Antioxidant activity and Pre-ADME study of new 1,3,4, thiadiazole derivatives

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Abstract

A new thiadiazole derivative [4-(5-amino-1,3,4 thiadiazole-2yl)-2,6-di-tert-butylphenol] (compound A1) and its Schiff base derivatives (compound A2-A3) were in silico evaluated for their pharmacokinetic properties, and the antioxidant activity was studied in vitro using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent as a simple, rapid assay method. The pre-ADME results referred to good oral absorption of compound A1 from the GIT with no CNS side effects after oral absorption. The liver dysfunction was not expected upon administration. The new derivatives have good antioxidant activity compared with ascorbic acid. The percent of free radical inhibition of compound A2 was 68.85% at a concentration of 250 µg/ml, while the IC50 value was 161.95 µg/ml.

Keywords: Thiadiazole, Schiff base, DPPH, ADME.

دراسة نظرية للتنبؤ عن الخصائص الحركية والتقييم الدوائي المختبري لمشتقات جديدة من1, 3, 4 ثايادايازول

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الخلاصة

مشتقات جديدة من الثايوديازول مركب A1 (4-(5-amino-1,3,4 thiadiazole-2yl)-2,6- di- tert-butylphenol) وقواعد الشف المحضرة منة (مركب A2 و A3) تم دراسة الخصائص الحركية لها نظريا. بالإضافة الى الدراسة المختبرية لفعالية مضاد الاكسدة باستخدام مادة1,1- -diphenyl-2-picrylhydrazyl(DPPH) كونها طريقة سهلة وسريعة. حيث اظهرت نتائج دراسة الخصائص الحركية الامتصاصية المعوية الجيدة للمركب A1 وعدم وجود تأثير جانبي على الجهاز العصبي المركزي بعد الامتصاص الغموي. ايضا توقع عدم وجود تأثير للمركبات على نشاط الكبد عند الاستخدام. المركبات الجديدة تمتلك فعالية جيدة كمضادات اكسدة عند المقارنة مع حمض الاسكوربك. حيث وصلت نسبة التثبيط للمركب A2 الى 68.85%)) عند التركيز(250مايكرو غم\ مل) بينما كانت قيمة التركيز النصفي له (161.95مايكرو غم\ مل).

الكلمات المفتاحية: ثايودايازول, قواعد الشف,DPPH, الخصائص الحركية

Introduction

Heterocyclic compounds are cyclic organic compounds that include at least one hetero atom in their structure. Nitrogen (N), sulfur (S), and oxygen (O) are the most commonly occurring heteroatoms **(1).**

They are regarded as crucial constituents in various naturally occurring organic substances such as alkaloids, proteins, medicines, enzymes, and natural colours. The chemicals are primarily categorized as saturated and unsaturated. The saturated compound behaves similarly to acyclic derivatives but with changes in steric effects. Unsaturated compounds, particularly those with 5- or 6-member rings, have been extensively researched because of their unstrained structure. Heterocyclic chemicals are extensively used in veterinary, medicinal, and agrochemical goods(1, **2).**

Heterocyclic compounds, particularly those containing nitrogen, play a crucial role in biological processes due to their widespread use as active agents in pharmaceutical frameworks **(3).** Thiadiazoles are a subset of azole compounds in chemistry, named according to the Hantzsch-Widman nomenclature. They are nitrogen and sulfur-containing five-membered heterocyclic compounds possess two double bonds and a ion pair of Sulphur with the chemical formula C2H3N2**(4, 5).**

Four distinct structures are present based on the different arrangements of the heteroatoms. These structures are considered structural isomers rather than tautomers because they do not convert between each other. The structures are 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole. **(6, 7).**

1,3,4-thiadiazole derivatives are believed to display diverse biological effects because of the presence of the =N-C-S- functional group **(8).** Some researchers believe that the biological effects of 1,3,4-thiadiazole derivatives are attributed to the ring's strong aromatic properties, which contribute to its in vivo stability and minimal toxicity in higher vertebrates, including humans **(9).** Many drugs with a thiadiazole ring are available in the market, including carbonic anhydrase inhibitors like acetazolamide and methazolamide for diuretic purposes, cefazolin as a first-generation cephalosporin, sulfamethizole as an antimicrobial sulfonamide, and the antiparasitic drug megazol**(10).** Schiff bases are compounds that include an imine or azomethine (–C=N–) functional group, first documented by Hugo Schiff **(11, 12).** They are the most commonly utilized chemical substances as pigments, dyes, catalysts, intermediates in organic synthesis, and polymer stabilizers**(13).**

Additionally, Schiff bases have been proven to exhibit a wide spectrum of biological activities such as antifungal, antibacterial **(14),** antimalarial **(15)**, antiproliferative, anti-inflammatory **(16)**, antiviral, and antipyretic effects (**17**). Schiff base is a major group in the structures of certain medications, such as dantrolene (a muscle relaxant), nifuroxazide (an antibiotic), and thiacetazone (an antituberculosis medication) **(18)**, as shown in **Figure 1**.

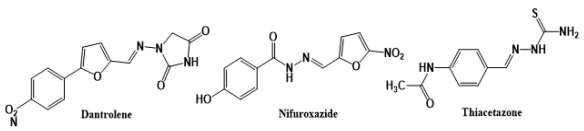
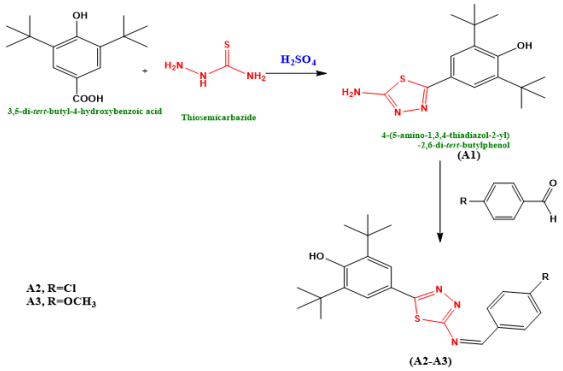


Figure 1: Chemical structure of some Schiff base-containing drugs.

Oxidative stress caused by reactive oxygen species (ROS) is considered a key contributing element to multiple inflammatory disorders. ROS are associated with cancer, coronary heart disease, and neurological illnesses, leading to DNA damage in cells. Antioxidants work by scavenging or inhibiting the production of ROS, which helps in preventing the formation of free radicals and slowdown the development of various chronic diseases like cancer, inflammation, and cardiovascular diseases **(19).** This study aimed to investigate the antioxidant activity of some thiadiazole-synthesized derivatives.

Material and Methods

The anhydrous solvent and reagent were all used as supplied by the commercial vendors (USA; BDH, Pool Dorset, England; and Sigma-Aldrich, Munich, Germany). The method of synthesis for the tested compounds was mentioned in our previous work**(20).** The chemical structure of the tested compounds is mentioned in **Scheme 1.**



Scheme 1: The chemical structure of tested compounds A1 and A2-A3.

1.In-vitro Antioxidant assay

The free radical scavenging ability of the new thiadiazole derivatives (A1 and A2-A3) has been measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH). This was achieved through the utilization of the Blois technique with some modifications (21). Different concentrations (50, 100, 150, 200, and 250 µg/mL) of vitamin C, and tested compounds (A1-A3) were prepared. A 0.1 mM DPPH solution in ethanol was prepared.

For each concentration, 2700 µl was mixed with 300µl of the DDPH solution. Each solution was prepared in triplicate. After that, the mixtures were stirred rapidly and left in the dark for 30 minutes at room temperature. Consequently, the absorbance of each prepared sample was measured at 517nm. A percent of free radical inhibition for each sample was calculated using the following equation:

Equation 1(22):

$$\%\ free\ radical\ inhibition = \left(\frac{Ac - At}{Ac}\right) \times 100$$

Where (Ac) represents the control absorbance (ethanol + DPPH without sample) while (At) is the tested sample absorbance (ethanol + DPPH + sample). The absorbance of each triplicate was measured. Then an average was calculated, as shown in **Table 1 and Figure 2.** Consequently, IC50 values (the concentration of an antioxidant-containing substance required to scavenge 50% of the initial DPPH radicals(23)) were calculated from the first-order linear equation.

2.Computational study of synthesis compounds

In-silico ADMET and Drug likeness prediction

The insilico drug screening method, besides drug-likeness prediction, has been commonly used to predict the pharmacokinetics, toxicities, and oral bioavailability of the synthesized compounds.

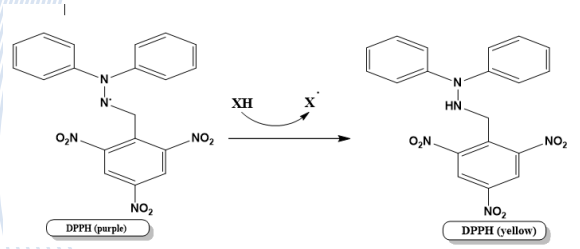
Consequently, absorption, distribution, metabolism, excretion, and toxicity (ADMET) estimation of the compounds has been predicted using Pre-ADME online software. These descriptors involve gastrointestinal absorption, plasma protein binding, blood-brain barrier penetration, and inhibition of liver enzymes. Furthermore, Lipinski's Rule was utilized to predict the drug-likeness of the compounds. Therefore, H-bond donors and acceptors, CLog p, and Topological Polar Surface Area (TPSA) were calculated. The study was carried out using the Swiss Institute of Bioinformatics on web site: **http://www.swissadme.ch/.**

Results and discussion

1.Free radical scavenger activity

The free radical DPPH is a simple, inexpensive method used for the evaluation of antioxidant capability**(24).** DPPH is a stable crystalline compound with free radical particles that have a dark visible purple color. Its absorbance is at 517nm. The delocalization of the electron over the whole DPPH molecule has contributed to its stability, consequently, the molecules don't dimerize **(25).** Once reduced, the profound dark violet color turns into yellow, either by proton or electron donated group; accordingly, any compound with such characteristics can be considered an antioxidant **(see scheme 2).**

The % inhibition and IC50 values of the tested compounds and ascorbic acid, which was considered a standard, were mentioned in T**able 1 and Figure 2.** It was found that compound A, which contains electron donated group (OH, NH2), showed moderate antioxidant activity as compared with the standard with IC50 (198.9µg/mL) and % inhibition (59.65) at concentration (250 µg/mL). In comparison, IC50of ascorbic acid was (101.43 µg/mL) and % inhibition (69.65) at concentration (250 µg/mL). Additionally, the presence of 1,3,4 thiadiazole ring has little effect on improving the antioxidant activity of the new derivatives (A1-A2) where IC50 values were (161.59 and 236.07µg/mL) respectively.



Scheme 2: Mechanism of antioxidant activity by DPPH

Concentration µg/ml	A1		A2		A3		Ascorbic acid	
	Abs.*	INH %	Abs.*	INH %	Abs.*	INH %	Abs.*	INH %
50	0.77	18.85	0.89	9.61	0.86	9.38	0.52	45.01
100	0.69	28.12	0.63	42.33	0.80	16.37	0.49	48.69
150	0.56	41.59	0.53	53.40	0.67	29.41	0.43	55.16
200	0.47	50.72	0.48	59.97	0.56	41.40	0.366	61.76
250	0.38	59.65	0.41	68.85	0.45	52.55	0.29	69.65
IC_{50}	198.90		161.59		236.07		101.43	

*absorbance represents the average of triplicate. INH%: inhibition %

Table 1:Antioxidant activity of compounds A1 and (A2-A3) using DPPH

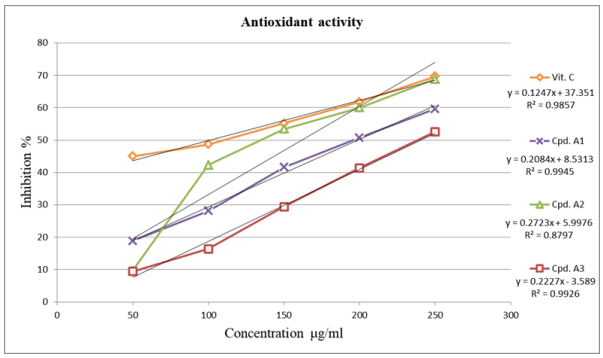


Figure 2: Antioxidant activity of tested compounds and Ascorbic acid by DPPH.

2.Physicochemical properties and Drug likeness prediction of synthesized compounds (A1 and A2-A3)

Hydrogen bonds (HBs) are attractions between a proton attached to an electronegative atom (a proton donor) and a lone pair of electrons on an electronegative atom (a proton acceptor) **(26).** According to data in Table 2, compound A1 has (2) HBD and (4) HBA, while its Schiff base derivatives (A2-A3) have (1) HBD and (4 and 5) HBA, respectively.

Clog P is an essential physicochemical property that demonstrates molecular lipophilicity. Whenever its value is increased, the lipophilic character is increased**(27).** It is worthwhile to note that the CLog p-value for compound A1 and its derivatives were (4.66, 6.90, and 6.26).Moreover, the total polar surface area (TPSA) is an important analysis that reveals drug bioavailability.

For passively absorbed molecules, the oral bioavailability is low when TPSA >140 **(28).** The synthesized compounds showed an acceptable value of TPSA, as shown in **Table 2**. Thus, the tested compounds are predicted to have good bioavailability. Moreover, the drug-likeness study indicated that compounds A1 and A2-A3 have most of Lipinski's Rule criteria for oral administration.

Parameter	Cpd. A1	Cpd. A2	Cpd. A3
Chemical formula	$C_{16}H_{23}N_3OS$	$C_{23}H_{26}ClN_3OS$	$C_{24}H_{29}N_3O_2S$
Mol. Wt. g/mol	305.44	427.99	423.58
NRB	3	5	6
NHA	4	4	5
NHD	2	1	1
CLog p	4.66	6.9	6.26
/	100.27	86.61	95.84
Water solubility	Moderate	poor	poor
Lipinski	Yes, 0 violation	Yes, 1 violation	Yes,1violation

Mol. Wt.: Molecular weight, NRB: No. of rotatable bond, NHA: No. of H bond acceptor, NHD: No. of H bond donor TPSA: total polar surface area.

Table 2: Physicochemical properties of compound A1 and its derivatives

3.In-silico pre-ADMET study of synthesized compounds (A1 and A2-A3)

The ADMET properties of drugs can predicted by P-glycoprotein binding, intestinal absorption, and skin permeability levels**(29).** The data displayed in Table 3 showed that compound A1 has a good GIT absorption, while compounds A2 and A3 were low. Additionally, it has been found that all tested compounds were unable to penetrate the blood-brain barrier (BBB). This indicates that central nervous system (CNS) side effects are not expected.

Furthermore, the metabolism profile was predicted depending on the CYP models, in particular (CYP1CA2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4). It was found that there was no inhibitory effect against most of the tested liver enzymes, as shown in **Table 3.** Thus, liver dysfunction is less expected upon administration.

Parameter	Cpd. A1	Cpd. A2	Cpd. A3
GI absorption	High	Low	Low
BBB penetration	-	-	-
p-gp subs.	-	-	-
CYP 1A2 inhibitor	-	-	-
CYP 2C19 inhibitor	-	+	+
CYP 2C9 inhibitor	-	+	+
CYP 2D6 inhibitor	-	-	-
CYP 3A4 inhibitor	-	-	-
Log k_p cm\s	-4.85	-4.05	-4.18

GI: gastrointestinal, BBB: Blood blood-brain barrier, p-gp: plasma protein, CYP: cytochrome, Log Kp: skin penetration.

Table 3: ADMET and Drug likeness prediction of compound A and its derivatives

Conclusion

The presence of an electron-donated group at the para position of Thidiazole –Schiff base derivative (A2) plays an important role in enhancing antioxidant activity and represents a promising ligand for the design of a new agent with the prediction of good bioavailability after oral administration with no CNS expected adverse effect upon uses.

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