Design, Synthesis, and Biological Evaluation of New 5-Substituted-1,3,4-thiadiazole-2-thiols as Potent Antioxidants

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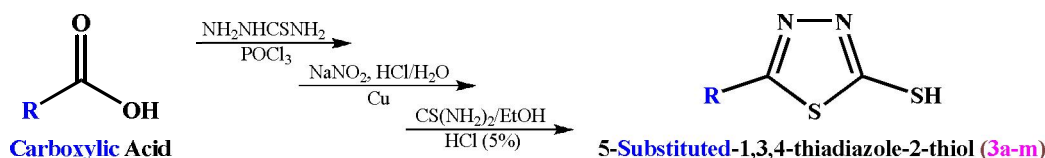*Corresponding Author: amgadpharmacist1@yahoo.com and pharm*org*chem1@mans.edu.eg

Abstract: A novel series of thirteen 5-substituted-1,3,4-thiadiazole-2-thiols (**3a-m**) was designed, synthesized, and evaluated for its potential antioxidant activities. Structural modifications at position 5 of the 1,3,4-thiadiazole scaffold (linked to a fixed antioxidant thiol group at position 2 of the ring) was expected to give new 1,3,4-thiadiazole derivatives with a broad spectrum of biological antioxidant activity. The synthesis of these new compounds was achieved through three different steps. Undoubted elucidation and confirmation of the chemical structures of all the newly synthesized compounds were accomplished using both the spectroscopic (IR, ^{1}H-NMR, and mass spectroscopy (MS)) and elemental (C, H, and N) analyses. The pharmacological screening for evaluation of the antioxidant activity of the new thirteen target thiol compounds was done using *in vitro* antioxidant screening by both 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay (ABTS test) and 2,2-diphenyl-1-picrylhydrazyl assay (DPPH test). The results of both assays showed that three compounds (**3b,d,h**) exhibited interestingly very high antioxidant activities and they could be very promising lead and parent compounds for the design and synthesis of new antioxidant agents by further *in vivo* biological evaluation, structural modifications, investigations, computational studies, and SAR establishment.

Graphical Abstract:

1. Introduction

Medicinally (as defined by the National Cancer Institute at the National Institutes of Health, U.S.A.), antioxidants are chemical compounds that may protect cells from the damage caused by unstable molecules known as free radicals. These antioxidant substances include those of a nonenzymatic as well as an enzymatic nature.[1-4] The oxidative stress and damage, caused by the attack of excess free radicals and other ROS/RNS (other nonradicals), is implicated in the pathogenesis and development (and also indicative) of various diseases in human, i.e., either as a primary cause or as a consequence of disease progression, specially, the chronic diseases and degenerative disorders,[2,5-7] such as neurodegenerative diseases,[2,3,5-14] cardiovascular diseases,[2,3,5,6,8,10,14-16] hepatic and pancreatic diseases,[2,3,5-8,10,14,16-18] renal and urological diseases,[2,5,6,8,10,14] respiratory diseases,[2,3,6,8,10] ocular (ophthalmic) diseases,[2,3,5,6,8,10,14] dermal/hair/nails diseases,[2,3,6,8,19] orthopedic diseases,[2,3,5,6,10] hematologic diseases,[2,3,6,8,14] gastrointestinal (gastroenterological) or digestive diseases,[2,3,6,8,10] immunological and infectious diseases,[2,3,6] otorhinolaryngological (ear, nose, and throat) and dental diseases,[2,3,5,8,10] andrological/gynecological/obstetrical (reproductive system) diseases,[2,3,6,8] and other (e.g., multiorgan or multisystem) diseases.[2,5-10,14,16-18] Antioxidants can be classified according to many items,[3,7,8,15] but, generally, they can be classified into natural antioxidants (including many enzymes such as superoxide dismutase and glutathione reductase; some vitamins such as vitamins E and C; carotenoids such as carotenes and lycopene; some polyphenols such as resveratrol and silymarin; some hormones such as melatonin; some coenzymes such as ubiquinol which is the reduced form of coenzyme Q_{10}; some inorganic nutrients/chemical elements such as selenium and copper; and various natural antioxidant compounds such as glutathione, bilirubin, and uric acid) and synthetic antioxidants (including many dietary or nutritional antioxidant supplements such as ebselen, trolox, disufenton sodium, and raxofelast; many food additives and preservatives such as propyl gallate and butylated hydroxytoluene; and other synthetic antioxidant medicines).[2-4,6-8,14] Figure 1 (below) shows the chemical structures of two of the most potent antioxidants (vitamin C and trolox).

The usefulness of 1,3,4-thiadiazole ring as a privileged structural system in medicinal chemistry has prompted the advances of the therapeutic potentials of this system.[20] The compounds containing 1,3,4-thiadiazole nucleus, in addition to being very important organic reaction intermediates for molecule planning as they undergo various chemical reactions, are one of the most biologically active classes of compounds as they possess an enormous spectrum of potent pharmacological activities.[20-25] Some researchers[26,27] proved and reported the antioxidant activity of 5-substituted-2-mercapto-1,3,4-thiadiazoles (5-substituted-1,3,4-thiadiazole-2-thiols), the class to which the target compounds (**3a-m**) of this new research belong. For example, in 2008, Caroline Prouillac and her coworkers[26,27] synthesized a new series of 5-substituted-2-mercapto-1,3,4-thiadiazoles (**3Thia**, Figure 2) with relatively potent antioxidant and radioprotective activities. They also reported the importance of thiol (SH) group for the antioxidation effect of thiol derivatives of 1,3,4-thiadiazoles. Based on these previous findings, we report here the synthesis of a new series of 5-substituted-2-mercapto-1,3,4-thiadiazoles with the objective to study the effect of changing the substitution at position 5 of the 1,3,4-thiadiazole ring on the antioxidant activities of these new target compounds.
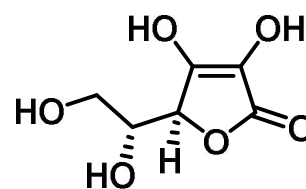
Vitamin C (L-ascorbic acid)
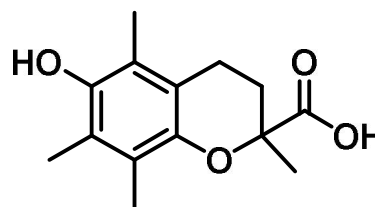
Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; a water-soluble analog of α-tocopherol form of vitamin E)

Figure 1. The chemical structures of vitamin C (a very strong natural antioxidant) and trolox (a strong synthetic antioxidant)
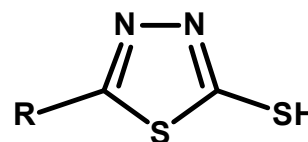
3Thia

R = SCH$_2$CH$_3$, CH$_2$CH$_3$

Figure 2. The general chemical structure and skeleton of the new antioxidant 5-substituted-2-mercapto-1,3,4-thiadiazoles (**3Thia**, with the substituents at position 5 shown below it) synthesized by Caroline Prouillac and her coworkers

2. Research Aims and Rationale

The large number of the diverse problems associated with the use of old antioxidant compounds and drugs (antioxidants, in general, and antioxidant 1,3,4-thiadiazoles, in particular) that are present in the previous art of literature and market, such as weak potency and efficiency, severe side (adverse) and toxic effects (due to other undesirable biological actions), low bioavailability, imbalanced water/lipid solubility (which leads to suboptimal pharmacokinetics like weak absorption from the gastrointestinal tract and difficulty in crossing the blood-brain barrier in human), drug-drug interactions, hypersensitivity, and expensive costs of synthesis (i.e., high price), triggered the need of new generations of antioxidants and antioxidant 1,3,4-thiadiazoles.[7,18,22,24-27]

In order to solve all the above-mentioned problems, we decided to design and synthesize a new series of 5-substituted-2-mercapto-1,3,4-thiadiazoles having simple uncomplicated structures (with low molecular weights), proposed to be biocompatible, with increasing water and/or lipid solubility of these compounds, according to need, to balance their solubility, improve their bioavailability, and solve all the other expected pharmacokinetic problems; also with masking and overcoming all unwanted side effects and decreasing nonselective cytotoxicity showed by old analogs of these 1,3,4-thiadiazole derivatives (by many ways, such as avoiding incorporation of certain functional groups and known pharmacophoric moieties, responsible for these undesirable severe side and toxic effects, in the structures of these compounds as much as possible); and also with increasing antioxidant activities of these 2,5-disubstituted-1,3,4-thiadiazoles (by, for example, trying incorporation of considerable number of diverse and different substituents at position 5 of the 1,3,4-thiadiazole scaffold and studying the effects of these structural differences and modifications on the observed physicochemical properties and, as a result, on the experimental antioxidation properties of these compounds to make interpretation and correlation of these results to elucidate and construct the structure-antioxidant activity relationship (SAR) for this type of compounds). Also, we designed cheaper and greener synthetic procedures and pathways (e.g., using green chemistry techniques in synthesis, mainly, MW-assisted methods, which are much more efficient than conventional traditional methods of synthesis), and we used much cheaper and readily available starting materials (e.g., cheap carboxylic acids) for synthesis of these 1,3,4-thiadiazole derivatives.

In order to build a good general antioxidant 2,5-disubstituted-1,3,4-thiadiazole model (to solve all the previously mentioned problems), the following general structural formula was firstly designed and given the number or general code **3** (Figure 3) to lead us in the synthesis of the new 1,3,4-thiadiazole series **3a-m**.

This proposed antioxidant 2,5-disubstituted-1,3,4-thiadiazole structural model consists of three important complementary parts (i.e., three parts acting in a complementary way to give the best antioxidant activities expected), which are:

a- *Aromatic 1,3,4-Thiadiazole Ring*: Many of the compounds containing 1,3,4-thiadiazole ring (the scaffold and main part in this model) efficiently exhibit electron donor-acceptor properties, specially when an EDG is attached to this ring, as the introduction of EDGs into the electron-withdrawing heterocyclic 1,3,4-thiadiazole ring affords excellent electron donor-acceptor compounds that are easily both oxidized and reduced (i.e., having excellent antioxidant activities by being easily oxidized by oxidants which include ROS/RNS and all other free radicals).[21]

1,3,4-thiadiazole moiety traps free radicals and ROS/RNS by potential conjugation of the aromatic structure, in addition, it is characterized by many unique properties that are not collectively present in most other ring systems, such as acting as a hydrogen-binding domain (this greatly increases the antioxidant properties of the compounds containing it), acting as a two-electron donor nitrogen system (due to the presence of two -N=C-S- moieties that exhibit a wide variety of bioactivities, mainly antioxidant activities), having one centered electronegative sulfur (S) atom (this also increases the antioxidant properties of the compounds containing it), being a constrained antioxidant pharmacophore, having a strong aromaticity and resonance effect of the ring system (which is required in most antioxidant pharmacophoric moieties and, also, gives the ring unusual great stability), having ambipolar characteristics (e.g., it can react with and be attacked by both nucleophiles and/or electrophiles), having a great *in vivo* stability (this makes most of the compounds, containing this scaffold, are very biocompatible and with very high bioavailability), lacking a toxicity for higher vertebrates (including human) and this makes most of its 2,5-disubstituted derivatives having very minor or negligible side/toxic effects and contraindications in human, its attachment at positions 2 and 5 of the ring to diverse functional groups that interact with biological receptors (specially those mediate and interfere with redox biochemical processes) affords compounds possessing outstanding pharmacological (specially antioxidant) properties, being a bioisostere of many moieties and ring systems (such as 1,3-thiazole) and thus it can be incorporated in the structures of many antioxidant

products which contain these moieties and ring systems (i.e., in place of them) to make use of the extraordinary characteristics of the 1,3,4-thiadiazole ring system and solve many problems associated with the use of these unfavorable bioisosteres in the structures of antioxidants, having good ultraviolet (UV) radiation-absorbing properties (this gives the compounds containing it additional radioprotective activities), being a heat resistant scaffold (this gives the compounds containing it additional stability), having a resistance to acids which makes it stable against and not affected by the strong acidic biological fluids (e.g., the gastric juice), having a great susceptibility to redox reactions even in acidic or alkaline medium, having an ability to form stable mesoionic betaine type compounds; having a good hydrolytic and metabolic stability, and its easy exposition to facile nucleophilic attack taking place at positions 2 and 5 of the ring (the two carbons of the ring) by many groups which are very reactive and exhibiting their typical reactions when present at these two positions.[20-28]

All these various useful properties of 1,3,4-thiadiazole ring dramatically aid and increase the net antioxidant biological activity of the ring derivatives to reach the optimal levels.

b- *Fixed Antioxidant Moiety (SH Group)*: It was very important in the design of this antioxidant model to have a fixed antioxidant moiety (at position 2 of the 1,3,4-thiadiazole ring) that is not changed along all the compounds of this series (1,3,4-thiadiazoles series) to establish the main moiety responsible for the occurrence of the principal *in vivo* redox cycle (among all the possible redox cycles carried out by each 1,3,4-thiadiazole derivative) in which the oxidized form of each 1,3,4-thiadiazole derivative is much more stable (i.e., favorable and predominant) than ROS/RNS and other free radicals (i.e., than most active *in vivo* oxidants).

This group moiety was chosen to have an electronegative heteroatom that is similar to the centered electronegative heteroatom of the heterocyclic 1,3,4-thiadiazole ring (i.e., to have S atom), and as a result, thiol (SH) group was chosen for the 1,3,4-thiadiazoles series. The SH group (attached to an aromatic ring) has very strong antioxidant properties as it characterizes by donating its hydrogen (or, first, giving an electron, then, the proton) to any oxidant or radical, to catch it, very easily (i.e., it is a very good hydrogen donor).[26,27,29]

c- *Changed Aiding Substituent (R)*: An important part, which has a complementary role in increasing the antioxidant activities of this model, is the changeable aiding moiety or substituent (R) at position 5 of the 1,3,4-thiadiazole scaffold. The main function of this aiding substituent R is to increase the net total antioxidant activities of the target compounds, directly, through helping their pharmacodynamic properties (i.e., through giving an additive antioxidant effect to the activity of the original parent 2-mercapto-1,3,4-thiadiazole compound and/or aiding the mechanism of the antioxidant action of the original parent 2-mercapto-1,3,4-thiadiazole compound) and/or, indirectly, through helping their pharmacokinetic parameters to reach the required optimal values according to the need, target site(s) of administration, and target human organs (e.g., by increasing their lipo(hydro)philicity, rate of absorption, and bioavailability).

Using and testing many structurally different R groups (e.g., alkyl/substituted alkyl, alkenyl/substituted alkenyl, and phenyl/substituted phenyl) as aiding substituents can undoubtedly help us in reaching the best ideal model which puts the principal base for any medicinal chemist, in the next designing/synthetic attempts, working on this type of antioxidant heterocyclic compounds.
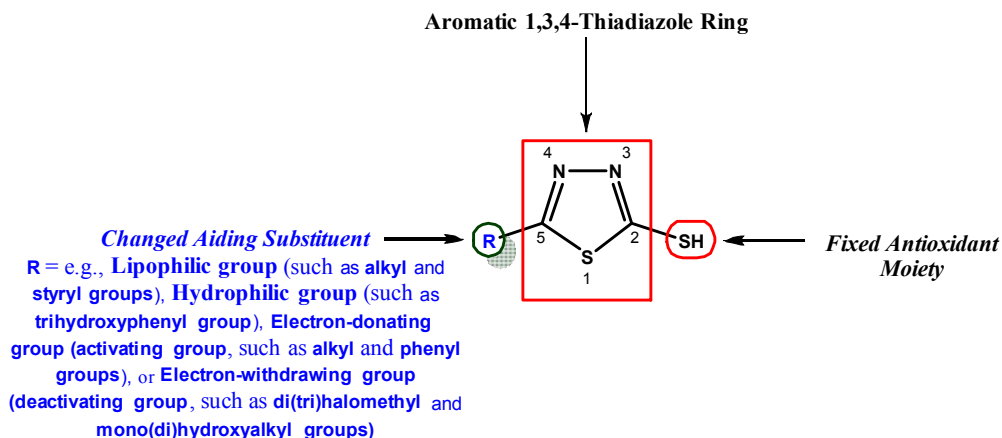
Figure 3. The Proposed general antioxidant 2,5-disubstituted-1,3,4-thiadiazole model

As a result, the substituent R was changed through the new thirteen target compounds of this series (i.e., the new target 1,3,4-thiadiazoles) and used as a tool (being the only changeable part among all the three parts in this series of compounds as the other two previously mentioned parts constitute the pharmacophore and are fixed in the series, hence, R (i.e., R modifications) is the only item to which the differences in the antioxidant activities, among the compounds of this series, could be attributed and correlated) to explore and study the effects of these diverse structural modifications on the experimental antioxidant activities of the target compounds in order to improve the proposed primary antioxidant model to a more advanced ideal one at the end of this new research study through building the structure-antioxidant activity relationship and investigating the structural requirements and pharmacophore for the design of novel potent antioxidant 2,5-disubstituted-1,3,4-thiadiazoles (the chemical structures of all the R groups of the thirteen target compounds **3a-m** are shown in Table 1).

In view of the above-mentioned facts, it is concluded that 1,3,4-thiadiazole scaffold and thiol group have been known to have antioxidant properties and, therefore, according to ''the combination principles'', if an aromatic 1,3,4-thiadiazole ring is directly linked with a thiol moiety at position 2 of the ring and with an aiding group at position 5 of the ring, the produced 2,5-disubstituted-1,3,4-thiadiazoles should be or are expected to be capable of scavenging free radicals, ROS, RNS, and all other types of oxidants in a potent ideal manner.

3. Results and Discussion
3.1. Chemical Syntheses
3.1.1. Synthesis of 5-Substituted-1,3,4-thiadiazol-2-amines (5-Substituted-2-amino-1,3,4-thiadiazoles, 1a-m)

The first step in Scheme 1 (illustrated below) is the synthesis of 5-substituted-1,3,4-thiadiazol-2-amines (intermediate compounds **1** or compounds **1a-m**) which was achieved using the oxidative cyclodehydration reaction of the starting material thiosemicarbazide (Th.) with various starting carboxylic acids (S.C.A.) using phosphorus oxychloride (POCl$_3$) as the dehydrating agent and applying both the conventional (Conv.) and new greener MW methods of synthesis with a very slight modification (just in heating time and/or temperature, and, sometimes, in the dehydrating agent or its excess amount) in most of the different original procedures (either conventional or MW-assisted ones) present in the literature (e.g., the original procedure of Mohamed

et al.,[30] Bingfang and Zengmin,[31] Remers *et al.*,[32] Adiguzel *et al.*,[33,34] Turan *et al.*,[35] Koparir *et al.*,[36] Jassim *et al.*,[37] Song *et al.*,[38-40] An *et al.*,[41] Tu *et al.*,[42] Salimon *et al.*,[43] and Mullick *et al.*[44] for the synthesis of compounds **1a,c,d,f,g,i,k**, respectively; Pattan *et al.*;[45] Mathew *et al.*,[46] Swamy *et al.*,[47] and Shiradkar *et al.*[48] which is applied on 1,2,4-triazoles containing thiosemicarbazide skeleton in their chemical structure; Amir *et al.*[49] and Kato and Ohta[50] which is applied on thiosemicarbazide derivatives; Demirbaş;[51] Sharba *et al.*[52] which is applied on thiosemicarbazide and dicarboxylic acids; Atta *et al.*;[53] Sharabasappa *et al.*;[54] Al-Gawady;[55,56] and Khan *et al.*[57] for intramolecular dehydrative cyclization of thiosemicarbazide derivatives) to make an approximation among these slightly different methods into just only one single highly successful and efficient procedure.

Table 1. List of the chemical structures, shown in blue, of all the diverse R substituents present in the target 1,3,4-thiadiazoles (**3a-m**)
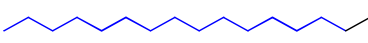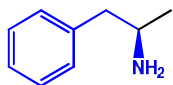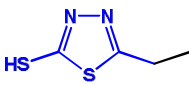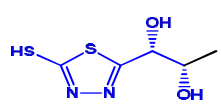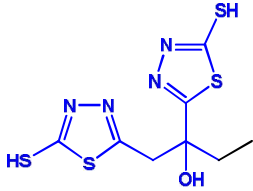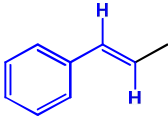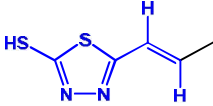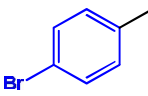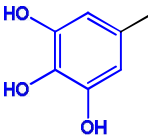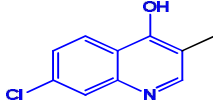
New Target 1,3,4-Thiadiazole	R
3a	
3b	
3c	
3d	
3e	
3f	
3g	
3h	

Table 1. *Continue*

New Target 1,3,4-Thiadiazole	R
3i	
3j	
3k	
3l	
3m	

As reported in and concluded from the Experimental Work Section, Table 2 shows the heating time (in hours or h and minutes or min, respectively) and power (in watts or W) needed by the Conv. method (method A) and MW method (method B), while Table 3 shows a comparative assessment of Conv. method versus MW method of synthesis of 5-substituted-2-amino-1,3,4-thiadiazole compounds (**1a-m**, thirteen compounds were synthesized) from their precursors in terms of overall yield percentage and heating (reaction) time and their improvement in the MW method relative to the Conv. method (this explains how the MW method is much more efficient, time-saving, energy-saving, productive, and more environmentally benign than the traditional Conv. method of heating). Scheme 1 shows that we used acidic alumina as the adsorbent for the reactants at room temperature (R.T.) in method B in which we used the domestic MW oven as the reactor and applying MWI (microwave irradiation) intermittently at intervals of 30 seconds (s) at a frequency of 2.45 GHz (gigahertz). Scheme 1 also shows that we used POCl$_3$ in excess (Xss.) amounts as the dehydrating agent for this reaction in both methods.

The structures of the newly synthesized compounds among compounds **1a-m** (six products are new, compounds **1b,e,h,j,l,m**) were confirmed from their spectral and elemental analyses as reported in the Experimental Work Section. In IR spectra, the general absence of any C=O stretching (no peaks in the region of 1730-1700 cm^{-1} which is very characteristic for any carboxylic acid carbonyl group) was a good indication of conversion of all the carboxylic acids (with thiosemicarbazide) to the heteroring (1,3,4-thiadiazole ring), in addition to, the presence of the common characteristic absorption peaks of NH stretching and bending at frequencies of 3468-3124 cm^{-1} and 1640-1500 cm^{-1}, respectively, was a good indication of the existence of the amino group attached to the 1,3,4-thiadiazole ring, and also the presence of absorption peak representing the C-N stretching (aryl) at frequencies 1351-1216 cm^{-1} indicated the attachment of the amino group to the position 2 of the 1,3,4-thiadiazole ring and not to any of the three heteroatoms of the ring, furthermore, the presence of clear absorption peaks representing the ring C=N stretching at frequencies 1628-1504 cm^{-1} confirmed the formation of nitrogenous heteroring (1,3,4-thiadiazole ring) which contains two -C=N- moieties.[58] In ^{1}H-NMR spectra, the general absence of any characteristic signal for the proton of the OH group of the carboxyl moiety in the range of 10.5-15.0 ppm was an excellent confirmation of conversion of all the carboxylic acids (with thiosemicarbazide) to the heteroring (1,3,4-thiadiazole ring), in addition to, the general presence of singlet signal at 6.971-7.131 ppm indicated the existence of the two protons of the primary aromatic amino group (this also confirms the existence of the amino group attached to the thiadiazole ring).[58] The specific values of MS (mass spectroscopy)[58] and elemental analyses gave a final confirmatory assignment and verification for each compound.

3.1.2. Synthesis of 2-Chloro-5-substituted-1,3,4-thiadiazoles (5-Substituted-2-chloro-1,3,4-thiadiazoles, 2a-m)

The second intermediate step in Scheme 1 is the synthesis of 2-chloro-5-substituted-1,3,4-thiadiazoles (intermediate compounds **2** or compounds **2a-m**) which was achieved using Gattermann reaction (this nucleophilic aromatic substitution reaction is a modified form of Sandmeyer reaction in which copper(I) chloride, i.e., CuCl, salt is replaced by finely divided copper powder which acts as a catalyst in the decomposition of the solution of diazonium salt)[59] in which the diazotization reaction of the corresponding amino derivative (compounds **1a-m**) was made and followed by substitution of the diazonium group by chloro group to obtain the required chloro derivative using the procedure of Caroline Prouillac *et al.*[26,27]
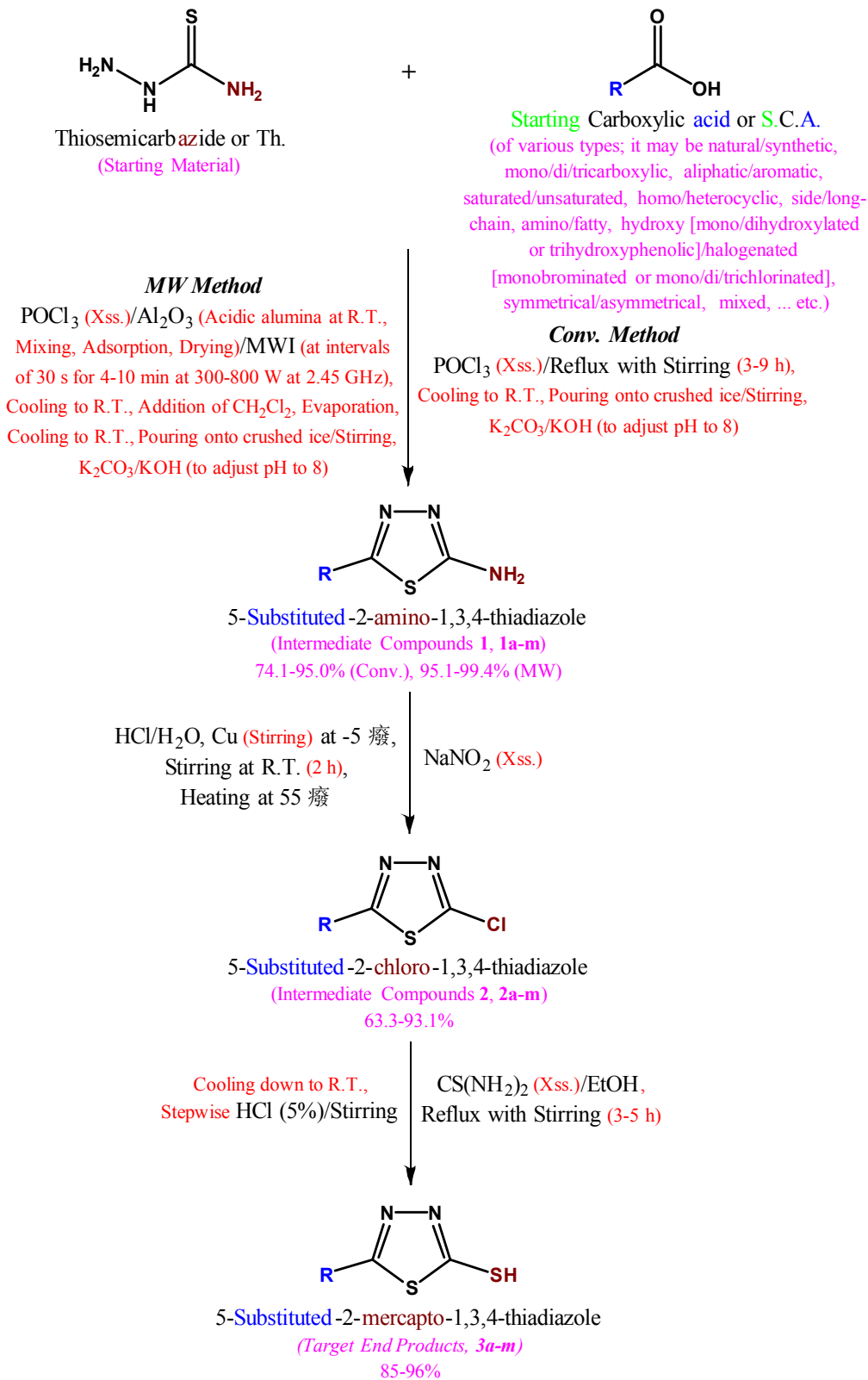
Scheme 1. Synthesis of the target 5-substituted-1,3,4-thiadiazole-2-thiols (**3a-m**)

Table 2. Reaction details (by both methods A and B) for the synthesis of compounds **1a-m**
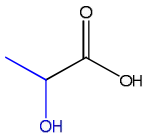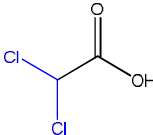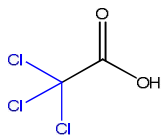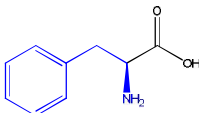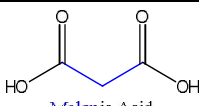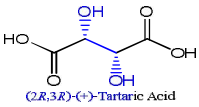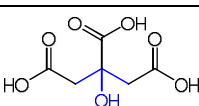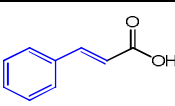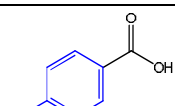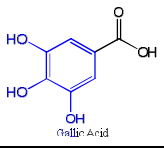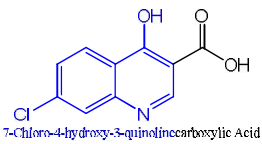
Compound Code	S.C.A. (R-COOH)[a] (Entry)	Molar Ratio of S.C.A.:Th.	Reaction Time & Heating Power	
			Conv. (h), Temperature	MW (min), Power Level (W)
1a	$CH_3-(CH_2)_{14}-COOH$ Palmitic Acid	1:1	4, under reflux	4, 800
1b	Lactic Acid (DL)	1:1	3, under reflux	4, 800
1c	Dichloroacetic Acid	1:1	3, under reflux	4, 300
1d	Trichloroacetic Acid	1:1	3, under reflux	4, 300
1e	(S)-2-Amino-3-phenylpropanoic Acid (L-Phenylalanine)	1:1	9, under reflux	6, 800
1f	Malonic Acid	1:2	8, under reflux	8, 600
1g	(2R,3R)-(+)-Tartaric Acid	1:2	3, under reflux	4, 600
1h	Citric Acid (Anhydrous)	1:3	4, under reflux	5, 600
1i	Cinnamic Acid	1:1	5, under reflux	5, 800
1j	Fumaric Acid	1:2	3, under reflux	4, 450
1k	4-Bromobenzoic Acid	1:1	5, under reflux	7, 600

Table 2. *Continue*

Compound Code	S.C.A. (R-COOH)[a] (Entry)	Molar Ratio of S.C.A.:Th.	Reaction Time & Heating Power	
			Conv. (h), Temperature	MW (min), Power Level (W)
1l	Gallic Acid	1:1	6, under reflux	9, 600
1m	7-Chloro-4-hydroxy-3-quinolinecarboxylic Acid	1:1	7, under reflux	10, 800

[a]The R substituents in di- and tricarboxylic acids are modified in the resulted amino compounds (**1a-m**) and their corresponding chloro derivatives (**2a-m**) and mercapto derivatives (**3a-m**) as previously shown in Table 1.

Table 3. Comparative assessment of Conv. method versus MW method of synthesis of 1,3,4-thiadiazole compounds (**1a-m**) from their precursors in terms of overall yield percentage, heating (reaction) time, and energy consumption, with their improvement percentages or times in the MW method relative to the Conv. method

Item	Conv. Method	MW Method	Improvement
Overall Yield (Range)	74.1-95.0%	95.1-99.4%	4.4-21.0% increase (more productive method)
Heating Time (Range)	3-9 h	4-10 min	40-90 times less (time-saving method)
Energy Consumption Range (KWh)[a]	6-18	0.02-0.13	About 1125 times less (energy-saving method)

[a]KWh: Kilowatt-hour(s); energy consumption (KWh) is equal to the power P (in watts or W) multiplied by time t (in h) divided by 1000 W per kilowatt (KW), i.e., energy consumption (KWh) = P (W) × t (h) / 1000 (W/KW), where, in this present work, P for the used laboratory heater or hot plate, i.e., for Conv. method, is 2000 W and for the used domestic MW oven, i.e., for MW method, is 300-800 W (P range of MW oven for the synthesis of **1a-m**).

Other reported procedures for the synthesis of 2-chloro-5-substituted-1,3,4-thiadiazoles from 5-substituted-1,3,4-thiadiazol-2-amines in the available researches from the previous literature (including the ones used for the previously synthesized 2-chloro-5-substituted-1,3,4-thiadiazoles such as **2k**) used the same idea of the original procedure of Sandmeyer or Gattermann (used in this present work),[26,27,59] but, sometimes, with very few and slight modifications like using Xss. amount of $NaNO_2$ (sodium nitrite) more than three times the amount of the starting corresponding amine (e.g., about four times Xss.),[60] using less amount of Cu powder (e.g., 0.45 g instead of 0.50 or 0.51 g for each 0.01 mole of the corresponding amine),[61] or using different periods of constant stirring at R.T. and/or heating at 55 °C in a water bath.[51,60,61]

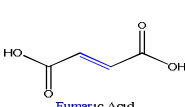Again, only one unified fixed procedure was used, in this present work, for the synthesis of all compounds **2a-m** to make an approach and approximation among these slightly different few methods into just only one single highly successful and efficient standardized procedure (as illustrated later in the Experimental Work Section).

The structures of the eleven newly synthesized compounds among compounds **2a-m** (only two products were previously synthesized among all these thirteen compounds, compounds **2d,k**) were confirmed from their spectral[58] and elemental analyses as reported in details in the Experimental Work Section.

3.1.3. Synthesis of 5-Substituted-1,3,4-thiadiazol-2-thiols (5-Substituted-2-mercapto-1,3,4-thiadiazoles, 3a-m)

The last step in Scheme 1 is the synthesis of the target new 5-substituted-1,3,4-thiadiazole-2-thiols (final new compounds **3** or compounds **3a-m**, i.e., target end products) which was achieved using normal nucleophilic aromatic substitution reaction in which substitution of the chloro (Cl) group, present in the corresponding chloro derivative (compounds **2a-m**), by mercapto (sulfanyl, SH) group (i.e., thiolation reaction) was made to obtain the required mercapto derivative (the thiol or end product) using the procedure of Caroline Prouillac *et al.*[26,27] Other reported procedures for the synthesis of 5-substituted-1,3,4-thiadiazole-2-thiols from 2-chloro-5-substituted-1,3,4-thiadiazoles in the previous literature used the same idea of the procedure of Caroline Prouillac *et al.* (used in this present work).[51,62,63]

The thiol derivatives **3a-m** (all of them are new compounds) were obtained in this final step by the reaction of the corresponding chlorinated compounds (**2a-m**) with Xss. thiourea ($CS(NH_2)_2$) in EtOH (ethanol) under reflux, then, the reaction mixture was cooled down to R.T. and a solution of HCl, i.e., hydrochloric acid, (5%) was added dropwise under stirring.[26,27] The heating (reflux) time for this reaction (in the present work) ranges from 3 to 5 h. Most of the target compounds (compounds **3a,b,c,d,g,j**) need only 3 h of heating (followed by cooling to R.T. and HCl addition) to be formed (i.e., for the reaction to reach completion), while some compounds (compounds **3h,i,k,l**) need 4 h of heating, and few compounds (compounds **3e,f,m**) need 5 h of heating. All the 1,3,4-thiadiazole compounds of series **3** (compounds **3a-m**) were extracted from the reaction mixture solutions by $CHCl_3$ (chloroform) except compounds **3f,g,h,l,m** which were extracted by DMSO (dimethylsulfoxide) due to their lower solubility in $CHCl_3$ (compared to their much higher solubility in DMSO), and all of them were synthesized in very good to excellent yields ranging from 85 to 96% (see Experimental Work Section).

Undoubted elucidation and confirmation of the chemical structures of the newly synthesized compounds **3a-m** were accomplished using both the spectroscopic (IR, ^{1}H-NMR, and MS) and elemental analyses (see Experimental Work Section for the detailed values[58] for each new compound). In IR spectra, the presence of the absorption peaks of SH stretching at frequencies of 2731-2451 cm^{-1} was an excellent indication of the existence of the mercapto group attached to the 1,3,4-thiadiazole ring at position 2, in addition to, the presence of absorption peaks representing the normal moieties that constitute the 1,3,4-thiadiazole ring (i.e., the characteristic bands for the 1,3,4-thiadiazole ring; as mentioned before under the corresponding amino derivatives, **1a-m**) and other varied absorption peaks representing the specific

different R groups that are attached to position 5 in the 1,3,4-thiadiazole ring in each compound in this series (**3a-m**).[58] In ^{1}H-NMR spectra, the signal appeared at 13.031-13.087 ppm was attributed to the SH group and was an excellent primary confirmation of conversion of all chlorothiadiazoles (in the thiolation reaction) to mercaptothiadiazoles, in addition to, the presence of other varied signals representing the protons of the specific different R groups that are attached to position 5 in the 1,3,4-thiadiazole ring in each compound in this series.[58] The specific values of MS[58] and elemental analyses gave a confirmatory assignment and a further final evidence for the characterization of the structures of all these newly synthesized compounds (**3a-m**).

From the previously mentioned facts and results, we could conclude that spectral data and elemental analyses of the samples of all the newly synthesized intermediate and target compounds in this research were in an ideal and full agreement with the proposed structures. On the other hand, the previously synthesized and known intermediate compounds were characterized in this research, mainly, by the physical constants of their synthesized samples.

3.2. Pharmacological Studies
3.2.1. Free Radical Scavenging Ability of the Target Compounds (Compounds 3a-m)
3.2.1.1. ABTS Test

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS$^{.+}$) is a free and stable radical cation which is able to react with any compound that can give a hydrogen atom or an electron (i.e., antioxidants, such as phenols and thiols). 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay (ABTS test) is usually used to evaluate the antioxidant capacity of the biological fluids and many pure organic compounds. Under these conditions, the ABTS$^{.+}$ radical cation shows a strong absorption at 734 nm and becomes colorless on reduction. Each new target compound (compounds **3a-m**) was tested in distilled water and/or pure EtOH at several concentrations varying from 0.01 to 0.30 mM (i.e., from 10 to 300 μM), a steady state was achieved after 15 min of beginning this redox reaction, and, the percent reduction in absorbance (which represents the ABTS$^{.+}$ radical cation scavenging activity of the test compound) was calculated in the following way:

ABTS$^{.+}$ radical cation scavenging activity of certain test compound (%) = $100(A_{blank}-A_{test})/A_{blank}$.

Where, A_{blank} or A_0 is the absorbance of ABTS$^{.+}$ radical cation in water and EtOH directly before

reaction time = 0 min, i.e., directly before adding the test compound to the ABTS$^{\cdot+}$ radical cation (A$_{blank}$ was adjusted to be 0.70), while A$_{test}$ or A$_{15}$ is the absorbance of ABTS$^{\cdot+}$ radical cation (or the reaction mixture) in water and EtOH at reaction time = 15 min, i.e., after 15 min of adding the test compound to the ABTS$^{\cdot+}$ radical cation. For each of the test compounds, the IC$_{50}$ (the inhibitory concentration 50%, it is the concentration of any test compound needed to inhibit or reduce the absorption or the amount of ABTS$^{\cdot+}$ radical cation by 50% at a wavelength of 734 nm) was determined after 15 min of reaction and compared to that of L-ascorbic acid (taken as the reference and standard antioxidant compound in this assay). The assay was carried out according to the original procedure of Re *et al.*[64] (with very slight modifications due to the different solubilities of test compounds) and for each test compound (along with the reference L-ascorbic acid), the IC$_{50}$ value was calculated using GraphPad Prism 6 software (U.S.A., 2015) and the lower the IC$_{50}$ value is, the more powerful the test compound as antioxidant is (i.e., the stronger the antioxidant capacity of the test compound). ABTS test results (antioxidant IC$_{50}$ values for the target compounds **3a-m**) are summarized in Table 4 below.

As shown in Table 4, the target 5-substituted-2-mercapto-1,3,4-thiadiazoles displayed various degrees of free radical scavenging activity towards the ABTS radical, with decreasing activity in the following order: **3h** > **3b** > **3d** > **3c** > **3l** > **3k** > **3i** > **3f** > **3e** > **3j** > **3m** > **3g** > **3a**. The most potent compounds are the three compounds **3h**, **3b**, and **3d** with *in vitro* antiradical effects (IC$_{50}$ = 22.36, 23.67, and 24.94 μM, respectively) higher than that of L-ascorbic acid (IC$_{50}$ = 30.08 μM), while other compounds demonstrated either close and comparable activity to that of L-ascorbic acid (such as compounds **3c**, **3l**, and **3k** which have IC$_{50}$ = 33.70, 33.87, and 36.67 μM, respectively), or lower and weaker activity than that of L-ascorbic acid (such as compounds **3m**, **3g**, and **3a** which have IC$_{50}$ = 95.84, 122.01, and 169.81 μM, respectively). These results demonstrate that 2-mercapto-1,3,4-thiadiazoles which have simple aliphatic R groups at position 5 (such as compounds **3b**, **3d**, and **3c**, respectively) are more effective antioxidants than those have aromatic complicated ones (such as compounds **3g**, **3m**, **3j**, **3e**, **3f**, and **3i**, respectively). This could be explained by the simpler structures (i.e., small low-molecular-weight chains), the absence of bulky groups (i.e., the absence of hindrance effect), the more hydrophilic characters (i.e. the more water solubility due to the presence of less number of hydrophobic benzene or aromatic rings), and the more stability of the ions formed (after the redox reaction) of the thiadiazole compounds having

aliphatic R groups relative to those having aromatic ones. Exceptions for this conclusion are only compounds **3a** and **3h**. Compound **3a** has relatively the lowest antioxidant activity among the thirteen compounds possibly because its R group is a very long straight aliphatic chain as it consists of fifteen carbon atoms (i.e., not a simple small aliphatic structure) and this extremely inhibits the electron-donating effect of R group on the thiadiazole ring which, in turn, drastically decreases the free radical scavenging activity of the compound. On the other hand, compound **3h** has relatively the highest antioxidant activity among the thirteen compounds possibly because although it has aromatic properties, but its structure has three 1,3,4-thiadiazole rings, three mercapto groups, and a central simple aliphatic chain (consists of only three carbon atoms) having a hydroxyl group at position 2 of it; and all of these collective several antioxidant moieties (which, as already mentioned, are present in relatively considerable numbers) greatly increase the overall antioxidant activity of this compound.

Table 4. Results of the antioxidant capacities (expressed as IC$_{50}$ values) of the target mercapto-thiadiazole compounds **3a-m** in the ABTS test (using L-ascorbic acid as a reference antioxidant)

Compound	IC$_{50}$ (μM)[a]
3a	169.81±0.79
3b	23.67±0.17[b]
3c	33.70±0.24
3d	24.94±0.18[b]
3e	46.74±0.33
3f	40.36±0.29
3g	122.01±0.72
3h	22.36±0.14[b]
3i	37.83±0.27
3j	50.67±0.36
3k	36.67±0.26
3l	33.87±0.24
3m	95.84±0.66
L-Ascorbic acid (Reference)	30.08±0.22

[a]IC$_{50}$ values are means of three independent determinations (measurements), so results are expressed as the mean (the average value) ± the standard deviation (SD) of triplicate analysis obtained from GraphPad Prism 6 software (U.S.A., 2015).
[b]Test compounds with the least IC$_{50}$ values (i.e., with IC$_{50}$ values less than that of L-ascorbic acid) are the most active antioxidant compounds in this assay.

3.2.1.2. DPPH Test

The ability of thiol and phenolic derivatives to donate a hydrogen atom was also evaluated by their ability to react with the stable 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH·). DPPH· free radicals show a strong absorption band at 516 nm and become colorless on reduction (i.e., their absorption band color fades away upon the absorption band reduction by a free radical scavenger compound). Assay of bleaching of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH test) is also used to evaluate the antioxidant capacity of the biological fluids and many pure compounds. Each new target compound (compounds **3a-m**) was tested in EtOH or EtOH solution at several concentrations varying from 0.005 to 0.15 mM (i.e., from 5 to 150 μM), the absorbance was measured at 516 nm at time = 0 min (i.e., directly before adding the test compound to the DPPH· free radical) (A_0) and after 30 min of incubation at R.T. (i.e., after 30 min of adding the test compound to the DPPH· free radical and beginning the reaction) (A_{30}), and, the percent reduction in absorbance or the percent inhibition of DPPH· free radical (which represents the DPPH· free radical scavenging activity of the test compound) (I%) was calculated in the following way:

I% = 100(A_0-A_{30})/A_0.

For each of the test compounds, the IC_{50} (the inhibitory concentration 50%, it is the concentration of any test compound needed to inhibit or reduce the absorption or the amount of DPPH· free radical by 50% at a wavelength of 516 nm) was determined after 30 min of reaction and compared to those of L-ascorbic acid and trolox (both were taken as the reference and standard antioxidant compounds in this assay). The assay was carried out by following the method described by Prouillac et al.[26,27] and for each of the test compounds (along with the two references L-ascorbic acid and trolox), the IC_{50} value was calculated using GraphPad Prism 6 software (U.S.A., 2015) and the lower the IC_{50} value is, the more powerful the test compound as antioxidant is. DPPH test results (antioxidant IC_{50} values for the target compounds **3a-m**) are summarized in Table 5 below.

As shown in Table 5, exactly as with the ABTS radical, the target 5-substituted-2-mercapto-1,3,4-thiadiazoles displayed various degrees of free radical scavenging activity towards the DPPH radical, with decreasing activity in the following order: **3h** > **3b** > **3d** > **3c** > **3l** > **3k** > **3i** > **3f** > **3e** > **3j** > **3m** > **3g** > **3a**. The most potent compounds are the three compounds **3h**, **3b**, and **3d** with in vitro antiradical effects (IC_{50} = 12.08, 14.17, and 14.93 μM, respectively) higher than that of both L-ascorbic acid and trolox (IC_{50} = 18.02 and 30.60 μM, respectively),

while other compounds demonstrated either close and comparable activity to that of L-ascorbic acid with higher activity than that of trolox (such as compounds **3c**, **3l**, and **3k** which have IC_{50} = 20.18, 20.28, and 21.96 μM, respectively), or lower and weaker activity than that of both L-ascorbic acid and trolox (only compounds **3m**, **3g**, and **3a** which have IC_{50} = 57.40, 73.08, and 94.92 μM, respectively). It was generally noted that most of the compounds **3a-m** have stronger antioxidant activities than the potent antioxidant trolox. Being very close and relatively similar, the differences in values in this assay can be explained by and attributed to the same effects of structural modifications (i.e., differences) that were previously mentioned under ABTS test. The results of this DPPH assay are very closely related to those of ABTS assay indicating that these new 1,3,4-thiadiazole derivatives behave with the same relative manner and efficiency against both radicals (DPPH· and ABTS·+), i.e., these new 1,3,4-thiadiazoles react with and scavenge both types of radicals in close efficient ways and this makes them having a wide spectrum of antioxidant activities against the different types of free radicals.

Table 5. Results of the antioxidant capacities (expressed as IC_{50} values) of the target mercapto-thiadiazole compounds **3a-m** in the DPPH test (using L-ascorbic acid and trolox as antioxidant references).

Compound	IC_{50} (μM)[a]
3a	94.92±0.64
3b	14.17±0.13[b]
3c	20.18±0.19
3d	14.93±0.14[b]
3e	26.12±0.26
3f	24.17±0.23
3g	73.08±0.58
3h	12.08±0.10[b]
3i	22.66±0.22
3j	30.35±0.29
3k	21.96±0.21
3l	20.28±0.19
3m	57.40±0.53
L-Ascorbic acid (Reference 1)	18.02±0.18
Trolox (Reference 2)	30.60±0.40

[a]IC_{50} values are means of three independent determinations, so results are expressed as the mean ± SD of triplicate analysis obtained from GraphPad Prism 6 software (U.S.A., 2015).
[b]Test compounds with the least IC_{50} values (i.e., with IC_{50} values less than those of both L-ascorbic acid and trolox) are the most active antioxidant compounds in this assay.

3.2.2. SAR of the Target Compounds (Compounds 3a-m)

On correlating the modifications of the chemical structure (substituent R change) of the new compounds of the 5-substituted-2-mercapto-1,3,4-thiadiazoles series (i.e., along the new series of compounds **3a-m**) with their *in vitro* antioxidant biological activity (in both ABTS and DPPH assays), it has been observed that:

- Simple short-chain aliphatic-R 5-substituted-2-mercapto-1,3,4-thiadiazole compounds are generally more active as antioxidants than large complicated aromatic-R ones (supposing that there are not any additional moieties that affect the overall antioxidant activity).
- As the length of the aliphatic straight chain (if present) at position 5 of the 1,3,4-thiadiazole compounds increases, the antioxidant activity of these compounds gradually decreases until it reaches certain limit (e.g., fifteen carbon atoms or more) above which, the compounds become much less active than those have short aliphatic straight chains (one to three carbon atoms) and also than aromatic-R compounds (i.e., their antioxidant activities are relatively very weak), on a condition that there are not any additional moieties on the aliphatic chain that impart and add any antioxidant activity.
- Aromatic-R 5-substituted-2-mercapto-1,3,4-thiadiazoles having complete resonating system (uninterrupted resonance effect) are generally more active as antioxidants than those having incomplete interrupted one.
- Aromatic-R 5-substituted-2-mercapto-1,3,4-thiadiazole compounds bearing more than one OH group (e.g., three OH groups) on the aromatic ring attached to position 5 of the thiadiazole ring are much more active as antioxidants than those bearing just one OH group (supposing that there are not any additional moieties that affect the overall antioxidant activity).
- As the number of halogens (e.g., Cl and Br substituents) attached to the aliphatic side chain which is present at position 5 of the thiadiazole ring in aliphatic-R 5-substituted-2-mercapto-1,3,4-thiadiazoles increases, the antioxidant activity of these compounds also increases in a relative way.
- Compounds that have considerable number of 1,3,4-thiadiazole rings and SH groups (i.e., three and more) are generally expected to be very potent antioxidant compounds and to

have much more antioxidant activities than those have one or two of these two moieties.
- 5-Substituted-2-mercapto-1,3,4-thiadiazoles that have balanced lipophilic/hydrophilic properties are much more active *in vitro* as antioxidants than extremely lipophilic ones.

3.3. Conclusions

On the basis of ''the combination principles'', we have designed and synthesized, in very good to excellent yields, a novel series of 5-substituted-2-mercapto-1,3,4-thiadiazole compounds (compounds **3a-m**) in which a bioactive aromatic 1,3,4-thiadiazole ring is directly linked with an antioxidant thiol moiety and an aiding substituent at the two carbons of the ring, the produced 2,5-disubstituted-1,3,4-thiadiazoles were characterized by most different spectral/elemental analytical methods. The synthesized compounds showed a wide range of potentially promising antioxidant activities. Depending on their pharmacological scavenging effects against the tested radicals *in vitro*, these target compounds can be categorized relative to L-ascorbic acid and trolox into three distinguished classes of antioxidants, as they can be classified into either very potent and excellent antioxidants (group I, compounds **3b,d,h**), moderately potent and good antioxidants (group II, compounds **3c,e,f,i,j,k,l**), or relatively less potent and mild antioxidants (group III, compounds **3a,g,m**). Compounds **3b,d,h** exhibited interestingly very potent antioxidant activity and they can be very promising lead and parent compounds for the design and synthesis of new antioxidant compounds and medicines by further *in vivo* biological evaluation, structural modifications, deep investigations, and advanced clinical studies.

4. Experimental Work

4.1. Chemical Syntheses

4.1.1. General Data (the Used Materials and Instruments)

All reactions were performed with commercially available reagents. All chemicals (reagents and solvents) were of analytical grade, purchased from commercial suppliers, and were used as received without further purification (if needed, some solvents were dried by standard methods). Acidic alumina (aluminum oxide; acidic; Brockmann I; ~ 150 mesh; 58 Å CAMAG 506-C-I; surface area = 155 m^2/g; pH = 6) was used as an efficient adsorbent for MW reactions. MWI for MW reactions was carried out in an unmodified domestic MW oven (*Samsung* type, model M1733N with T.D.S. (Triple Distribution System) property, and having a power level of 100-800 W) operated at 2.45 GHz. Thin-layer

chromatography (TLC) was used to monitor the progress of all the reactions to reach their completion (reaction times) and to check the purity of the compounds synthesized, it was carried out on TLC silica gel 60 F_{254} plates (plates of aluminum sheets pre- coated with unmodified silica gel 60 F_{254} to a layer thickness of 0.20 mm, purchased from E. Merck, Merck Millipore Division or Merck Chemicals, Merck KGaA, Darmstadt, Germany) as the stationary phase using petroleum ether/ethyl acetate/absolute EtOH (6:3:2, v/v/v) mixture as the eluting solvent system and the chromatograms spots were visualized and observed under UV light at wavelengths of 254 (mainly) and 366 nm to detect the produced components. The pH of reaction mixtures solutions was measured (to be adjusted) by a portable waterproof pH/ORP meter with smart electrodes and log-on-demand (HI 98150, HANNA instruments, Hungary Kft., Hungary) and this was done mainly to get the neutral pH (about 8) in the neutralization step for each reaction mixture solution contained $POCl_3$ as Xss. with the product. Evaporation and concentration was carried out by using rotary evaporator (rotavap) under reduced pressure (for the efficient and gentle removal of solvents from reaction mixtures). Melting points ($^\circ$C) of all the synthesized compounds were measured and recorded in open glass capillaries using *Fisher-Johns* melting point apparatus and were uncorrected. IR spectra were recorded on *Mattson* 5000 FT-IR spectrometer (υ in cm^{-1}) using KBr (potassium bromide) disks at the Spectral Analysis Unit (Faculty of Science, Mansoura University, Mansoura, Egypt) (*Abbreviations in IR characterization data*: Str. = Strong (if not mentioned, this means that the peak is weak to medium in intensity); Bro. = Broad (if not mentioned, this means that the peak is sharp, not broad enough, or overlapped with other peaks); Aliph. = Aliphatic; Arom. = Aromatic). ^{1}H-NMR spectra were recorded on *Varian Gemini*-300 spectrometer (Mercury-300BB "NMR300") at about 300 MHz (megahertz) using tetramethylsilane (TMS) as an internal standard at the Microanalytical Center (Faculty of Science, Cairo University, Cairo, Egypt) and their chemical shifts values (δ) were given in ppm downfield from TMS at a temperature of 30 °C using either $CDCl_3$ (deuterated chloroform) or DMSO-d_6 (deuterated dimethyl-sulfoxide) as a solvent (according to the solubility of each analyzed compound) (*Abbreviations in ^{1}H-NMR characterization data*: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; dd = double doublet (a doublet of doublets); *J* = Coupling Constant (expressed in Hz (hertz)); Aliph. = Aliphatic; Arom. = Aromatic; *o* = ortho; *m* = meta; *p* = para). MS analyses were performed on *Shimadzu* Qp-2010 Plus at 70 eV (electron volts) and results were represented

by *m/z* (Rel. Int. in %), i.e., mass/charge (relative intensity in %), at the Microanalytical Center (Faculty of Science, Cairo University, Cairo, Egypt). Elemental analyses were performed at the Microanalytical Center (Faculty of Science, Cairo University, Cairo, Egypt) in order to determine C, H, and N contents of all the newly synthesized compounds (they, all, were in full agreement with the calculated values). *Other abbreviations in synthetic procedures and characterization data below*: Recryst. = Recrystallized; Col. & App. = Color & Appearance; M.P. = Melting Point; abs. = absolute; v/v = volume per volume; M.Wt. = Molecular Weight; Elem. Anal. = Elemental Analyses; EtOAc = ethyl acetate; DEE = diethyl ether; DMF = dimethylformamide; dec. = with decomposition; MeOH = methanol.

4.1.2. General Procedures for the Synthesis of 5-Substituted-1,3,4-thiadiazol-2-amines (5-Substituted-2-amino-1,3,4-thiadiazoles, 1a-m)

• **General Conventional Procedure (Method A):** An ice-cooled mixture of thiosemicarbazide (0.01 mole, 0.9114 g if the carboxylic acid is monocarboxylic acid; 0.02 mole, 1.8228 g if the carboxylic acid is dicarboxylic acid; or 0.03 mole, 2.7342 g if the carboxylic acid is tricarboxylic acid) and the respective carboxylic acid (0.01 mole; see Table 2) was dissolved in dry $POCl_3$ (5 mL if the carboxylic acid is monocarboxylic acid, 10 mL if the carboxylic acid is dicarboxylic acid, or 15 mL if the carboxylic acid is tricarboxylic acid; by dropwise addition of $POCl_3$ to the mixture) and the resulted solution was gently heated under reflux (i.e., the temperature of the resulted solution was gradually raised till the solution was to be refluxed, at about 105-110 °C) with constant magnetic stirring for 3-9 h (see Table 2). The reaction of the mixture was followed up by using TLC (which was used to monitor reaching the completion of the reaction and to determine the purity of the product). When the reaction was over as indicated by TLC, the reaction mixture solution was concentrated in rotavap under reduced pressure, cooled to R.T., and then gradually (slowly and carefully) poured onto crushed ice with stirring. The least amount required of finely powdered K_2CO_3 (potassium carbonate) and the required amount of solid KOH (potassium hydroxide) were added, with stirring, to the mixture solution till the pH of the solution was raised to 8 (it was measured by using pHmeter) to remove the Xss. of $POCl_3$. The mixture solution was allowed to stand overnight till the solid was separated and settled down. The precipitated crude solid was filtered, washed thoroughly with cold distilled H_2O, dried, and purified by recrystallization from an appropriate solvent or mixture of solvents

(see for each compound below) to give the pure product **1** as shown below in details.

   • **General MW-assisted Procedure (Method B):** An ice-cooled mixture of thiosemicarbazide (0.01 mole, 0.9114 g if the carboxylic acid is monocarboxylic acid; 0.02 mole, 1.8228 g if the carboxylic acid is dicarboxylic acid; or 0.03 mole, 2.7342 g if the carboxylic acid is tricarboxylic acid) and the respective carboxylic acid (0.01 mole; see Table 2) was dissolved in dry $POCl_3$ (5 mL if the carboxylic acid is monocarboxylic acid, 10 mL if the carboxylic acid is dicarboxylic acid, or 15 mL if the carboxylic acid is tricarboxylic acid; by dropwise addition of $POCl_3$ to the mixture); acidic alumina (acidic Al_2O_3; 5 g if the carboxylic acid is monocarboxylic acid, 10 g if the carboxylic acid is dicarboxylic acid, or 15 g if the carboxylic acid is tricarboxylic acid) was added to the above-resulted solution at R.T.; and the resulted paste of reaction mixture was well mixed, adsorbed, dried, kept inside the alumina bath, covered with aluminum foil, and subjected to MWI (in the domestic MW oven which has the traditional MW frequency of 2.45 GHz) intermittently at intervals of 30 s for 4-10 min at a power level of 300-800 W (see Table 2). The reaction of the mixture was followed up by using TLC till it was over. After cooling the reaction mixture to R.T., a suitable amount of anhydrous dichloromethane (methylene chloride, CH_2Cl_2) was added to this mixture to efficiently dissolve the crude product and isolate it from the acidic alumina; the CH_2Cl_2 layer was then separated and evaporated in rotavap under reduced pressure; the remaining crude paste was cooled to R.T. and then gradually (slowly and carefully) poured onto crushed ice with stirring. The least amount required of finely powdered K_2CO_3 and the required amount of solid KOH were added, with stirring, to the mixture solution till the pH of the solution was raised to 8 (it was measured by using pHmeter) to remove the Xss. of $POCl_3$. The mixture solution was allowed to stand overnight till the solid was separated and settled down. The separated solid was filtered, washed thoroughly with cold distilled H_2O, dried, and purified by recrystallization from an appropriate solvent(s) (see for each compound below) to give the pure product **1** as shown below in details.

4.1.2.1. 5-Pentadecyl-1,3,4-thiadiazol-2-amine (1a, *old*):[30,31] Recryst. from *benzene*; Col. & App.: white to buff solid mass and/or crystalline plates; Yield: 95.0% (Conv.), 99.4% (MW); M.P.: 130 °C.

4.1.2.2. 1-(5-Amino-1,3,4-thiadiazol-2-yl)ethanol (1b, *new*): Recryst. from *abs. EtOH/H_2O (3:1, v/v)*; Col. & App.: greenish grey fine powder; Yield: 92.2% (Conv.), 98.0% (MW); M.P.: 217-219 °C; IR (υ in cm^{-1}):

Str. & Bro. 3400 (O-H), Str. 3282 & Str. 3124 (2 N-H, i.e., NH_2), Str. 2922 & 2851 (C-H, Aliph.), Str. 1628 & Str. 1513 (C=N), 1331 (C-N, Arom.), 1140 (C-O), 1045 (N-N), 686 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 1.491 (d, J = 6.8 Hz, 3H, CH$_3$), 3.626 (s, 1H, Aliph. OH), 4.687 (q, J = 6.8 Hz, 1H, CH), 6.993 & 7.131 (s, 2H, Arom. NH$_2$); MS (*m/z* (Rel. Int. in %), M.Wt. = 145.18): 145.20 (5.41), 128.20 (86.58), 100.10 (4.05), 85.15 (22.73), 60.05 (100.00), 57.10 (70.57); Elem. Anal. (%, for C$_4$H$_7$N$_3$OS): *Calculated* (*Found*): C: 33.09 (33.11), H: 4.86 (4.80), N: 28.94 (28.98).

4.1.2.3. 5-(Dichloromethyl)-1,3,4-thiadiazol-2-amine (1c, *old*):[31,32] Recryst. from *EtOAc*; Col. & App.: pale yellow to brown crystals; Yield: 90.0% (Conv.), 97.5% (MW); M.P.: 181-183 °C.

4.1.2.4. 5-(Trichloromethyl)-1,3,4-thiadiazol-2-amine (1d, *old*):[31,32] Recryst. from *EtOAc*; Col. & App.: snow white to yellow crystals; Yield: 89.0% (Conv.), 97.3% (MW); M.P.: 179-180 °C.

4.1.2.5. (*S*)-5-(1-Amino-2-phenylethyl)-1,3,4-thiadiazol-2-amine (1e, *new*): Recryst. from *DEE/abs. EtOH (3:1, v/v)*; Col. & App.: buff-brown fine powder; Yield: 82.5% (Conv.), 95.9% (MW); M.P.: 186-188 °C; IR (υ in cm^{-1}): Str. 3372 & Str. 3288 & Str. 3249 & Str. 3222 (4 N-H, i.e., 2 NH$_2$), Str. 3062 & Str. 3029 (C-H, Arom.), Str. 2925 (C-H, Aliph.), Str. 1621 & Str. 1542 (C=N), Str. 1511 & Str. 1496 & 1452 (C=C, Arom.), 1236 (C-N), 1076 (N-N), 700 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 3.010 & 3.218 (2 dd, J = -12.4 Hz & 7.0 Hz, 2 Diastereotopic H, CH$_2$), 4.287 (t, J = 7.0 Hz, 1H, CH), 5.131 (s, 2H, Aliph. NH$_2$), 6.993 (s, 2H, Arom. NH$_2$), 7.271 & 7.295 & 7.440 (m, 5H, 1 *p*- & 2 *o*- & 2 *m*-Benzene-H); MS (*m/z* (Rel. Int. in %), M.Wt. = 220.29): 220.15 (97.13), 129.05 (68.71), 91.05 (100.00), 85.10 (16.70), 77.05 (24.51), 57.05 (73.46); Elem. Anal. (%, for C$_{10}$H$_{12}$N$_4$S): *Calculated* (*Found*): C: 54.52 (54.50), H: 5.49 (5.49), N: 25.43 (25.44).

4.1.2.6. 5,5'-Methylenebis(1,3,4-thiadiazol-2-amine) (1f, *old*):[33-35,50,65] Recryst. from *DMF/abs. EtOH (2:1, v/v)*; Col. & App.: orange-brown crystalline powder and pellets (creamy precipitate before the purification and recrystallization steps); Yield: 86.0% (Conv.), 96.0% (MW); M.P.: 251-252 °C.

4.1.2.7. (1*R*,2*R*)-1,2-Bis(5-amino-1,3,4-thiadiazol-2-yl)ethane-1,2-diol (1g, *old*):[36,66] Recryst. from *DMF/abs. EtOH (2:1, v/v)*; Col. & App.: white crystalline powder; Yield: 77.0% (Conv.), 97.0% (MW); M.P.: 138 °C.

4.1.2.8. 1,2,3-Tris(5-amino-1,3,4-thiadiazol-2-yl)propan-2-ol (1h, *new*): Recryst. from *abs. EtOH/H_2O (3:1, v/v)*; Col. & App.: yellowish brown fine powder;

Yield: 90.3% (Conv.), 99.1% (MW); M.P.: 286 °C (dec.); IR (υ in cm^{-1}): Str. & Bro. 3400 (O-H), Str. 3277 & Str. 3157 (2 N-H, i.e., NH$_2$), 2921 (C-H, Aliph.), Str. 1609 & Str. 1541 (C=N), 1216 (C-N, Arom.), 1135 (C-O), 1076 (N-N), 660 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 2.994 (s, 4H, 2 CH$_2$), 3.663 (s, 1H, Aliph. OH), 6.992 (s, 6H, 3 Arom. NH$_2$); MS (*m/z* (Rel. Int. in %), M.Wt. = 357.44): 357.30 (46.86), 340.30 (28.99), 241.30 (7.73), 212.30 (7.73), 136.20 (100.00), 59.10 (78.74); Elem. Anal. (%, for C$_9$H$_{11}$N$_9$OS$_3$): *Calculated* (*Found*): C: 30.24 (30.24), H: 3.10 (3.10), N: 35.27 (35.25).

4.1.2.9. (*E*)-5-Styryl-1,3,4-thiadiazol-2-amine (1i, *old*):[37-41,67] Recryst. from *abs. EtOH/H$_2$O (3:1, v/v)*; Col. & App.: white to grey amorphous crystals; Yield: 74.1% (Conv.), 95.1% (MW); M.P.: 220 °C.

4.1.2.10. (*E*)-5,5'-(Ethene-1,2-diyl)bis(1,3,4-thiadia-zol-2-amine) (1j, *new*): Recryst. from *abs. MeOH*; Col. & App.: buff amorphous powder; Yield: 93.7% (Conv.), 99.0% (MW); M.P.: >300 °C; IR (υ in cm^{-1}): 3468 & Str. 3285 (2 N-H, i.e., NH$_2$), 3093 (=C-H, Alkene), Str. 1625 (C=C, Alkene), Str. 1504 (C=N), 1331 & 1237 (C-N, Arom.), Str. 1129 & Str. 1067 (N-N), 698 & 620 (C-S); ^{1}H-NMR (DMSO-*d$_6$*, δ in ppm): 6.646 (d, *J* = 15.1 Hz, 2H, *trans* H̲C=CH̲), 6.971 (s, 4H, 2 Arom. NH$_2$); MS (*m/z* (Rel. Int. in %), M.Wt. = 226.28): 226.40 (0.58), 126.30 (4.11), 114.30 (1.05), 100.20 (1.28), 85.20 (39.08), 57.15 (100.00); Elem. Anal. (%, for C$_6$H$_6$N$_6$S$_2$): *Calculated* (*Found*): C: 31.85 (31.88), H: 2.67 (2.65), N: 37.14 (37.17).

4.1.2.11. 5-(4-Bromophenyl)-1,3,4-thiadiazol-2-ami-ne (1k, *old*):[42-44,68,69] Recryst. from *abs. EtOH*; Col. & App.: white to light brown crystalline powder; Yield: 84.1% (Conv.), 96.6% (MW); M.P.: 222-224 °C.

4.1.2.12. 5-(5-Amino-1,3,4-thiadiazol-2-yl)benzene-1,2,3-triol (1l, *new*): Recryst. from *abs. EtOH/H$_2$O (3:1, v/v)*; Col. & App.: colorless to white transparent crystalline masses and/or plates; Yield: 88.0% (Conv.), 98.0% (MW); M.P.: 299 °C (dec.); IR (υ in cm^{-1}): Str. 3406 (O-H), Str. 3294 & Str. 3198 (2 N-H, i.e., NH$_2$), 3056 (C-H, Arom.), Str. 1621 & Str. 1557 (C=N), Str. 1500 & Str. 1475 & Str. 1440 (C=C, Arom.), Str. 1349 & Str. 1264 (C-N, Arom.), Str. 1206 (C-O), Str. 1013 (N-N), 695 & 651 (C-S); ^{1}H-NMR (DMSO-*d$_6$*, δ in ppm): 5.335 (s, 3H, 3 Arom. OH), 6.737 (s, 2H, 2 Benzene-H), 6.997 (s, 2H, Arom. NH$_2$); MS (*m/z* (Rel. Int. in %), M.Wt. = 225.22): 225.30 (2.17), 125.30 (14.23), 100.20 (1.72), 85.15 (39.36), 77.10 (2.90), 57.15 (100.00); Elem. Anal. (%, for C$_8$H$_7$N$_3$O$_3$S): *Calculated* (*Found*): C: 42.66 (42.64), H: 3.13 (3.15), N: 18.66 (18.66).

4.1.2.13. 3-(5-Amino-1,3,4-thiadiazol-2-yl)-7-chloro-quinolin-4-ol (1m, *new*): Recryst. from *hexane*; Col.

& App.: reddish orange fine powder; Yield: 88.2% (Conv.), 95.8% (MW); M.P.: 250 °C (dec.); IR (υ in cm^{-1}): Str. & Bro. 3454 (O-H), Str. 3216 & Str. 3193 (2 N-H, i.e., NH$_2$), 3073 (C-H, Arom.), Str. 1616 (C=N), Str. 1496 & Str. 1452 (C=C, Arom.), Str. 1351 (C-N, Arom.), 1204 (C-O), Str. 1082 (N-N), 773 (C-Cl), 737 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 5.367 (s, 1H, Arom. OH), 6.993 (s, 2H, Arom. NH$_2$), 7.740-7.760 (dd, *J* = 7.5 Hz & 1.5 Hz, 1H, Quinoline-H-7), 7.940 (d, *J* = 1.5 Hz, 1H, Quinoline-H-9), 8.414 & 8.424 (d & s, J_{H-6} = 7.5 Hz, 2H, Quinoline-H-6,2); MS (*m/z* (Rel. Int. in %), M.Wt. = 278.72 & 280.72): 281.05 (22.44), 279.00 (67.50), 100.10 (3.31), 85.15 (9.54), 74.05 (100.00), 57.10 (16.57); Elem. Anal. (%, for C$_{11}$H$_7$ClN$_4$OS): *Calculated* (*Found*): C: 47.40 (47.44), H: 2.53 (2.50), N: 20.10 (20.10).

4.1.3. General Procedure for the Synthesis of 2-Chloro-5-substituted-1,3,4-thiadiazoles (5-Substitu-ted-2-chloro-1,3,4-thiadiazoles, 2a-m)

A mixture of the corresponding amine (the corresponding compound **1**; 0.01 mole) and an Xss. of NaNO$_2$ (0.03 mole, 2.07 g if the starting carboxylic acid in the previous synthetic step **4.1.2** is monocarboxylic acid; 0.06 mole, 4.14 g if the starting carboxylic acid in the previous synthetic step **4.1.2** is dicarboxylic acid; or 0.09 mole, 6.21 g if the starting carboxylic acid in the previous synthetic step **4.1.2** is tricarboxylic acid) was slowly added at -5 °C to a stirred solution of HCl/H$_2$O (31.5 mL/13.5 mL if the starting carboxylic acid in the previous synthetic step **4.1.2** is monocarboxylic acid, 63 mL/27 mL if the starting carboxylic acid in the previous synthetic step **4.1.2** is dicarboxylic acid, or 94.5 mL/40.5 mL if the starting carboxylic acid in the previous synthetic step **4.1.2** is tricarboxylic acid) containing Cu powder (0.008 mole, about 0.511 g if the starting carboxylic acid in the previous synthetic step **4.1.2** is monocarboxylic acid; 0.016 mole, 1.022 g if the starting carboxylic acid in the previous synthetic step **4.1.2** is dicarboxylic acid; or 0.024 mole, 1.533 g if the starting carboxylic acid in the previous synthetic step **4.1.2** is tricarboxylic acid), the resulted mixture was stirred at R.T. for 2 h, and then it was heated at 55 °C (preferably in a water bath) until the evolution of gas was ceased (i.e., no more N$_2$ gas evolution). The reaction mixture was left to cool to R.T., then it was extracted by either CHCl$_3$ or DMSO (all the products were extracted by CHCl$_3$ except compounds **2b,e,f,k** which were extracted by DMSO) for three times (3 × 60 mL), the combined organic extracts were washed with 10% sulfuric acid (10% H$_2$SO$_4$, 30 mL), dried over anhyd. Na$_2$SO$_4$ (anhydrous sodium sulfate), concentrated by evaporation in rotavap under reduced pressure, and filtered to give the crude solid product which was further purified by recrystallization from

abs. EtOH/DEE (2:1, v/v; except compound **2k** which was recryst. from abs. EtOH only) to give the pure product **2** as shown below in details.

4.1.3.1.   2-Chloro-5-pentadecyl-1,3,4-thiadiazole (2a, *new*): Col. & App.: yellowish green fine powder; Yield: 90.0%; M.P.: 94-96 °C; IR (υ in cm^{-1}): Str. 2917 & Str. 2848 (C-H, Aliph.), Str. 1637 & 1596 (C=N), 1079 (N-N), 717 (C-Cl), 646 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 0.864-0.888 (t, J = 8.0 Hz, 3H, Terminal CH$_3$), 1.266 & 1.298 & 1.312 (m, 24H, All Other 12 CH$_2$), 1.612 (m, 2H, β-CH$_2$ to Thiadiazole Ring), 2.824 (t, J = 7.1 Hz, 2H, α-CH$_2$ to Thiadiazole Ring); MS (*m/z* (Rel. Int. in %), M.Wt. = 330.96 & 332.96): 332.20 (23.65), 330.25 (72.00), 295.30 (27.56), 134.00 (100.00), 85.10 (13.05), 57.05 (58.34); Elem. Anal. (%, for C$_{17}$H$_{31}$ClN$_2$S): *Calculated* (*Found*): C: 61.69 (61.70), H: 9.44 (9.45), N: 8.46 (8.46).

4.1.3.2.   1-(5-Chloro-1,3,4-thiadiazol-2-yl)ethanol (2b, *new*): Col. & App.: dark brown fine powder; Yield: 63.3%; M.P.: 251-253 °C (dec.); IR (υ in cm^{-1}): Str. & Bro. 3400 (O-H), Str. 2922 & 2851 (C-H, Aliph.), Str. 1628 & Str. 1513 (C=N), 1140 (C-O), 1045 (N-N), 700 (C-Cl), 686 (C-S); ^{1}H-NMR (DMSO-d_6, δ in ppm): 1.471-1.481 (d, J = 6.8 Hz, 3H, CH$_3$), 3.647 (s, 1H, Aliph. OH), 4.670 & 4.677 & 4.680 (q, J = 6.8 Hz, 1H, CH); MS (*m/z* (Rel. Int. in %), M.Wt. = 164.61 & 166.61): 167.00 (12.48), 165.00 (38.41), 119.20 (4.34), 85.15 (32.72), 64.00 (100.00), 57.10 (86.37); Elem. Anal. (%, for C$_4$H$_5$ClN$_2$OS): *Calculated* (*Found*): C: 29.19 (29.17), H: 3.06 (3.06), N: 17.02 (17.03).

4.1.3.3. 2-Chloro-5-(dichloromethyl)-1,3,4-thiadiazole (2c, *new*): Col. & App.: brown amorphous powder; Yield: 79.0%; M.P.: 44-46 °C; IR (υ in cm^{-1}): 2921 (C-H, Aliph.), Str. 1623 & 1531 (C=N), Str. 1085 (N-N), 779 (C-Cl), 654 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 6.751 (s, 1H, CH); MS (*m/z* (Rel. Int. in %), M.Wt. = 203.48 & 205.48 & 207.48 & 209.48): 209.15 (1.53), 207.25 (13.71), 205.20 (40.99), 203.30 (41.19), 85.15 (39.36), 57.15 (100.00); Elem. Anal. (%, for C$_3$HCl$_3$N$_2$S): *Calculated* (*Found*): C: 17.71 (17.71), H: 0.50 (0.50), N: 13.77 (13.75).

4.1.3.4. 2-Chloro-5-(trichloromethyl)-1,3,4-thiadiazole (2d, *old*):[70,71] Col. & App.: colorless transparent plates and amorphous masses; Yield: 91.0%; M.P.: 40-41 °C.

4.1.3.5. (*S*)-1-(5-Chloro-1,3,4-thiadiazol-2-yl)-2-phenylethanamine (2e, *new*): Col. & App.: brown amorphous powder; Yield: 77.5%; M.P.: 192-194 °C; IR (υ in cm^{-1}): Str. 3382 & Str. 3367 (2 N-H, i.e., NH$_2$), Str. 3060 & Str. 3029 (C-H, Arom.), Str. 2956 & Str. 2923 (C-H, Aliph.), Str. 1623 & Str. 1554

(C=N), Str. 1494 & 1452 (C=C, Arom.), 1236 (C-N, Aliph.), 1076 (N-N), 748 (C-Cl), Str. 700 (C-S); ^{1}H-NMR (DMSO-d_6, δ in ppm): 3.003 & 3.213 (2 dd, J = -12.4 Hz & 7.0 Hz, 2 Diastereotopic H, CH$_2$), 4.281 (t, J = 7.0 Hz, 1H, CH), 5.130 (s, 2H, Aliph. NH$_2$), 7.257 & 7.286 & 7.403 (m, 5H, 1 *p*- & 2 *o*- & 2 *m*-Benzene-H); MS (*m/z* (Rel. Int. in %), M.Wt. = 239.72 & 241.72): 241.25 (24.00), 239.25 (72.00), 134.00 (100.00), 85.10 (13.05), 77.05 (2.23), 57.00 (58.34); Elem. Anal. (%, for C$_{10}$H$_{10}$ClN$_3$S): *Calculated* (*Found*): C: 50.10 (50.10), H: 4.20 (4.21), N: 17.53 (17.51).

4.1.3.6.   Bis(5-chloro-1,3,4-thiadiazol-2-yl)methane (2f, *new*): Col. & App.: brown fine powder; Yield: 88.0%; M.P.: 250 °C (dec.); IR (υ in cm^{-1}): 2958 (C-H, Aliph.), Str. 1668 & Str. 1652 (C=N), Str. 1097 (N-N), 780 (C-Cl), 740 (C-S); ^{1}H-NMR (DMSO-d_6, δ in ppm): 3.816 (s, 2H, CH$_2$); MS (*m/z* (Rel. Int. in %), M.Wt. = 253.13 & 255.13 & 257.13): 256.40 (0.13), 254.60 (0.77), 252.40 (1.14), 99.20 (13.49), 85.15 (25.49), 57.10 (100.00); Elem. Anal. (%, for C$_5$H$_2$Cl$_2$N$_4$S$_2$): *Calculated* (*Found*): C: 23.72 (23.74), H: 0.80 (0.80), N: 22.13 (22.11).

4.1.3.7. (1*R*,2*R*)-1,2-Bis(5-chloro-1,3,4-thiadiazol-2-yl)ethane-1,2-diol (2g, *new*): Col. & App.: brown fine powder; Yield: 75.7%; M.P.: >300 °C; IR (υ in cm^{-1}): Str. & Bro. 3405 (O-H), 2952 (C-H, Aliph.), 1660 & Str. 1623 (C=N), 1230 (C-O), Str. 1085 (N-N), 777 (C-Cl), 654 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 3.665 (s, 2H, 2 Aliph. OH), 4.996 (d, J = 7 Hz, 2H, 2 CH); MS (*m/z* (Rel. Int. in %), M.Wt. = 299.16 & 301.16 & 303.16): 303.00 (7.23), 301.10 (43.43), 299.00 (65.17), 254.00 (100.00), 229.00 (76.40), 55.10 (41.57); Elem. Anal. (%, for C$_6$H$_4$Cl$_2$N$_4$O$_2$S$_2$): *Calculated* (*Found*): C: 24.09 (24.11), H: 1.35 (1.33), N: 18.73 (18.71).

4.1.3.8. 1,2,3-Tris(5-chloro-1,3,4-thiadiazol-2-yl)propan-2-ol (2h, *new*): Col. & App.: dark brown fine powder; Yield: 90.0%; M.P.: 235-237 °C; IR (υ in cm^{-1}): Str. & Bro. 3430 (O-H), 2958 & 2923 (C-H, Aliph.), Str. 1668 & Str. 1652 & 1506 (C=N), 1097 (C-O), 1025 (N-N), 780 & 740 (C-Cl), 669 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 2.992 (s, 4H, 2 CH$_2$), 3.669 (s, 1H, Aliph. OH); MS (*m/z* (Rel. Int. in %), M.Wt. = 415.73 & 417.73 & 419.73 & 421.73): 421.95 (0.39), 420.00 (3.29), 417.95 (9.89), 416.00 (9.91), 79.95 (100.00), 57.05 (73.13); Elem. Anal. (%, for C$_9$H$_5$Cl$_3$N$_6$OS$_3$): *Calculated* (*Found*): C: 26.00 (26.00), H: 1.21 (1.22), N: 20.22 (20.20).

4.1.3.9. (*E*)-2-Chloro-5-styryl-1,3,4-thiadiazole (2i, *new*): Col. & App.: yellowish brown fine powder; Yield: 80.0%; M.P.: 280-282 °C; IR (υ in cm^{-1}): 3082 & 3058 (=C-H, Alkene), 3027 (C-H, Arom.), Str.

1690 (C=C, Alkene), Str. 1632 (C=N), Str. 1560 & 1497 & Str. 1439 (C=C, Arom.), 1075 (N-N), Str. 752 (C-Cl), 689 & 648 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 6.956 & 6.996 (2 d, J = 15.1 Hz, 2H, *trans* HC=CH), 7.300 & 7.411 & 7.603 (m, 5H, 1 *p*- & 2 *m*- & 2 *o*-Benzene-H); MS (*m/z* (Rel. Int. in %), M.Wt. = 222.69 & 224.69): 225.00 (32.32), 223.05 (97.13), 120.15 (97.13), 91.05 (100.00), 77.05 (24.51), 55.00 (76.20); Elem. Anal. (%, for C$_{10}$H$_7$ClN$_2$S): *Calculated* (*Found*): C: 53.93 (53.93), H: 3.17 (3.14), N: 12.58 (12.55).

4.1.3.10. (*E*)-1,2-Bis(5-chloro-1,3,4-thiadiazol-2-yl)ethene (2j, *new*): Col. & App.: white crystalline powder; Yield: 87.5%; M.P.: 244-246 °C; IR (υ in cm^{-1}): 3062 (=C-H, Alkene), Str. 1637 (C=C, Alkene), 1596 (C=N), 1079 (N-N), 717 (C-Cl), 646 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 6.999-7.001 (d, J = 15.1 Hz, 2H, *trans* HC=CH); MS (*m/z* (Rel. Int. in %), M.Wt. = 265.14 & 267.14 & 269.14): 269.15 (5.33), 267.00 (35.30), 265.10 (53.03), 114.15 (50.72), 64.00 (100.00), 57.10 (29.73); Elem. Anal. (%, for C$_6$H$_2$Cl$_2$N$_4$S$_2$): *Calculated* (*Found*): C: 27.18 (27.17), H: 0.76 (0.77), N: 21.13 (21.12).

4.1.3.11. 2-Chloro-5-(4-bromophenyl)-1,3,4-thiadiazole (2k, *old*):[60,68] Col. & App.: yellowish brown crystalline powder; Yield: 91.0%; M.P.: 127-128 °C.

4.1.3.12. 5-(5-Chloro-1,3,4-thiadiazol-2-yl)benzene-1,2,3-triol (2l, *new*): Col. & App.: dark brown fine powder; Yield: 93.1%; M.P.: 282 °C (dec.); IR (υ in cm^{-1}): Str. 3406 (O-H), 3056 (C-H, Arom.), Str. 1621 & Str. 1557 (C=N), Str. 1500 & Str. 1475 & Str. 1440 (C=C, Arom.), Str. 1206 (C-O), Str. 1013 (N-N), 751 (C-Cl), 695 & 651 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 5.351 & 5.367 & 5.381 (3 s, 3H, 3 Arom. OH), 6.743 (s, 2H, 2 Benzene-H); MS (*m/z* (Rel. Int. in %), M.Wt. = 244.65 & 246.65): 246.40 (0.10), 244.40 (0.28), 125.25 (11.26), 119.20 (5.28), 85.15 (25.49), 57.10 (100.00); Elem. Anal. (%, for C$_8$H$_5$ClN$_2$O$_3$S): *Calculated* (*Found*): C: 39.27 (39.29), H: 2.06 (2.06), N: 11.45 (11.44).

4.1.3.13. 7-Chloro-3-(5-chloro-1,3,4-thiadiazol-2-yl)quinolin-4-ol (2m, *new*): Col. & App.: light brown fine powder; Yield: 85.8%; M.P.: 270 °C (dec.); IR (υ in cm^{-1}): Str. & Bro. 3413 (O-H), 3060 & 3027 (C-H, Arom.), Str. 1629 & 1554 (C=N), 1540 & 1521 & 1494 & 1452 (C=C, Arom.), 1190 (C-O), 1076 (N-N), 750 (C-Cl), 700 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 5.347 (s, 1H, Arom. OH), 7.740-7.761 (dd, J = 7.5 Hz & 1.5 Hz, 1H, Quinoline-H-7), 7.946 (d, J = 1.5 Hz, 1H, Quinoline-H-9), 8.416 & 8.428 (d & s, J_{H-6} = 7.5 Hz, 2H, Quinoline-H-6,2); MS (*m/z* (Rel. Int. in %), M.Wt. = 298.15 & 300.15 & 302.15): 302.00 (1.56), 300.05 (9.44), 298.10 (14.42), 179.10 (41.59), 64.00 (100.00), 59.05 (71.88); Elem. Anal. (%, for

C$_{11}$H$_5$Cl$_2$N$_3$OS): *Calculated* (*Found*): C: 44.31 (44.33), H: 1.69 (1.67), N: 14.09 (14.07).

4.1.4. General Procedure for the Synthesis of 5-Substituted-1,3,4-thiadiazole-2-thiols (5-Substituted-2-mercapto-1,3,4-thiadiazoles, 3a-m)

A reaction mixture containing the corresponding chlorinated derivative (the corresponding compound **2**; 0.01 mole), an Xss. of thiourea (0.03 mole, 2.2836 g if the starting carboxylic acid in the first synthetic step **4.1.2** is monocarboxylic acid or if the structure of the corresponding chlorinated derivative contains only one 1,3,4-thiadiazole ring that contains only one chloro group at position 2 and irrespective of any other chloro groups or halogens present in other sites of the whole molecule, i.e., in case of all chlorinated derivatives **2** except compounds **2f,g,h,j**; 0.06 mole, 4.5672 g if the starting carboxylic acid in the first synthetic step **4.1.2** is dicarboxylic acid or if the structure of the corresponding chlorinated derivative contains only two 1,3,4-thiadiazole rings that contain only one chloro group at position 2 in each one of them and irrespective of any other chloro groups or halogens present in other sites of the whole molecule, i.e., in case of compounds **2f,g,j**; or 0.09 mole, 6.8508 g if the starting carboxylic acid in the first synthetic step **4.1.2** is tricarboxylic acid or if the structure of the corresponding chlorinated derivative contains only three 1,3,4-thiadiazole rings that contain only one chloro group at position 2 in each one of them and irrespective of any other chloro groups or halogens present in other sites of the whole molecule, i.e., in case of compound **2h**), and 25 mL (for each 0.03 mole of thiourea) of EtOH was refluxed for 3-5 h (compounds **3a,b,c,d,g,j** need only 3 h of heating, compounds **3h,i,k,l** need 4 h of heating, and compounds **3e,f,m** need 5 h of heating). Then the mixture was cooled down to R.T. and 50 mL (for each 0.03 mole of thiourea) of a solution of HCl (5%) was added drop by drop under stirring. After filtration of the reaction mixture (to remove impurities including the remaining unreacted Xss. thiourea), the aqueous layer was extracted by either CHCl$_3$ or DMSO (all the products were extracted by CHCl$_3$ except compounds **3f,g,h,l,m** which were extracted by DMSO) for three times (3 × 200 mL), the combined organic extracts were washed with 10% H$_2$SO$_4$ (90 mL), dried over anhyd. Na$_2$SO$_4$, concentrated by evaporation in rotavap under reduced pressure, and filtered to give the crude solid product which was further purified by recrystallization from an appropriate solvent(s) (see for each compound below) to give the pure target product **3** as shown below in details.

4.1.4.1. 5-Pentadecyl-1,3,4-thiadiazole-2-thiol (3a, *new*): Col. & App.: Recryst. from *DEE*; greenish brown amorphous powder; Yield: 96.0%; M.P.: 102-104 °C; IR (υ in cm^{-1}): Str. 2919 & Str. 2850 (C-H, Aliph.), 2522 (S-H), 1635 & 1521 (C=N), 1079 (N-N), 719 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 0.863-0.903 (t, J = 8.0 Hz, 3H, Terminal CH$_3$), 1.263 & 1.298 & 1.312 (m, 24H, All Other 12 CH$_2$), 1.621-1.704 (m, 2H, β-CH$_2$ to Thiadiazole Ring), 2.853-2.877 (t, J = 7.1 Hz, 2H, α-CH$_2$ to Thiadiazole Ring), 13.075 (s, 1H, Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 328.58): 328.25 (88.05), 211.20 (3.33), 134.00 (100.00), 118.10 (0.34), 85.10 (13.05), 57.05 (68.34); Elem. Anal. (%, for C$_{17}$H$_{32}$N$_2$S$_2$): *Calculated* (*Found*): C: 62.14 (62.14), H: 9.82 (9.81), N: 8.53 (8.55).

4.1.4.2. 1-(5-Mercapto-1,3,4-thiadiazol-2-yl)ethanol (3b, *new*): Recryst. from *abs. EtOH/H$_2$O (3:1, v/v)*; Col. & App.: brown fine powder; Yield: 89.0%; M.P.: 202-204 °C; IR (υ in cm^{-1}): Str. & Bro. 3372 (O-H), Str. 2922 & Str. 2850 (C-H, Aliph.), 2578 (S-H), Str. 1678 & Str. 1578 (C=N), Str. 1122 (C-O), Str. 1051 (N-N), 720 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 1.481-1.492 (d, J = 6.8 Hz, 3H, CH$_3$), 3.646 (s, 1H, Aliph. OH), 4.666 & 4.670 & 4.676 & 4.694 (q, J = 6.8 Hz, 1H, CH), 13.053 (s, 1H, Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 162.23): 162.20 (32.72), 130.00 (7.59), 117.20 (2.39), 85.15 (32.72), 64.00 (100.00), 57.10 (86.37); Elem. Anal. (%, for C$_4$H$_6$N$_2$OS$_2$): *Calculated* (*Found*): C: 29.61 (29.62), H: 3.73 (3.71), N: 17.27 (17.27).

4.1.4.3. 5-(Dichloromethyl)-1,3,4-thiadiazole-2-thiol (3c, *new*): Recryst. from *DEE*; Col. & App.: white crystalline plates; Yield: 86.5%; M.P.: 114 °C; IR (υ in cm^{-1}): Str. 2921 (C-H, Aliph.), 2574 (S-H), Str. 1629 & 1554 (C=N), 1076 (N-N), 755 (C-Cl), Str. 700 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 6.733 (s, 1H, CH), 13.035 (s, 1H, Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 201.10 & 203.10 & 205.10): 205.15 (2.94), 203.20 (17.71), 201.30 (26.74), 131.30 (3.48), 85.15 (39.36), 57.15 (100.00); Elem. Anal. (%, for C$_3$H$_2$Cl$_2$N$_2$S$_2$): *Calculated* (*Found*): C: 17.92 (17.97), H: 1.00 (1.00), N: 13.93 (13.99).

4.1.4.4. 5-(Trichloromethyl)-1,3,4-thiadiazole-2-thiol (3d, *new*): Recryst. from *DEE*; Col. & App.: brown amorphous crystals; Yield: 85.0%; M.P.: 112 °C; IR (υ in cm^{-1}): Str. 2567 (S-H), Str. 1623 & 1531 (C=N), Str. 1085 (N-N), 779 (C-Cl), 654 & 604 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 13.055 (s, 1H, Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 235.54 & 237.54 & 239.54 & 241.54): 242.00 (2.61), 240.25 (24.00), 238.00 (71.09), 236.25 (72.00), 134.00 (100.00), 55.00 (77.01); Elem. Anal. (%, for C$_3$HCl$_3$N$_2$S$_2$):

Calculated (*Found*): C: 15.30 (15.33), H: 0.43 (0.42), N: 11.89 (11.88).

4.1.4.5. (*S*)-5-(1-Amino-2-phenylethyl)-1,3,4-thiadiazole-2-thiol (3e, *new*): Recryst. from *abs. EtOH/H$_2$O (3:1, v/v)*; Col. & App.: dark brown fine powder; Yield: 87.5%; M.P.: 200-202 °C; IR (υ in cm^{-1}): Str. 3413 & Str. 3388 (2 N-H, i.e., NH$_2$), 3060 & 3027 (C-H, Arom.), 2952 & Str. 2921 (C-H, Aliph.), 2574 (S-H), Str. 1629 & 1554 (C=N), 1540 & 1521 & Str. 1494 & 1452 (C=C, Arom.), 1186 (C-N, Aliph.), 1076 (N-N), 750 & Str. 700 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 3.010 & 3.218 (2 dd, J = -12.4 Hz & 7.0 Hz, 2 Diastereotopic H, CH$_2$), 4.287 (t, J = 7.0 Hz, 1H, CH), 5.131 (s, 2H, Aliph. NH$_2$), 7.271 & 7.295 & 7.440 (m, 5H, 1 *p*- & 2 *o*- & 2 *m*-Benzene-H), 13.081 (s, 1H, Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 237.34): 237.25 (38.75), 161.05 (9.03), 134.00 (100.00), 105.10 (2.06), 77.05 (2.23), 57.00 (58.34); Elem. Anal. (%, for C$_{10}$H$_{11}$N$_3$S$_2$): *Calculated* (*Found*): C: 50.60 (50.66), H: 4.67 (4.62), N: 17.70 (17.77).

4.1.4.6. 5,5'-Methylenebis(1,3,4-thiadiazole-2-thiol) (3f, *new*): Recryst. from *abs. EtOH/H$_2$O (3:1, v/v)*; Col. & App.: reddish brown fine powder; Yield: 90.0%; M.P.: 288-290 °C; IR (υ in cm^{-1}): 2952 & Str. 2921 (C-H, Aliph.), 2566 & 2537 (S-H), Str. 1629 & 1554 & 1544 & 1521 (C=N), 1079 (N-N), 645 (C-S); ^{1}H-NMR (DMSO-d_6, δ in ppm): 3.826 (s, 2H, CH$_2$), 13.055 (s, 2H, 2 Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 248.37): 248.40 (0.58), 132.20 (1.12), 118.15 (6.20), 99.20 (13.49), 85.15 (25.49), 57.10 (100.00); Elem. Anal. (%, for C$_5$H$_4$N$_4$S$_4$): *Calculated* (*Found*): C: 24.18 (24.14), H: 1.62 (1.66), N: 22.56 (22.55).

4.1.4.7. (1*R*,2*R*)-1,2-Bis(5-mercapto-1,3,4-thiadiazol-2-yl)ethane-1,2-diol (3g, *new*): Recryst. from *abs. EtOH*; Col. & App.: greenish brown amorphous crystals; Yield: 88.0%; M.P.: 172-174 °C; IR (υ in cm^{-1}): Str./Bro. 3486 & Str./Bro. 3473 (O-H), 2942 & 2912 (C-H, Aliph.), 2621 & 2542 (S-H), 1654 & 1610 (C=N), 1152 (C-O), 1085 (N-N), 715 & Str. 647 & Str. 619 (C-S); ^{1}H-NMR (DMSO-d_6, δ in ppm): 3.626 (s, 2H, 2 Aliph. OH), 4.957 (d, J = 7 Hz, 2H, 2 CH), 13.048 (s, 2H, 2 Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 294.40): 294.10 (12.57), 177.10 (10.10), 161.10 (26.20), 117.10 (9.13), 64.00 (100.00), 59.05 (71.88); Elem. Anal. (%, for C$_6$H$_6$N$_4$O$_2$S$_4$): *Calculated* (*Found*): C: 24.48 (24.48), H: 2.05 (2.06), N: 19.03 (19.01).

4.1.4.8. 1,2,3-Tris(5-mercapto-1,3,4-thiadiazol-2-yl)propan-2-ol (3h, *new*): Recryst. from *abs. EtOH*; Col. & App.: yellowish orange fine powder; Yield: 95.5%; M.P.: 244-245 °C; IR (υ in cm^{-1}): Str. & Bro. 3494 (O-H), Str. 2922 (C-H, Aliph.), 2451 (S-H), Str. 1709 & Str. 1579 (C=N), Str. 1263 (C-O), Str. 1048

(N-N), Str. 767 & 699 & Str. 618 (C-S); ^{1}H-NMR (DMSO-d_6, δ in ppm): 2.972 (s, 4H, 2 CH$_2$), 3.622 (s, 1H, Aliph. OH), 13.031 (s, 3H, 3 Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 408.59): 408.20 (17.75), 392.20 (21.16), 328.20 (19.80), 100.20 (11.95), 85.15 (28.67), 57.10 (100.00); Elem. Anal. (%, for C$_9$H$_8$N$_6$OS$_6$): *Calculated* (*Found*): C: 26.46 (26.46), H: 1.97 (1.95), N: 20.57 (20.55).

4.1.4.9. (*E*)-5-Styryl-1,3,4-thiadiazole-2-thiol (3i, new): Recryst. from *abs*. *EtOH*; Col. & App.: brownish orange fine powder; Yield: 91.0%; M.P.: 292-295 °C; IR (υ in cm^{-1}): 3080 & 3057 (=C-H, Alkene), 3035 (C-H, Arom.), 2731 (S-H), Str. 1691 (C=C, Alkene), Str. 1635 (C=N), Str. 1560 & 1497 & Str. 1441 (C=C, Arom.), 1070 (N-N), 705 & 684 & 648 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 6.956 & 6.996 (2 d, *J* = 15.1 Hz, 2H, *trans* HC=CH), 7.300 & 7.373 & 7.411 & 7.589 & 7.603 (m, 5H, 1 *p*- & 2 *m*- & 2 *o*-Benzene-H), 13.065 (s, 1H, Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 220.31): 220.10 (85.35), 115.15 (100.00), 103.15 (14.30), 85.15 (5.03), 77.10 (26.66), 55.10 (7.63); Elem. Anal. (%, for C$_{10}$H$_8$N$_2$S$_2$): *Calculated* (*Found*): C: 54.52 (54.54), H: 3.66 (3.66), N: 12.72 (12.73).

4.1.4.10. (*E*)-5,5'-(Ethene-1,2-diyl)bis(1,3,4-thiadiazole-2-thiol) (3j, new): Recryst. from *abs. EtOH/H$_2$O (3:1, v/v)*; Col. & App.: pale white crystalline powder; Yield: 93.3%; M.P.: 277 °C; IR (υ in cm^{-1}): 3056 (=C-H, Alkene), 2550 (S-H), Str. 1621 (C=C, Alkene), Str. 1557 (C=N), Str. 1013 (N-N), 695 & 651 & 607 (C-S); ^{1}H-NMR (CDCl$_3$, δ in ppm): 6.997-7.001 (d, *J* = 15.1 Hz, 2H, *trans* HC=CH), 13.077 (s, 2H, 2 Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 260.38): 260.10 (4.76), 227.10 (5.34), 143.10 (5.48), 117.10 (6.20), 64.00 (100.00), 55.10 (25.32); Elem. Anal. (%, for C$_6$H$_4$N$_4$S$_4$): *Calculated* (*Found*): C: 27.68 (27.65), H: 1.55 (1.57), N: 21.52 (21.55).

4.1.4.11. 5-(4-Bromophenyl)-1,3,4-thiadiazole-2-thiol (3k, new): Recryst. from *abs. EtOH/H$_2$O (3:1, v/v)*; Col. & App.: yellow to orange crystalline powder; Yield: 92.0%; M.P.: 172-175 °C; IR (υ in cm^{-1}): 3070 & 3001 (C-H, Arom.), 2707 (S-H), 1634 & 1602 (C=N), 1589 & 1508 & Str. 1487 & 1460 (C=C, Arom.), Str. 1069 (N-N), 706 & 659 & 627 (C-S), 528 (C-Br); ^{1}H-NMR (CDCl$_3$, δ in ppm): 7.669-7.867 (m, 4H, 4 Benzene-H), 13.084 (s, 1H, Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 273.17 & 275.17): 275.05 (51.77), 273.05 (52.52), 117.15 (20.60), 77.05 (7.44), 74.05 (100.00), 57.10 (16.57); Elem. Anal. (%, for C$_8$H$_5$BrN$_2$S$_2$): *Calculated* (*Found*): C: 35.17 (35.19), H: 1.84 (1.88), N: 10.25 (10.22).

4.1.4.12. 5-(5-Mercapto-1,3,4-thiadiazol-2-yl)benzene-1,2,3-triol (3l, new): Recryst. from *abs. EtOH/*

H_2O *(3:1, v/v)*; Col. & App.: yellowish brown crystalline powder; Yield: 95.0%; M.P.: 265 °C (dec.); IR (υ in cm^{-1}): Str. & Bro. 3413 (O-H), 3060 & 3027 (C-H, Arom.), 2575 (S-H), Str. 1629 & 1554 (C=N), 1540 & 1521 & Str. 1494 & 1455 (C=C, Arom.), 1191 (C-O), 1076 (N-N), 750 & Str. 700 (C-S); ^{1}H-NMR (DMSO-d_6, δ in ppm): 5.331 (s, 3H, 3 Arom. OH), 6.733 (s, 2H, 2 Benzene-H), 13.083 (s, 1H, Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 242.27): 242.40 (0.27), 209.30 (1.57), 118.15 (6.20), 109.20 (11.01), 77.10 (0.58), 57.10 (100.00); Elem. Anal. (%, for C$_8$H$_6$N$_2$O$_3$S$_2$): *Calculated* (*Found*): C: 39.66 (39.63), H: 2.50 (2.52), N: 11.56 (11.57).

4.1.4.13. 7-Chloro-3-(5-mercapto-1,3,4-thiadiazol-2-yl)quinolin-4-ol (3m, new): Recryst. from *abs. EtOH*; Col. & App.: yellowish orange crystalline powder; Yield: 94.0%; M.P.: 290-291 °C; IR (υ in cm^{-1}): Str. & Bro. 3454 (O-H), 3073 (C-H, Arom.), 2531 (S-H), Str. 1616 & Str. 1554 (C=N), Str. 1496 & Str. 1452 (C=C, Arom.), 1204 (C-O), 1082 (N-N), 773 (C-Cl), 737 (C-S); ^{1}H-NMR (DMSO-d_6, δ in ppm): 5.335 (s, 1H, Arom. OH), 7.703 (dd, *J* = 7.5 Hz & 1.5 Hz, 1H, Quinoline-H-7), 7.947 (d, *J* = 1.5 Hz, 1H, Quinoline-H-9), 8.406 & 8.426 (d & s, *J*$_{H-6}$ = 7.5 Hz, 2H, Quinoline-H-6,2), 13.087 (s, 1H, Arom. SH); MS (*m/z* (Rel. Int. in %), M.Wt. = 295.77 & 297.77): 298.00 (4.86), 296.00 (14.61), 254.00 (100.00), 178.10 (87.64), 164.10 (58.43), 55.10 (41.57); Elem. Anal. (%, for C$_{11}$H$_6$ClN$_3$OS$_2$): *Calculated* (*Found*): C: 44.67 (44.66), H: 2.04 (2.02), N: 14.21 (14.24).

4.2. Pharmacological Assays
4.2.1. ABTS Test

All reagents and L-ascorbic acid were purchased from Aldrich Chemical Co., U.S.A.; while pure EtOH (of very high analytical grade) was purchased from El-Nasr Co. for Pharmaceutical Chemicals, Egypt. This assay was done according to the original idea of Re *et al.*[64] The ABTS$^{\cdot+}$ radical cation (blue-dark green) was prepared by reacting (i.e., mixing) equal volumes of ABTS stock solution (colorless; 7 mM in pure distilled H$_2$O) and potassium persulfate stock solution (K$_2$S$_2$O$_8$; 3.5 mM in pure distilled H$_2$O) (ABTS and K$_2$S$_2$O$_8$ react stoichiometrically at a ratio of 2:1, respectively). The mixture was kept and allowed to stand in the dark at R.T. overnight (i.e., for about 12-16 h in the darkness) until the reaction was complete and the strong spectrophotometric absorbance (under UV) at a wavelength of 734 nm reaches the maximal stable value to obtain the ABTS$^{\cdot+}$ stock solution which is valid for use in this form for about 2-3 days when stored in the dark at R.T. The ABTS$^{\cdot+}$ working solution was prepared by diluting the ABTS$^{\cdot+}$ stock solution in pure EtOH to have an absorbance (A$_{blank}$)

of 0.7±0.02 (after 3 times of measurement) at a wavelength of 734 nm and the solution was equilibrated with a temperature control set at 30 °C in an incubator (A_{blank} was adjusted in our assay to be exactly 0.7 before measuring the absorbance for all the test compounds). Free radical scavenging activity was assessed by mixing 1.5 mL of the blue-green $ABTS^{\cdot+}$ working solution with 10 µL of the solutions of the target test compounds (1,3,4-thiadiazoles) at various concentrations ranging from 10 to 300 µM (in distilled H_2O, pure EtOH, or mixture of both of them according to the solubility of each compound). The change in absorbance at 734 nm was immediately monitored at 0, 0.5, 1 min after the addition (i.e., after the mixing) and again at 5 min intervals until a steady-state value was obtained. The steady state was achieved after 15 min in our assay, so the absorbance value for each test compound after its addition to $ABTS^{\cdot+}$ solution (A_{test}) was taken after 15 min of their mixing. Values are means of 3 independent determinations (as all the measurements were taken 3 independent times after each period for each concentration of each test compound). The $ABTS^{\cdot+}$ radical cation scavenging activity of the test compound was calculated according to the equation shown before in the Pharmacological Studies Section. The antioxidant capacity of the different target test compounds **3a-m** was expressed as IC_{50} which is the concentration leading to a 50% decrease of the amount of $ABTS^{\cdot+}$ radical cation (see the Pharmacological Studies Section). L-Ascorbic acid was taken as a reference standard antioxidant.

4.2.2. DPPH Test

DPPH˙, L-ascorbic acid, and trolox were purchased from Aldrich Chemical Co., U.S.A.; while pure EtOH (of very high analytical grade) was purchased from El-Nasr Co. for Pharmaceutical Chemicals, Egypt. This assay was done according to the procedure described by Prouillac et al.[26,27] The stable DPPH˙ free radical has an absorption band at 516 nm, which disappears upon its reduction by a free radical scavenger compound. Briefly, the target test 1,3,4-thiadiazole derivatives (compounds **3a-m**) were added at various concentrations, varying from 5 to 150 µM in pure EtOH or aqueous EtOH solution (according to the solubility of each test compound), to a freshly prepared ethanolic solution of DPPH˙ (80 µM). The absorbance was firstly measured at 516 nm at time = 0 min (i.e., directly before adding the solution of the test compound to the DPPH˙ solution) (A_0) and after 30 min of incubation at R.T. (i.e., after 30 min of adding the test compound to the DPPH˙ solution and beginning the reaction) (A_{30}), and, the percent reduction in absorbance or the percent inhibition of DPPH˙ free radical by each test compound (I%) was calculated according to the equation shown before in the Pharmacological Studies Section. The concentration providing 50% inhibition (IC_{50}) was determined as shown before in the Pharmacological Studies Section. All experiments were carried out in triplicate. L-Ascorbic acid and trolox were taken as antioxidant references.

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Supplementary Materials:

Free samples of all the synthesized compounds (including starting & intermediate materials) and supplementary data associated with this research article can be requested directly from the principal investigator and author (Amgad Mohammed Rabie Hamed Fouda, Home Address: 16 Magliss El-Madina Street, Dikernis City, Dikernis, Dakahlia Governorate, Egypt; Departmental Address: Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura City, 35516, Mansoura, Dakahlia Governorate, Egypt; Mobile Numbers: +2-01019733188 & +2-01112900494; Telephone Number: +2-0503482471; E-mail Addresses: amgadpharmacist1@yahoo.com & dr.amgadrabie@gmail.com & pharm*org*chem1@mans.edu.eg).

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