR: (KBR cm-1):3051.27(aromatic C-H stretching), 1591.27 (N-H bend), 1490.97, 1425.40 (aromatic C=C ring stretching), 1321.24 (secondary aromatic C-N stretching), 1111.00(N-N=C), 1014.56 (N-N stretching), 761 (C6H5), 16401667 (aliphatic C=C), 2500-3000 (aliphatic C-C).

^{1}HNMR: (400 MHz, DMSO-d6) 8.854-Singlet,-NH (1H) 7.58-7.54, Triplet,-HC=CH (2H) 7.656-7.396, Multiplet, aromatic protons (10H) Total protons–13.

^{13}CNMR (100 MHz, DMSO-d6)**:** 164.48, 144.27 (C-thiadiazole ring), 119.53 (aromatic ring) 117.23(C=C)

MS: 279.3595(M+)

Elemental analysis: calcd**:** C(68.79%) H(4.69%) N(15.04%) S(11.48%)

Found: C(68.66 %) H(4.64%), N(15.12 %) S(11.36 %)

c) 2-(5-anilino-1, 3, 4-thiadiazol-2-yl)-2, 4-dichloro benzene (TD3)

N-Phenyl thiosemicarbazide (0.01 mol, 1.67g), and 2, 4-dichloro phenoxy acetic acid (0.01 mol) in DMF (25 ml) subjected to the steps explained under materials and methods to yield yellowish orange crystals of compound TD3. M. P, 136 °C-138 °C Yield: 60%W/W.

IR: (KBR cm-1): 3065.27(aromatic C-H stretching), 1586.27 (N-H bend), 1489.97, 1429.40 (aromatic C=C ring stretching),1330.24 (secondary aromatic C-N stretching), 1117.00(N-N=C), 1019.56 (N-N stretching), 759 (C6H5), 1644,1663 (aliphatic C=C), 2500-3000 (aliphatic C-C).

^{1}HNMR: (400 MHz, DMSO-d6) 8.754-Singlet,-NH (1H) 7.64-7.58, Triplet,-HC=CH (2H) 7.656-7.496, Multiplet,

^{13}CNMR (100 MHz, DMSO-d6)**:** 164.48, 144.27 (C-thiadiazole ring), 119.53 (aromatic ring) 49.23(1 C)

MS: 253.3672 (M+)

Elemental analysis: calcd**:** C(51.15%), H(3.15%), N(11.93%) O(4.54%), S(9.10%) Cl(20.13%)

Found: C(51.18%) H(3.23%) N(11.88 %) O(4.15 %) S(9.23 %) Cl (20.21%)

d)2-(5-anilino-1,3,4-thiadiazol-2-yl) benzene (TD4)

N-Phenyl thiosemicarbazide (0.01 mol, 1.67g), and benzoic acid (0.01 mol) in DMF (25 ml) subjected to the steps explained under materials and methods to yield off-whit ecrystals of compound TD4. M. P.135 °C-136 °C, Yield: 70%W/W.

IR: (KBR cm-1)3071.35 (aromatic C-H stretching),1567.87 (N-H bend),1500.85,1432.45 (aromatic C=C ring stretching),1326.24 (secondary aromatic C-N stretching), 1108.00(N-N=C), 1020.72 (N-N stretching), 772 (C6H5), 1650,1677 (aliphatic C=C), 2500-3000 (aliphatic C-C).

^{1}HNMR: (400 MHz, DMSO-d6) 8.854-Singlet,-NH (1H)7.58-7.54, Triplet,-HC=CH (2H)7.656-7.396, Multiplet,

^{13}CNMR (100 MHz, DMSO-d6)**:** 164.48, 144.27 (C-thiadiazole ring), 119.53 (aromatic ring)

MS: 267.7324 (M+)

Elemental analysis: calcd**:** C(66.38%) H(4.38%) N(16.59%) S(12.66%)

Found: C(66.23%) H(4.19%) N(16.32 %) S(12.55 %)

e) 2-(5-anilino-1,3,4-thiadiazol-2-yl)-4-methyl Benzene (TD5)

N-Phenyl thiosemicarbazide (0.01 mol, 1.67g), and p-toluic acid (0.01 mol) in DMF (25 ml) were taken and subjected to the steps explained under materials and methods to yield off-white crystals of compound TD5. 132 °C-134 °C Yield: 78%W/W.

IR: (KBr cm-1)3068.72 (aromatic C-H stretching), 1600.83 (N-H bend), 1496.97,1452.40 (aromatic C=C ring stretching), 1334.24 (secondary aromatic C-N stretching), 1125.00(N-N=C), 1072.56 (N-N stretching), 773 (C6H5), 1638, 1663 (aliphatic C=C), 2500-3000 (aliphatic C-C).

^{1}HNMR: (400 MHz, DMSO-d6) 8.754-Singlet,-NH (1H) 7.32-7.28, Triplet,-HC=CH (2H)7.854-7.597, Multiplet,

^{13}CNMR (100 MHz, DMSO-d6)**:** 164.48, 144.27 (C-thiadiazole ring), 119.53 (aromatic ring)

MS: 343.3267(M+)

Elemental analysis: calcd**:** C(67.39%) H(4.90%) N(15.72%) S(11.99%)

Found: C(67.42 %) H(4.73 %) N(15.77 %) S(12.09%).

f) 3-(5-anilino-1.3,4-thiadiazol-2-yl)-2,5-dinitro benzene (TD6)

N-Phenyl thio semicarbazide (0.01 mol, 1.67g), and 2,5-Dinitro benzoic acid (0.01 mol) in DMF(25 ml) were taken and subjected to the steps explained under materials and methods to yield white crystals of compound TD7. M. P-149 °C-152 °C Yield: 62%W/W.

IR: (KBR cm-1):3050.27(aromatic C-H stretching), 1580.27 (N-H bend),1456.97,1425.40 (aromatic C=C ring stretching),1356.24 (secondary aromatic C-N stretching), 1160.00(N-N=C), 1014.56 (N-N stretching), 758 (C6H5 str), 1637, 1670 (aliphatic C=C), 2500-3000 (aliphatic C-C).

^{1}HNMR: (400 MHz, DMSO-d6) 8.850-Singlet,-NH (1H) 7.58-7.54, Triplet,-HC=CH (2H) 7.652-7.400,

^{13}CNMR (100 MHz, DMSO-d6)**:** 168.48, 142.27 (C-thiadiazole ring), 123.34 (aromatic ring)

MS: 275.4359(M+)

Elemental analysis: calcd**:** C(46.80%) H(2.52%) N(19.49%) O(22.26%) S(8.92%)

Found: C(46.73%) H(2.56%), N(19.23 %),O(22.35 %), S(8.74%)

g) 3-(5-anilino-1.3,4-thiadiazol-2-yl)phenyl acetate (TD7)

N-Phenyl thio semicarbazide (0.01 mol, 1.67g), and acetylsalicylic acid (0.01 mol) in DMF (25 ml) were taken and subjected to the steps explained under materials and methods to yield white crystals of compound TD7. M. P-147 °C-150 °C Yield: 68%W/W.

IR: (KBR cm-1) 3058.72 (aromatic C-H stretching), 1587.16 (N-H bend), 1478.97, 1434.52 (aromatic C=C ring stretching), 1365.38 (secondary aromatic C-N stretching), 1138.00(N-N=C), 1020.66 (N-N stretching), 774 (C6H5), 1627, 1684 (aliphatic C=C), 2500-3000 (aliphatic C-C).

^{1}HNMR: (400 MHz, DMSO-d6) 8.746-Singlet,-NH (1H)7.54-7.50, Triplet,-HC=CH (2H)7.6562-7.392, Multiplet

^{13}CNMR (100 MHz, DMSO-d6)**:** 164.48, 144.27 (C-thiadiazole ring), 119.53 (aromatic ring)

MS: 283.36(M+)

Elemental analysis: calcd: C(61.72%), H(4.21%), N(13.50%) O(10.28%), S(10.30%)

Found: C(61.79%), H(4.18 %), N(13.54%), O(10.32%), S(10.35%).

h) (5-anilino-1,3,4-thiadiazol-2-yl)(phenyl) methanol (TD8)

N-Phenyl thiosemicarbazide (0.01 mol, 1.67g), and mandelic acid (0.01 mol) were subjected to the steps explained under materials and methods to yield yellow crystals of compound TD8. M. P-132 °C-135 °C Yield: 64%W/W.

IR: (KBR cm-1) 3045.23(aromatic C-H stretching), 1591.27 (N-H bend), 1490.97, 1425.40 (aromatic C=C ring stretching), 1321.24 (secondary aromatic C-N stretching), 1111.00(N-N=C), 1014.56 (N-N stretching), 761 (C6H5), 1640-1667 (aliphatic C=C), 2500-3000 (aliphatic C-C).

^{1}HNMR: (400 MHz, DMSO-d6) 8.854-Singlet,-NH (1H)7.53-7.48, Triplet,-HC=CH (2H)7.651-7.392, Multiplet

^{13}CNMR (100 MHz, DMSO-d6)**:** 164.48, 144.27 (C-thiadiazole ring), 119.53 (aromatic ring) 50.12(CH2)

MS: 354.25(M+2)

Elemental analysis: calcd**:** C(63.64%) H(4.65%) N(14.84%) O(5.59%) S(11.27 %)

Found: C(63.58%) H(4.62%) N(14.83%)O(5.65%) S(11.32%)

Table 1: Physical data of synthesized compounds

Compound code	R (substituent group)	Molecular formula	Molecular Weight	Colour	Yield (%)
TD1	3,5-dinitro-2-hydroxy	$C_{14}H_9N_5SO_5$	279.37	Pale yellow	76
TD2	2-ethinyl	$C_{16}H_{13}N_3S$	359.52	yellow	72
TD3	4-methyl	$C_{14}H_{11}N_3S$	253.33	Off white	74
TD4	-H	$C_{15}H_{13}N_3S$	267.36	Off white	75
TD5	4-methyl	$C_{14}H_{13}N_3S$	343.32	Creamy white	74
TD6	2,5-dinitro	$C_{16}H_{13}N_3SO_2$	311.37	white	73
TD7	2-acetoxy	$C_{15}H_{13}N_3SO$	283.36	yellow	70
TD8	1-hydroxy methyl	$C_{15}H_{11}N_3S$	352.25	Yellowish orange	72

Table 2: Solubility of synthesized derivatives in various solvents

Compound code	DMSO	DMF	Acetate	Hexane	Chloroform	Methanol	Ethanol	Ethyl acetate	Water
TD1	++	++	++	++	+	+	+	+	-
TD2	++	++	++	++	+	+	+	+	-
TD3	++	++	++	++	+	+	+	+	-
TD4	++	++	++	++	+	+	+	+	-
TD5	++	++	++	++	+	+	+	+	-
TD6	++	++	++	++	+	+	+	+	-
TD7	++	++	++	++	+	+	+	+	-
TD8	++	++	++	++	+	+	+	+	-

Table 3: Percentage inhibition of synthesized compounds against *Myco bacterium tuberculosis* h37 rv strain

S. No.	Compound code	Concentration (µg/ml)	Absorbance	Percentage inhibition
1	TD1	6.25	2.18	5.357
		12.25	1.40	37.50
		25	0.90	59.82
		50	0.80	64.28
		100	0.41	81.69
		150	0.31	86.16
2	TD2	6.25	1.60	28.57
		12.5	1.21	45.98
		25	0.60	73.21
		50	0.50	77.68
		100	0.32	85.714
		150	0.22	90.17
3	TD3	6.25	2.23	0.44
		12.5	2.18	2.67
		25	1.15	48.66
		50	0.98	56.25
		100	0.90	59.82
		150	0.75	66.51
4	TD4	6.25	2.23	0.44
		12.5	2.18	2.67
		25	1.85	17.41

S. No.	Compound code	Concentration (µg/ml)	Absorbance	Percentage inhibition
		50	1.45	35.26
		100	1.16	48.21
		150	1.05	53.12
5	TD5	6.25	2.21	1.33
		12.5	1.63	27.23
		25	1.47	34.37
		50	1.28	42.85
		100	1.08	51.78
		150	0.98	56.25
6	TD6	6.25	2.23	0.44
		12.5	2.18	2.67
		25	1.96	12.5
		50	1.35	39.73
		100	0.93	58.48
		150	0.64	71.42
7	TD7	6.25	2.22	0.8928
		12.5	2.12	5.35
		25	2.05	8.48
		50	1.82	18.75
		100	0.98	56.24
		150	0.86	61.60
8	TD8	6.25	2.23	0.44
		12.5	2.12	5.35
		25	1.85	17.41
		50	1.55	30.80
		100	1.05	53.15
		150	0.92	58.92
9	DMSO	------	2.24	------
10	Streptomycin	6.25 µg/ml	------	100

Anti-mycobacterial screening [19, 20]

All the synthesized derivatives were tested for their activity against *Mycobacterium tuberculosis*. As the potency of the newly synthesized analogues was unknown, various concentrations were used in this study. The concentrations of the standard (Streptomycin) were also the same as that of the test compounds. Anti-tubercular susceptibility testing was performed in black, clear-bottomed, 96-well microplates Initial drug dilutions were prepared in dimethylsulfoxide, and subsequent two fold dilutions were performed in 0.1 ml of 7H9GC media in the microplates. 100 ml of 2000CFU/ml of *Mycobacterium tuberculosis* H37 Rv in 7H9GC were added to each well of 96 well microtitre plate containing test compounds. Three control well plates containing drug and medium, bacteria and medium and medium only were prepared and micro titre plates were incubated at 37 °C. At day 7 of incubation Alamar Blue dye solution (20 µl Alamar Blue solution and 12.5 ml added to all the wells and plates were re-incubated at 37 °C. for 24 h. Readings were taken at 365 nm. The results are presented in table 3.

RESULTS

Eight different novel derivatives of 1,3,4-thiadiazole were synthesized by treating N-Phenyl thiosemicarbaziode with eight different aromatic acids by two-step synthesis. The structures of all the synthesized derivatives were confirmed using IR, NMR, mass spectral and elemental analysis.

All the synthesized compounds were evaluated for their anti-tubercular activity. Synthesized derivatives TD1 and TD2 showed comparatively good anti-tubercular activity against *Mycobacterium Tuberculosis* H37Rv strain. All the other compounds (TD3-TD8) synthesized exhibited negligible anti-tubercular activity.

DISCUSSION

The strucutures of all the synthesized derivatives were confirmed using IR, NMR and mass spectroscopy techniques. The IR spectra of the synthesized compounds had shown peaks corresponding to C-N strecting, N-H streching as well as C-C double bond stretching. The ^{13}C NMR spectra of the synthesized derivatives exhibited peaks at 142-146 which is indicative of thiadiazole ring formation. The proton NMR spectra also had triplets at 7.58-7.63 which is indicative of C-C double bonds. The mass spectra of all compounds have produced M+peaks which corresponds to the molecular weight of the synthesized compounds, whereas TD8, which posess dichloro substituents in the aromatic ring produces M+2 peaks.

As we found that out of eight compounds synthesized, only two compounds exhibited reasonable antitubercular activity when compared with the standard. Out of the two compounds synthesized TD2 exhibited higher activity than TD1. TD2 is chemically 5-(2-phenyl ethenyl)-N-phenyl-1,3,4-thiadiazole-2-amine.

Comparatively enhanced antitubercular activity of TD2 can be attributed to the structural features of the compounds where an aromatic ring is attached to the thiadiazole moiety through an ethylene side chain. None of the other synthesized derivatives, possess similar chemical structure and hence did not exhibit appreciable antitubercular activity. Attachment of electron-rich aromatic ring to the thiadiazole moeity by unsaturated group has un doubtedly caused enhanced *in vitro* antitubercular activity in the present work.

CONCLUSION

Out of eight novel thiadiazole deriviatives synthesized one compound produced appreciable antitubercular activity, and this can be attributed to the substitutent group attached to the 2nd position of the pyrazole nucleus. Phenethyl group is attached to the second position of the pyrazole ring and from this fact it can be concluded that an unsaturated group with phenyl ring attached to the β carbon with respect to the pyrazole ring creates a good pharmacophore for anti tubercular activity. This moiety should be taken up for further evaluations so that ultimately a novel and effective anti tubercular agent will get delivered to the global healthcare community so as to eradicate the deadly disease TB from the world. Efforts must be undertaken to synthesize more such derivatives and both *in vitro* as well *as in vivo* studies of such derivatives may ultimately deliver a blockbuster agent to the therapeutic world.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Cinu TA, Sidhartha SK, Indira B, Varadaraj BG, Vishnu PS, Shenoy GG. Design, synthesis and evaluation of antitubercular activity of Triclosan analogues. Arab J Chem. 2019;12(8):3316-23. doi: 10.1016/j.arabjc.2015.09.003.
2. Global Tuberculosis Repot; 2020. Available from: www.who/int/i/item/9789240013131.
3. Coxon GD, Craig D, Corrales RM, Vialla E, Gannoun Zaki L, Kremer L. Synthesis, antitubercular activity and mechanism of resistance of highly effective thiacetazone analogues. PLOS ONE. 2013;8(1):e53162. doi: 10.1371/journal.pone.0053162. PMID 23301038.
4. Nguyen L. Antibiotic resistance mechanisms in M. tuberculosis: an update. Arch Toxicol. 2016;90(7):1585-604. doi: 10.1007/s00204-016-1727-6, PMID 27161440.
5. Bandodkar B, Shandil RK, Bhat J, Balganesh TS. Two decades of TB drug discovery efforts-what have we learned? Appl Sci. 2020;10(16):5704. doi: 10.3390/app10165704.
6. Cherian B, Kumar RA, Vinod B. Pyrazole carbaldehydes as novel antiinflammatory agents. Synthesis and *in vitro* membrane stabilization method. J Pharm Sci Res. 2020;12(2):252-7.
7. Raj V, Rai A, Singh AK, Keshari AK, Trivedi P, Ghosh B. Discovery of novel 2-Amino-5-(Substituted)-1,3,4-Thiadiazole derivatives: new utilities for colon cancer treatment. Anticancer Agents Med Chem. 2018;18(5):719-38. doi: 10.2174/1871520617666170419122916, PMID 28425852.
8. Eskandari D, Zou D, Grote L, Hoff E, Hedner J. Acetazolamide reduces blood pressure and sleep-disordered breathing in patients with hypertension and obstructive sleep apnea: a randomized controlled trial. J Clin Sleep Med. 2018;14(3):309-17. doi: 10.5664/jcsm.6968, PMID 29510792.
9. Millichap JG, Millichap JJ. Mechanism of action of acetazolamide and idiopathic intracranial hypertension. Front Neurol. 2015;6(13):1. doi: 10.3389/fneur.2015.00013.
10. Shlim DR. The use of acetazolamide for the prevention of high-altitude illness. J Travel Med. 2020 Aug 06;27:taz106. doi: 10.1093/jtm/taz106, PMID 31897486.
11. Nunez J, Heredia R, Paya A, Sanchis I, Del Prado S, Minana G. Use of acetazolamide in the treatment of patients with refractory congestive heart failure. Cardiovasc Ther. 2018;36(6):e12465. doi: 10.1111/1755-5922.12465, PMID 30191652.
12. Serban G, Stanasel O, Serban E, Bota S. 2-Amino-1,3,4-thiadiazole as a potential scaffold for promising antimicrobial agents. Drug Des Devel Ther. 2018;12:1545-66. doi: 10.2147/DDDT.S155958. PMID 29910602.
13. Tahghighi A, Babalouei F. Thiadiazoles: the appropriate pharmacological scaffolds with leishmanicidal and antimalarial activities: a review. Iran J Basic Med Sci. 2017;20(6):613-22. doi: 10.22038/IJBMS.2017.8828, PMID 28868117.
14. Akhter MW, Hassan MZ, Amir M. Synthesis and pharmacological evaluation of 3-diphenylmethyl-6-substituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles: a condensed bridgehead nitrogen heterocyclic system. Arab J Chem. 2014;7(6):955-63. doi: 10.1016/j.arabjc.2014.05.036.
15. Sharma B, Verma A, Prajapati S, Sharma UK. Synthetic methods, chemistry, and the anticonvulsant activity of thiadiazoles. Int J Med Chem. 2013. doi: 10.1155/2013/348948, PMID 25405032.
16. Sharma B, Verma A, Prajapati S, Sharma UK. Synthetic methods, chemistry, and the anticonvulsant activity of thiadiazoles. International Journal of Medicinal Chemistry. 2013;2013:1-16. doi: 10.1155/2013/348948.
17. Szeliga M. Thiadiazole derivatives as anticancer agents. Pharmacol Rep. 2020;72(5):1079-100. doi: 10.1007/s43440-020-00154-7, PMID 32880874.
18. Datar PA. Design and synthesis of thiadiazole derivatives as antidiabetic agents. Med Chem. 2014;4(4). doi: 10.4172/2161-0444.1000170.
19. Sadat Ebrahimi SE, Mirmohammadi M, Mojallal Tabatabaei Z, Azimzadeh Arani M, Jafari Ashtiani S, Hashemian M. Novel 5-(nitrothiophene-2-yl)-1,3,4-thiadiazole derivatives: synthesis and antileishmanial activity against promastigote stage of Leishmania major. Iran J Pharm Res. 2019;18(4):1816-22. doi: 10.22037/ijpr.2019.14547.12476, PMID 32184848.
20. Gupta P, Bhatter P, D'souza D, Tolani M, Daswani P, Tetali P. Evaluating the anti Mycobacterium tuberculosis activity of Alpinia galanga (L.) Willd. Axenically under reducing oxygen conditions and in intracellular assays. BMC Complement Altern Med. 2014;14:84. doi: 10.1186/1472-6882-14-84, PMID 24592852.
21. Sonar VN, Crooks PA. Synthesis and antitubercular activity of a series of hydrazone and nitrovinyl analogs derived from heterocyclic aldehydes. J Enzyme Inhib Med Chem. 2009;24(1):117-24. doi: 10.1080/14756360801915526. PMID 18629678.