DESIGN, SYNTHESIS, CHARACTERIZATION, ANTICANCER ACTIVITY AND IN SILICO ADME STUDY OF SOME NOVEL ALKYLTHIO TRIAZOLO THIADIAZOLE

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***Abstract**: A sequence of 6-(6-(methylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5a], 6-(6-(ethylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5b], 3-phenyl-6-(propylthio)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5c] has been designed. The spectral analyses validated the synthesis of novel 6-(Alkylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, which was examined using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. The ADME profiles demonstrated favorable oral bioavailability and pharmacokinetic characteristics. These parameters were investigated because many synthesized compounds often fail at later stages of drug discovery and development due to poor pharmacokinetic behavior. The SwissADME analysis provided theoretical insights into pharmacokinetics, revealing that most of the designed compounds exhibit high gastrointestinal absorption and do not cross the blood–brain barrier (BBB). This finding is particularly relevant for oral drugs targeting the gastrointestinal system, indicating their potential for good oral bioavailability. Overall, the newly synthesized compounds show promising pharmacokinetic properties, being well absorbed, distributed, metabolized, and excreted according to the SwissADME predictions. Therefore, these compounds may serve as viable candidates for further drug development. An in silico ADME evaluation was also conducted to assess their drug-likeness and support their potential as novel therapeutic agents. Anticancer evaluations demonstrated that the compounds had action against HepG2 and WRL68 cell lines. The synthesized chemical exhibited a pronounced cytotoxic impact on the HepG2 cell line (μg/mL 99.73), whereas little cytotoxicity was detected in normal human cells (WRL68 cell line).*

***Keywords:** ADME, Anticancer, Characterization, Design, In Silico, Triazolo and Thiadiazole.*
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1. Introduction

In recent years, heterocyclic molecules and their analogs have garnered significant attention owing to their extensive array of beneficial biological and pharmacological characteristics. Consequently, several classes of heterocyclic systems form the basis of most current pharmaceuticals or molecules nearing the completion of preclinical studies [1]. This topic examines heterocyclic compounds with an azole core with considerable synthetic and pharmacological promise. The distinctiveness of these heterocyclic systems lies in their facile functionalization, allowing for the synthesis of diverse condensed and non-condensed derivatives. Moreover, using contemporary methodologies for investigating this class of heterocycles, such as target-oriented and diversity-oriented synthesis (DOS) strategies, provides an opportunity to efficiently generate combinatorial libraries to identify novel medications or drug candidates [2].

1,2,4-triazoles, 1,3,4-thiadiazole, and triazolothiadiazolethione exhibit diverse bioactivities, including neuroprotection, antimalarial, antileishmanial, antiviral, and anticonvulsant effects [3]. Numerous pharmaceuticals incorporating 1,2,4-triazole and 1,3,4-thiadiazole moieties include acetazolamide (a diuretic and anticonvulsant), fluconazole (antifungal), methazolamide (a carbonic anhydrase inhibitor), trazodone (an antidepressant), rizatriptan (an analgesic for

headache relief), hexaconazole (an antifungal agent), and alprazolam (a sedative and tranquilizer). Moreover, triazolothiadiazole and triazolothiadiazine, hybrid structures resulting from the amalgamation of triazole with thiadiazole or thiadiazine, are crucial owing to their extensive uses as potential medicines. They may be synthesized from 4-amino-1,2,4-triazole-3-thiol by using the nucleophilic properties of its amino and mercapto groups [4], as shown in Fig. 1.

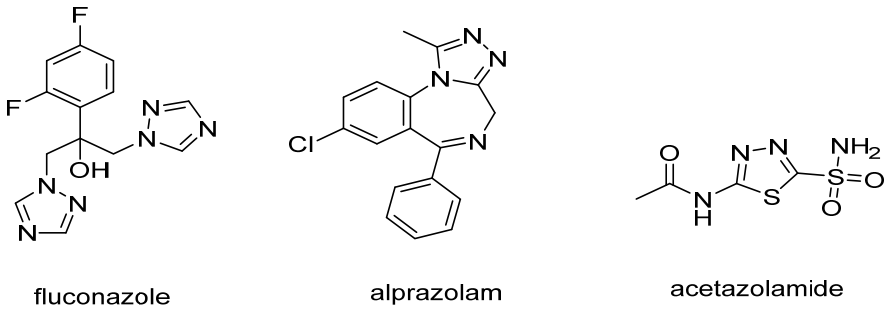


Fig. 1. Heterocyclic drugs containing 1,3,4-thiadiazole moieties and 1,2,4-triazole

In recent years, researchers have focused on triazolothiadiazolethione**,** a fused heterocyclic compound, because of its favorable physicochemical characteristics and remarkable pharmacological activities, including anticancer, antibacterial, antiviral, and antifungal effects. Moreover, it has recently found applications in computational chemistry.

Triazolothiadiazolethione - [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole thione - is synthesized by the fused combination of a member heterocyclic system 1,2,4-triazole molecule with a five-member heterocyclic ring 1,3,4-thiadiazole-2(3H)-thione [6-7] as shown in Fig. 2.

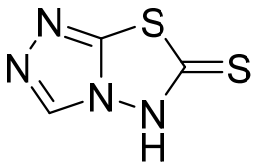
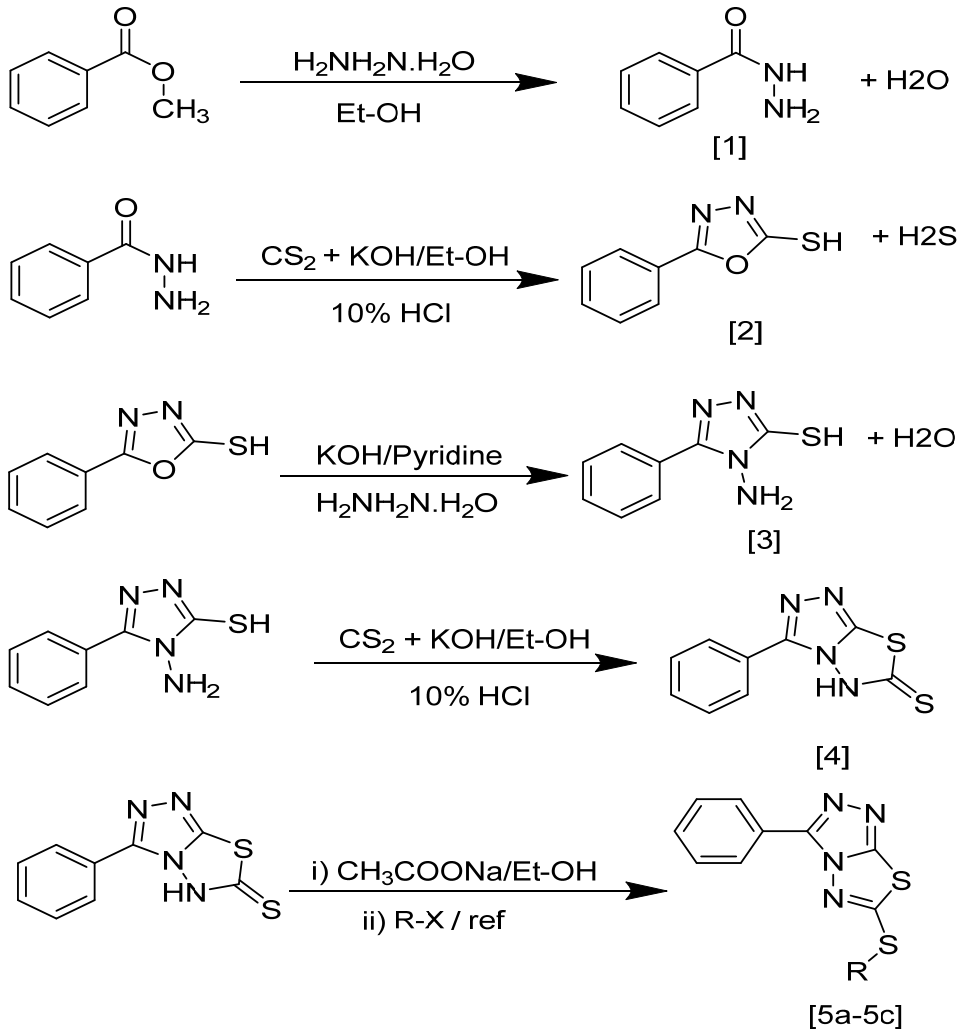


Fig. 2. Chemical structure of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione

The pharmacokinetic properties were evaluated because many synthesized compounds tend to fail in the later stages of drug discovery and development. To address this, theoretical pharmacokinetic investigations were carried out using the SwissADME software and the pre-ADMET predictor, an online platform designed to assess pharmacokinetic parameters and drug-likeness. These tools were employed to analyze the synthesized naphthalene-based derivatives, providing insights into their potential as promising drug candidates.

The objective of the research was to generate innovative 6-(alkylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole as new fused heterocyclic compounds and to investigate their pharmacokinetic characteristics to address the challenges associated with the synthesis of new chemical entities in the drug discovery and development process. Information on synthesizing and assessing the biological activity of compounds containing triazole and thiadiazole fragments. The ADME study indicates that almost all synthesized derivatives exhibit favorable theoretical pharmacokinetics and drug-likeness properties, rendering them appropriate as orally administered medicines [12]. The synthesized chemical compounds are higher activity drugs compared to captopril with natural heterocyclic compounds like alkaloids and glycosides, which are extracted by hot and cold methods like maceration [13-17].



Scheme 1. Preparation of 6-(alkylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole

2. Experimental part

The materials used were methyl benzoate (Merck, ≥98%), carbon disulfide (Aldrich, ≥99.9%), pyridine (Merck, ≥99.5%), methyl chloride (Aldrich, 99%), ethyl bromide (Aldrich, 99%), propyl bromide (Aldrich, 99%), potassium hydroxide (Thomas Baker, 85%), sodium acetate (Thomas Baker, 85%), and deionized water.

Instrumentations used in this research are infrared spectroscopy (FT-IR), Perkin Elmer toner 27 (Bruker, Germany),
Nuclear magnetic resonance spectrometer (1H-NMR, 13C-NMR) BioSpin GmbH 400,100 MHz (Bruker, Germany), mass spectrometer U3500 (mass spectrometer), and mass selective detector (5973) (Agilent Technologies, USA).

2.1 Synthesis of benzohydrazide [1]. In the round-bottom flask, hydrazine hydrate (0.48 ml, 0.01 mole) was dissolved in absolute ethanol (50 ml), and methyl benzoate (1.36 g, 0.01 mole) was added dropwise. The mixture was heated under reflux for 5 hrs. The solvent was evaporated gently under a moderate temperature till forming the precipitate. The formed solid crystals were filtered, dried, and purified using the ethanol solvent, m.p. 138-140°C, yield 92%, Rf 0.84, and color white needle (Table 1) [18].

2.2 Synthesis of 5-phenyl-1,3,4-oxadiazole-2-thiol [2]. To synthesize the titled compound, a quantity of benzohydrazide (0.68 g, 0.005 moles) and potassium hydroxide (0.28 g, 0.005 moles) was dissolved in 50 ml of absolute ethanol in the round-bottom flask. CS2 (0.3 ml, 0.005 mole) was added slowly at 0°C to the mixture. The mixture was heated for 72 hrs under

reflux until H_2S evolved (H_2S was tested by soaked lead acetate paper, which was a black color due to the formation of blackish lead sulfide). The solvent was removed, poured into crushed ice, and then acidified with 10% HCl. The precipitate formed was filtered, washed with water, and recrystallized from ethanol solvent. m.p. 220-222°C, yield 91%, Rf 0.92, color white (Table 1) [19].

2.3 Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol [3]. A mixture of compound 2 (1 g, 0.0056 mole) and potassium hydroxide (0.3 g, 0.0056 mole) in a round-bottom flask in 50 ml of pyridine. Then, hydrazine hydrate (0.24 ml, 0.005 mole) was added to the mixture. The resulting mixture was refluxed for 5 hrs. The complete reaction was monitored by TLC using eluent hexane:ethyl acetate (6:4). After that, the solvent was concentrated and then acidified with 10% HCl. The formed precipitated materials were filtered, washed with water, and recrystallized at 92-94 m.p. 189-191°C, yield 90%, Rf 0.66, and color pink, as illustrated in Table 1 [20].

2.4 Synthesis of 3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione [4]. A mixture of compound 3 (1 g, 0.0052 mole) and potassium hydroxide (0.29 g, 0.0052 mole) in 50 ml of absolute ethanol in a round-bottom flask. Then CS_2 (0.3 ml, 0.005 mole) was added slowly at 0°C to the mixture. The mixture was heated under reflux for 5 hrs. The reaction was monitored by TLC using eluent hexane ethyl acetate (6:4). The solvent was concentrated and then acidified with 10% HCl. The formed precipitated materials were filtered, washed with water, and recrystallized from 98-101% ethanol, m.p. 190-192°C, yield 81%, Rf 0.95, and color pale brown, as illustrated in Table 1 [21].

2.5 Synthesis of 6-(alkylsulfanyl)-3-phenyl[1,2,4]triazolo [3,4-b] [1,3,4]thiadiazole [5a-5c].

In a round-bottom flask, a mixture of 5 (2.34 g, 0.01 mole) and sodium acetate (0.82 g, 0.01 mole) in 50 ml of absolute ethanol was heated for 15 min. Alkyl halide (0.01 mole) was added to the mixture and heated under reflux. The reaction was monitored by TLC using eluent (7:3) hexane:ethyl acetate. The solvent was concentrated. The formed precipitated materials were filtered, washed with water, and recrystallized from ethanol. 5a; m.p. 210-212°C, yield 73%, Rf 0.65, color white; 5b; m.p. 214-216°C, yield 71%, Rf 0.62, color white; 5c; m.p. 218-220°C, yield 73%, Rf 0.65, color white, as illustrated in Table 1 [22].

Table 1. Physical properties of design compounds [1, 2, 3, 4, 5a-5c].

Comp No	Molecular formula	M.Wt	M.P °C.	% Yield	R_f 70% exane 30% ethyl acetate	Color
1	$C_7H_8N_2O$	136	138-140	94%	0.86	white
2	$C_8H_6N_2OS$	178	220-222	91%	0.92	white
3	$C_8H_8N_4S$	192	190-192	88 %	0.70	pink
4	$C_9H_6N_4S_2$	234	190-192	81 %	0.95	pale brown
5a	$C_{10}H_8N_4S_2$	248	210-212	73%	0.65	white
5b	$C_{11}H_{10}N_4S_2$	262	214-216	71%	0.62	white
5c	$C_{12}H_{12}N_4S_2$	276	218-220	73%	0.65	white

3. Results and discussion

3.1. Characterization of benzohydrazide [1]. The FT-IR spectra of the benzohydrazide showed an appearance absorption band at 3299, 3197, 3048, and 1662 cm^{-1} due to stretching of NH_2, NH, ArC-H, and carbonyl of benzohydrazide, respectively [23].

3.2. Characterization of 5-phenyl-1,3,4-oxadiazole-2-thiol [2]. The FT-IR spectrum of the 5-phenyl-1,3,4-oxadiazole-2-thiol showed an appearance absorption band at 3095, 2759, 1609, 1570, 1397, 1609,1570,1397,1345 and 694 cm^{-1} due to stretching vibration of C-H aromatic, S.H.,

C=N of oxadiazole ring, Asy C-O-C, sym C-O-C, and C-S, respectively. The ^{1}H-NMR spectrum of compound [2] showed singlet signals for the proton of the S.H. group at δ14.78 ppm. Furthermore, the signal at 7.89–7.57 ppm of aromatic protons proved the formation of 5-phenyl-1,3,4-oxadiazole-2-thiol. The ^{13}C-NMR spectrum of compounds [2] showed a signal at 77.42 and 160.47 ppm of C=N-S and C=N-Ar, respectively, of oxadiazole ring, and 132.26, 129.45, 126.06, 122.48 ppm of aromatic carbon. Mass spectra of [2] showed a molecular ion peak at 177 of $[C_8H_6N_2OS]^+$, and 118 corresponded to the basic peak $[C_7H_5NO]^+$ and others [24].

3.3. Characterization of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol [3]. The FT-IR spectrum of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiolate showed appearance absorption band at 3300, 3191, 3027, 1633, 1532, 1480, and 688 cm^{-1} due to stretching vibration of NH_2, N-H, ArC-H, C=N of triazole ring, Asy C-O-C, Sym C-O-C, and C-S, respectively. The ^{1}H-NMR spectrum of compounds [2] showed a singlet signal for S.H. at δ13.98 ppm and multiple signals at 8.02–7.51 ppm of aromatic protons and also showed new singlet signals at 5.81 ppm for NH_2, providing good information on synthesized compound [3]. The ^{13}C-NMR spectrum of compounds [3] showed a signal at 166.84 149.47 ppm corresponding to C=N-SH and C=N-Ar of triazole ring, in that order, signals at 130.48, 128.53, 128.07, and 125.77 ppm of aromatic carbon. Mass spectra of [3] showed a molecular ion peak at 192 of $[C_8H_8N_4S]^+$, and 104 corresponded to the basic peak $[CH_4N_4S]^+$ and others [25].

3.4. Characterization of 3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione [4]. The FT-IR spectrum of [4] (Fig. 3) showed the appearance of the new absorption band at 3157 cm^{-1} conforming to the stretching vibration of N-H and the absorption band at 3037 cm^{-1} attributable to the stretching vibration of C-H aromatic. On the other hand, bands at 1573-1609 cm^{-1} are due to C=N of the triazole ring, 1226 cm^{-1} to C=S of thiadiazolethion, and 640 cm^{-1} to C-S. The ^{1}H-NMR spectrum of compounds [4] (Fig. 4) showed singlet signals at δ 9.77 ppm exhibit to NH, while signals 7.82–δ 7.48 ppm refer to aromatic protons (ArH), The ^{13}C-NMR spectrum of compound [4] (Fig. 5) showed a signal at 177.87, 160.95, and 155.03 ppm corresponding to C=S, C=N-S, and C=N-Ar, respectively. In addition, signals 132.74, 129.68, 126.53, and 122.92 ppm are attributable to aromatic carbon [24].

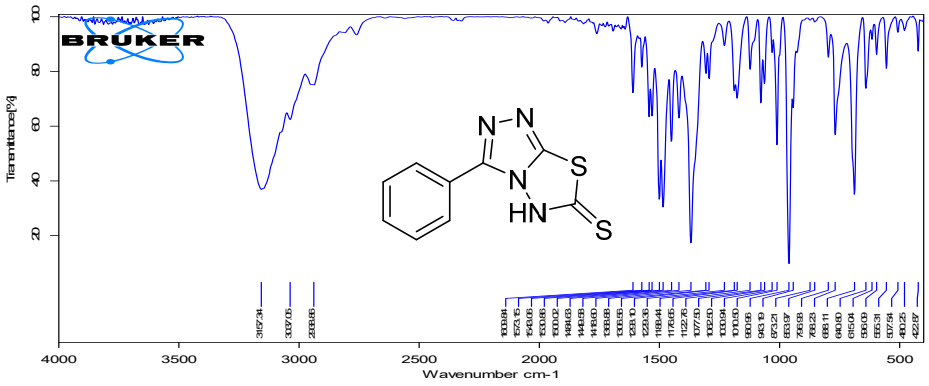


Fig. 3. FT-IR Spectrum of [4]

3.5. Characterization of 6-(6-(methylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5a]. ^{1}H-NMR (Fig. 6) (400 MHz, DMSO-d_6) δ 8.32 – 7.90 (m, J = 8.4 Hz, 2H), 7.70 – 7.55 (m, J = 8.1 Hz, 2H), 7.40 (s, J = 8.0 Hz, 1H), 1.70 (s, 3H) [25].

3.6. Characterization of 6-(ethylsulfanyl)-3-phenyl[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole [5b]. ^{1}H NMR (Fig. 7) (400 MHz, DMSO-d_6) δ 7.93 – 7.81 (m, J = 8.1 Hz, 2H), 7.56 – 7.51 (m, J = 8.2 Hz, 2H), 7.51 (s, J = 7.9 Hz, 1H), 3.24 – 3.19 (m, 2H), 1.69 (s, 3H) [26].

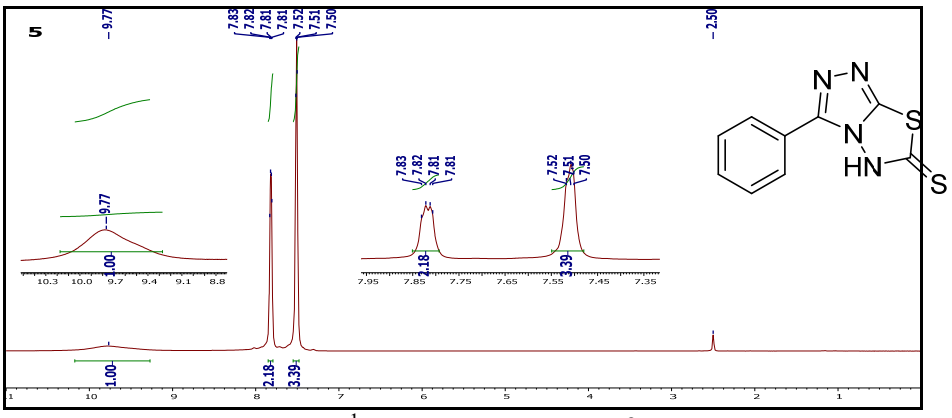


Fig. 4. ^{1}H-NMR Spectrum of [4]

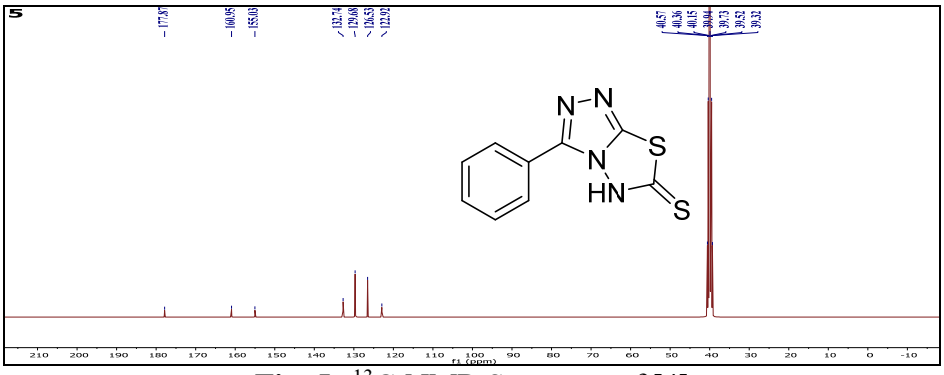


Fig. 5. ^{13}C-NMR Spectrum of [4]

3.7. Characterization of 3-phenyl-6-(propylthio)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5c]. ^{1}H NMR (Fig. 8) (400 MHz, DMSO-d$_6$) δ 8.00–7.98 (m, J = 6.7 Hz, 2H), 7.95–7.75 (m, J = 7.4 Hz, 2H), 7.44–7.38 (s, J = 7.9 Hz, 1H), 2.61–2.43 (m, 2H), 1.37–1.16 (m, 2H), 0.92–0.86 (s, 3H) [27].

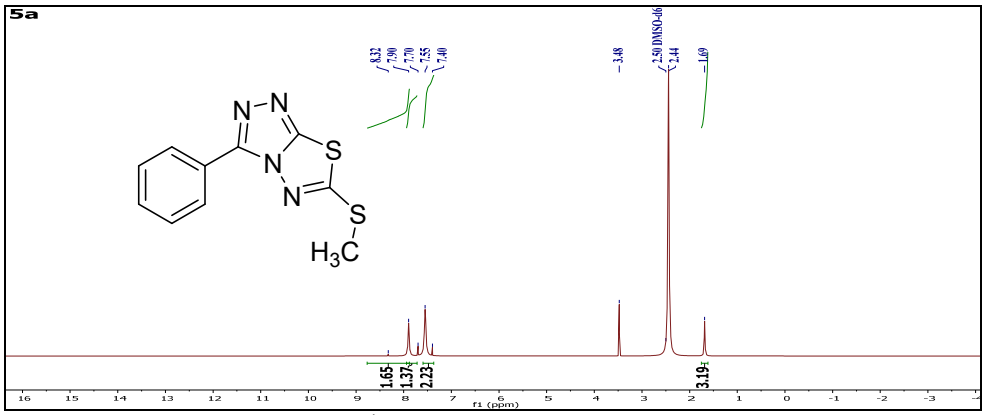


Fig. 6. ^{1}H-NMR Spectrum of [5a]

3.8. In Silico ADME Study. The SwissADME web tool was employed to determine the key physicochemical, lipophilic, and pharmacokinetic parameters, as well as to assess water solubility, drug-likeness, and medicinal chemistry properties. A rapid evaluation of drug-likeness can be visualized through the bioavailability radar shown in Fig. 9. This radar plot examines six critical physicochemical descriptors: lipophilicity, size, polarity, solubility, unsaturation, and flexibility. For a molecule to be considered drug-like, its radar profile must be entirely enclosed within the designated pink zone, which represents the optimal range for oral bioavailability. Compounds falling within this area are predicted to possess favorable bioavailability and thus hold potential for oral administration (Fig. 9) [28–33].

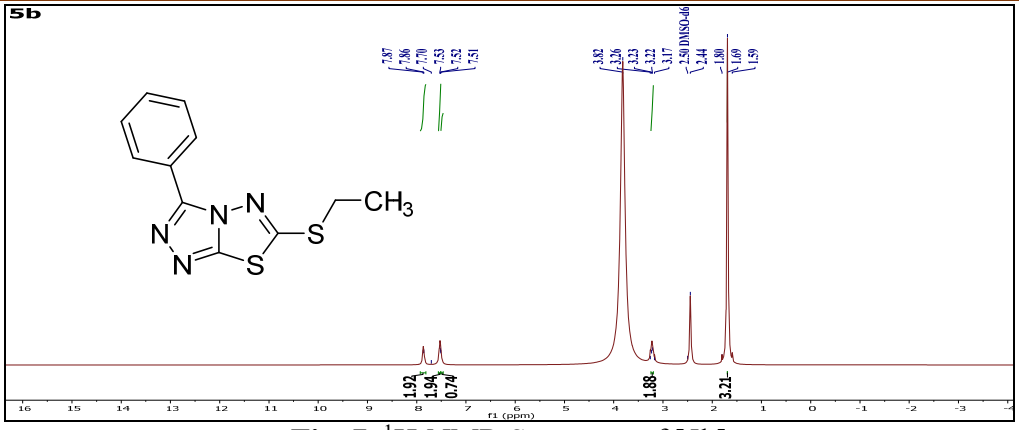


Fig. 7. ^{1}H-NMR Spectrum of [5b]

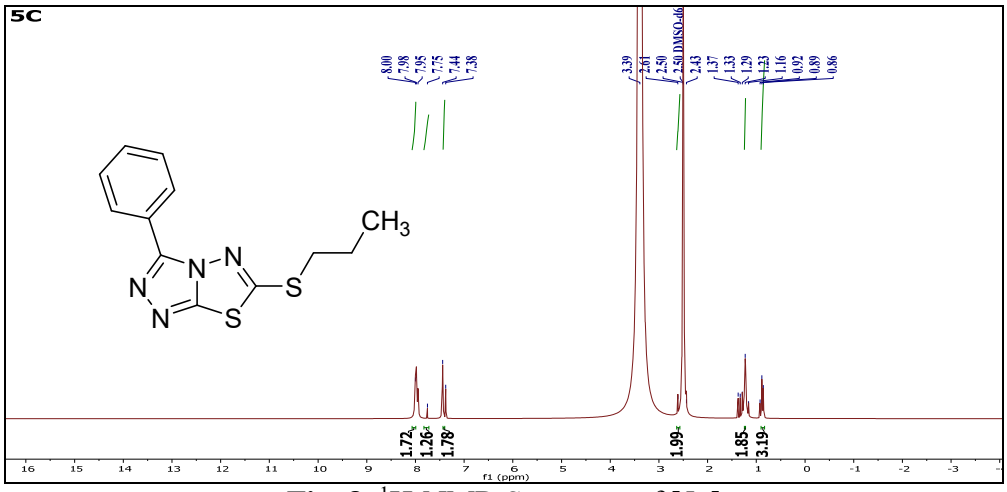


Fig. 8. ^{1}H-NMR Spectrum of [5c]

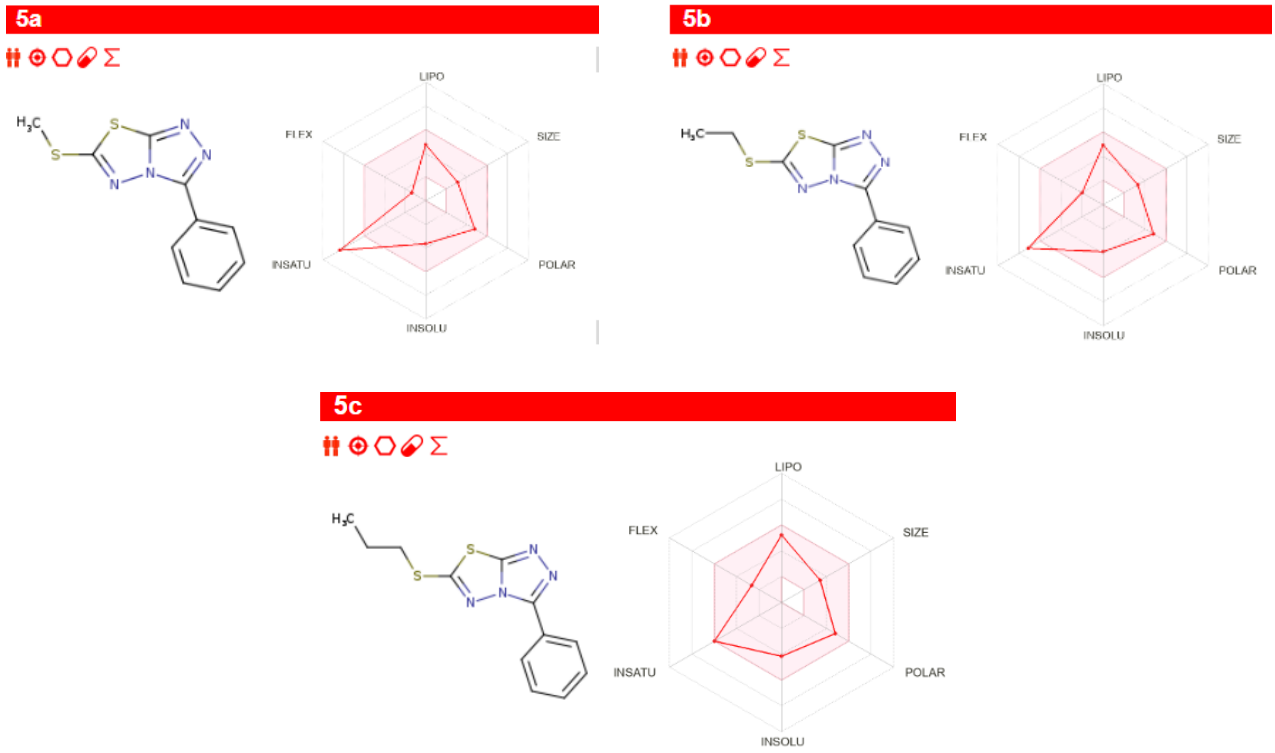


Fig. 9. Bioavailability radar enables a first glance at the drug-likeness of a molecule

Essential physiochemical characteristics, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and medicinal chemistry may be determined using the Swiss ADME Web application to speed drug-likeness and radar evaluation. The six physicochemical qualities considered are lipophilicity, size, polarity, solubility, unsaturation, and flexibility. There is a pink region in which the radar plot of the molecule must fall entirely for it to be termed drug-like, and it is projected that the chemical is bioavailable when taken orally, as illustrated in Table 2 [34-40].

Table 2. Physicochemical properties of synthesized compounds (5a-5c)

Compound	**5a**	**5b**	**5c**
Formula	$C_{10}H_8N_4S_2$	$C_{11}H_{10}N_4S_2$	$C_{12}H_{12}N_4S_2$
Molecular weight	248.33 g/mol	262.35 g/mol	276.38 g/mol
Num. heavy atoms	16	17	18
Num. from. heavy atoms	14	14	14
Fraction Csp3	0.10	0.18	0.25
Num. rotatable bonds	2	3	4
Num. H-bond acceptors	3	3	3
Num. H-bond donors	0	0	0
Molar Refractivity	65.82	70.62	75.43
Log K_p (skin permeation)	-5.85 cm/s	-5.68 cm/s	-5.39 cm/s

Lipinski's rule of five is a framework used in pharmaceutical research to assess the probability of a compound's viability as a therapeutic agent. The guideline indicates that a chemical is more probable to exhibit oral bioactivity and have favorable pharmacokinetic characteristics if it satisfies the following criteria: Molecular weight is less than or equal to 500. MLogP $\leq$ 4.15, N or O $\leq$ 10, NH or OH < 5. The ADME results of synthesized compounds [5a-5c] indicate compliance with Lipinski's criterion, demonstrating no violations of drug-likeness, and are closely aligned with drug-likeness in silico. ADME predictions were conducted on these compounds, revealing that most adhered to the Lipinski, Veber, and Egan guidelines, demonstrating favorable drug-likeness, certain oral bioavailability characteristics, and robust pharmacokinetic profiles. This investigation verifies that these compounds are non-hepatotoxic, as illustrated in Table 3.

Table 3. Drug likeness, bioactivity, and synthetic accessibility score (5a-5c)

Drug likeness	**5a**	**5b**	**5c**
Lipinski	yes, 0 violations	yes, 0 violations	yes, 0 violations
Ghose	yes, 0 violations	yes, 0 violations	yes, 0 violations
Veber	yes, 0 violations	yes, 0 violations	yes, 0 violations
Egan	yes, 0 violations	yes, 0 violations	yes, 0 violations
Muegge	yes, 0 violations	yes, 0 violations	yes, 0 violations

Bioavailability Score	0.55	0.55	0.55

ADME result of synthesized compounds [5a-5c] showed the solubility parameter in water that the log S (ESOL) range is soluble for all compounds except 5c, which is moderately soluble; solubility refers to the ability of a molecule to dissolve in a particular solvent. This property is important because many biological processes occur in aqueous environments, and solubility affects how well a molecule can be transported. An important parameter in the ADME study is solubility in water measured by log S. The solubility of compounds is classified according to the following log S scale: "insoluble <−10<poorly<−6<moderately<−4<soluble<−2< very<0<highly".

According to the ADME result, the solubility parameter in water showed a log S (ESOL) range of synthesized compounds [5a-5c] between -4.17 and -3.64, which are soluble depending on the log S scale, except 3c is moderately soluble.

Topological Surface Area (TPSA). The results of the Swiss ADME tool showed the TPSA of the most synthesized compounds 6-(6-(methylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5a], 6-(6-(ethylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5b], 3-phenyl-6-(propylthio)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5c] have a TPSA < 121 Å² (96.62 Å²). This indicates favorable characteristics for membrane permeability and oral bioavailability, making the compound potentially more suitable for development as an orally administered drug. The compounds do not face membrane permeability and oral bioavailability challenges; GIT absorption occurs.

The compounds [5a–5c] exhibited high gastrointestinal (GIT) absorption, which is an important attribute for ensuring effective oral bioavailability. However, the results also indicated that these compounds do not cross the blood–brain barrier (BBB)**,** suggesting that they are unlikely to reach the central nervous system (CNS)**.** Consequently, while these compounds may be well suited for peripheral or gastrointestinal therapeutic **targets**, they may not be appropriate for drugs intended to act on the CNS**,** where BBB permeability is a critical determinant of pharmacological efficacy.

The BOILED-Egg plot generated by the SwissADME web tool (Figure 10) and the data summarized in Table 4 further support these findings for the synthesized compounds **[**5a–5c**]**, illustrating their predicted absorption and BBB-penetration profiles.

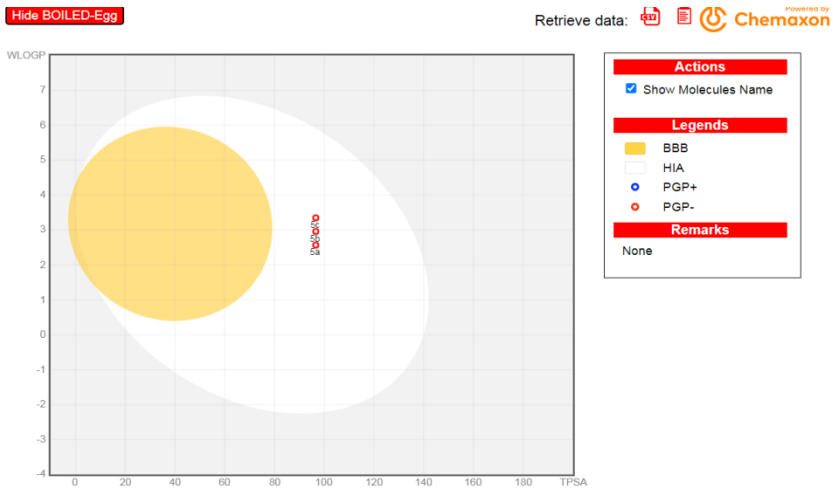


Fig. 10. BOILED egg plot of synthesized compound [5a-5c] from the Swiss ADME web tool

Table 4. Predicted of pharmacokinetics, water solubility of compounds [5a-5c]

Pharmacokinetics	5a	5b	5c
Log Po/w (WLOGP)	2.81	3.46	3.57
TPSA	96.62 Å²	96.62 Å²	96.62 Å²
GI absorption	High	High	High
BBB permeant	No	No	No
Log S (ESOL)	-3.64	-3.85	-4.17
Water Solubility Class	Soluble	Soluble	Moderately soluble

3.9. Anticancer activity. Hep G2 (or HepG2) is a human liver cancer cell line. Hep G2 [HEPG2] is a cell line distinguished by its epithelial-like morphology, originating from a hepatocellular carcinoma of a 15-year-old Caucasian male from Argentina diagnosed with liver cancer. The Wistar Institute supplied the cell line and a suitable transfection host.

The human hepatic cell line WRL-68 has morphology similar to that of hepatocytes and primary hepatic cultures. Cells have shown the production of albumin and alpha-fetoprotein, along with the expression of liver-specific enzymes such as alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, and alkaline phosphatase. Previously infected with Mycoplasma; treated and resolved at ECACC. The WRL 68 cell line was previously thought to derive from embryonic liver tissue. The cell line is identical to HeLa based on STR PCR DNA profiling. Therefore, the cell line should be considered as derived from HeLa [41]. The cytotoxicity of the synthesized molecule 3-phenyl-6-(propylthio)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5c] was tested against HepG2 and WRL68 cell lines by using the MTT assay, which is based on the changing in the color of (3-(4,5-dimethyl-2-thiazolyl)bromide-2,5-diphenyl-2Htetrazolium) from yellow to purple when the living cells are exposed to apoptosis. After keeping the platters that were used for 1 day at body temperature and in the presence of CO_2, specific concentrations of the produced compound 3-phenyl-6-(propylthio)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5c] are added as shown (25, 50, 100, 200, and 400 μg mL^{-1}), and the results of IC_{50}, which is half maximal inhibitory concentration, revealed that synthesized compounds [5c] have anticancer activity but at different levels based on the functional substitution group of the compounds, and the number of doses that were mainly used indicate that the activity of the anticancer is directly proportional to increasing the concentration of the drugs that were used. It demonstrates little efficacy at low dosages. The IC50 values for WRL68 (238.5) and HEPG2 (87.22) are influenced by several parameters, including lipophilicity and van der Waals volume. Figure 11 illustrates the outcomes of the anticancer activities of [5c] [42-49].

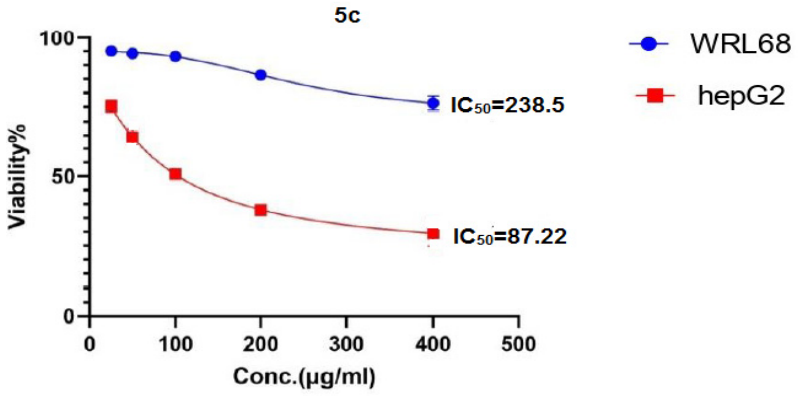


Fig. 11. Dose-dependent cytotoxic effect of 4a on hepG2 and WRL68 cell lines.

Conclusion

This study examines derivatives of a sequence of 6-(6-(methylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]hiadiazole [5a], 6-(6-(ethylthio)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5b], 3-phenyl-6-(propylthio)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole [5c]. The produced compounds were validated using various spectral techniques, including FT-IR, ^{1}H-NMR, and ^{13}C-NMR. The ADME profiles indicate good oral bioavailability. The pharmacokinetic properties of the synthesized compounds were investigated because many newly developed molecules tend to fail in the later stages of drug discovery and development due to poor ADME behavior. Theoretical pharmacokinetic analyses were therefore performed using the SwissADME tool, which suggested that the compounds possess favorable oral bioavailability, particularly suitable for drugs targeting the gastrointestinal system**.** According to the SwissADME predictions, the newly synthesized compounds exhibit desirable absorption, distribution, metabolism, and excretion (ADME) characteristics, indicating their potential for further drug development**.** An in silico ADME assessment was conducted to evaluate the drug-likeness of the synthesized molecules. The results revealed that most compounds complied with the Lipinski**,** Veber**,** and Egan rules, demonstrating favorable drug-likeness**,** oral bioavailability, and pharmacokinetic profiles. Furthermore, this analysis confirmed that the compounds are non-hepatotoxic. Anticancer evaluations showed that the synthesized compounds exhibited significant activity against HepG2 cancer cells, while displaying minimal cytotoxicity toward normal human liver cells (WRL68**).** Notably, one of the synthesized compounds demonstrated a strong cytotoxic effect on the HepG2 cell line (IC_{50} = 99.73 µg/mL), confirming its potential as a promising anticancer agent.

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