Synthesis, Characterization, and Anti-Inflammatory Evaluation of 1,2,4-Oxadiazoles Combined with Thiosemicarbazide and 1,3,4-Oxadiazole Moieties

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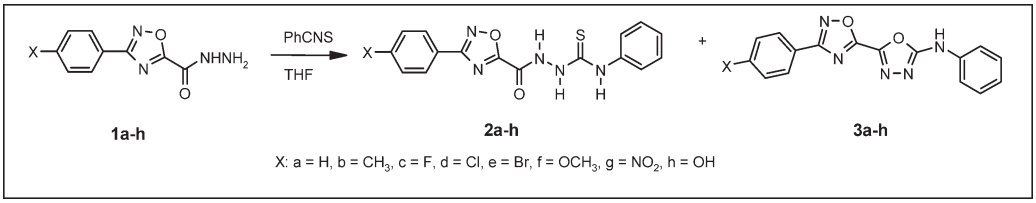

The reaction of 1,2,4-oxadiazole carbohydrazides **1a-h** with phenyl isothiocyanate led to an unexpected ring cyclisation of the thiosemicarbazide derivatives **2a-h**, giving compounds **3a-h** as side products. These two new series were preliminarily evaluated for their anti-inflammatory activity, using the carrageenin induced edema protocol.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used remarkably widely in the treatment of pain, fever, and inflammatory diseases, in particular rheumatoid arthritis [1–3]. However, severe side effects like gastrointestinal (GI) ulceration, bleeding, and nephrotoxicity are associated with the chronic use of NSAIDs [4,5]. Since most NSAIDs possess a carboxylic group or a 1,3-diketone moiety capable of forming an enol, they have the potential to have a topical effect on the stomach walls, leading to GI damage [6,7]. In addition, the inhibition of enzyme cyclooxygenases (COXs), which is the main NSAID action mechanism, also inhibits tissue prostraglandin production, undermining its physiological cytoprotective role in maintaining GI health [8–11].

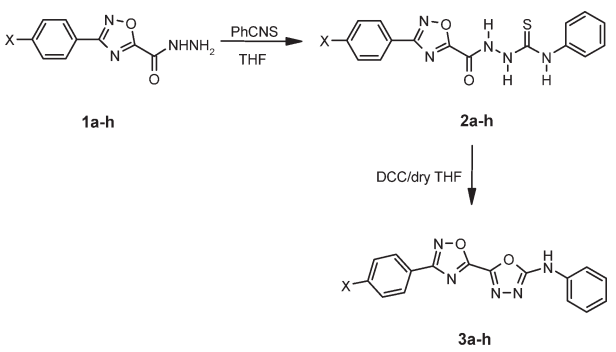
Substances containing heterocyclic and/or heteroaliphatic moieties have been found to exhibit a large spectrum of biological responses, including anti-inflammatory activity. It has been reported in the literature that certain compounds possessing 1,2,4-oxadiazole [12–14], 1,3,4-oxadiazole [15–19] or thiosemicarbazide [20–22] moieties exhibit significant anti-inflammatory properties. This led us to investigate the planning and synthesis of new series of substances whose structures fuse two of these groups with the consequent study of their biological responses. In our attempt to discover new and useful agents for the treatment of inflammation diseases, we selected the 1,2,4-oxadiazole ring as the main pharmacophoric group in our studies. Starting with 1,2,4-oxadiazole carbohydrazides **1a-h**, it was possible to introduce thiosemicarbazide and 1,3,4-oxadiazole residues at the C-5 position of the 1,2,4-oxadiazole ring, so as to obtain the designed substances. On the basis of the approach to the synthesis of 1,2,4-oxadiazole derivatives largely explored in our group, it was possible to synthesize compounds **1a-h**, which are the adequate starting materials for our purposes [23].

During the synthesis of the thiosemicarbazide derivatives **2a-h**, their spontaneous ring closure was observed, leading to 1,3,4-oxadiazole derivatives **3a-h**. To our best knowledge, this unusual outcome for this condensation reaction was never observed before and is possible due to the structural features of compounds **2a-h**. All isolated compounds were preliminary tested for their anti-inflammatory activity as part of our studies on new NSAIDs drugs.

RESULTS AND DISCUSSION

1,2,4-Oxadiazole hydrazides **1a-h** provided access to the designed heterogeneous chains and heterocyclic systems. Their synthesis was already described by our group and can be achieved in three easy and efficient steps [23]. Preparation of thiosemicarbazide derivatives has usually been carried out by condensation of hydrazides with appropriate isothiocyanates in a polar protic

Scheme 1



solvent under reflux. However, it was not possible to transform 1,2,4-oxadiazole hydrazides **1a-h** into their thiosemicarbazide derivatives **2a-h** under these conditions. First, educts were poorly soluble in ethanol or methanol even at their boiling points. Solubility was increased after addition of phenyl isothiocyanate and reflux. After reaction times, a complex mixture of products was observed when using TLC, but none of them could be isolated or identified. Access to the desired thiosemicarbazides **2a-h** was possible using dry THF as solvent and refluxing for only short periods of time (Scheme 1). Despite the satisfactory outcome, this methodology led to a result different from that usually reported in the literature. Thiosemicarbazide adducts **2a-h** have been formed in a mixture with their corresponding ring closed 1,3,4-oxadiazole derivatives **3a-h** as by-products in yields ranging from 2 to 10%, as determined after isolation (Tables 1 and 2).

Cyclisation of thiosemicarbazide moiety exclusively into the 1,3,4-oxadiazole nucleus has been reported under oxidative iodine-mediated conditions in the presence of base [24] or selective activation of the sulfur moiety by coupling reagents such as DCC [25] under reflux. No references to a spontaneous process have been found in the literature. This suggests that the ring closure process is possibly associated with the specific structural features of the thiosemicarbazides **2a-h**. In order to investigate the effect of the solvent on this cyclisation, the reaction of hydrazides **1a-h** with phenyl isothiocyanate was carried out in THF/MeOH 7:3. No difference in the results was observed, indicating that the reaction doesn't depend on using an aprotic or protic solvent. On the other hand, solutions of pure phenyl thiosemicarbazides **2a-h** in THF produce 1,3,4-oxadiazole derivatives **3a-h** very slowly at room temperature. By means of TLC control, traces of 1,3,4-oxadiazole were identified only after 10 days. Refluxing these solutions over several hours under argon, a mixture of decomposition products could be observed, confirming the thermal instability of the thiosemicarbazides. However, transformation into tetracyclic compounds **3a-h** was not increased, as observed by ^{1}H-NMR analysis. Hence, the cyclisation process should occur mainly during the formation of the thiosemicarbazides. Analysing the mechanism of this reaction, depicted in Scheme 2, it can be assumed that the formation of intermediate **A** is a critical step for understanding its outcome. After the breaking of the C=N bond, an electron pair of thiol group (pathway **a**) easily establishes the thiocarbonyl bond, leading to the thiosemicarbazide products **2a-h**, as normally described. Ring closure should otherwise occur as a consequence of a nucleophilic attack by the carbonyl oxygen on the carbon atom of the imine group, as shown in pathway **b**. However, this oxygen atom is usually not basic enough to undergo such a nucleophilic addition. It is reasonable to assume that the conjugated 3-aryl-1,2,4-oxadiazol-5-yl substituent attached to the

Table 1

Physical and pharmacological data for the derivatives N^1-[3-(4-substituted-aryl)-1,2,4-oxadiazol-5-yl carbonyl]-N^4-phenyl thiosemicarbazides **2a-h**.

Comp.	X	Molecular formula	Molecular weight	Yield (%)	M.p. (°C)	CFE, % inhibn ± S.E.M.[a,b]
2a	H	$C_{16}H_{13}N_5SO_2$	339	81	194–196	29.5 ± 7.2
2b	CH_3	$C_{17}H_{15}N_5SO_2$	353	75	192–193	28.9 ± 2.5
2c	F	$C_{16}H_{12}N_5SO_2F$	357	88	193–194	20.7 ± 2.5
2d	Cl	$C_{16}H_{12}N_5SO_2Cl$	373	82	193–194	23.5 ± 1.5
2e	Br	$C_{16}H_{12}N_5SO_2Br$	418	80	203–204	26.4 ± 2.5
2f	OCH_3	$C_{17}H_{15}N_5SO_3$	369	85	184–186	18.3 ± 1.8
2g	NO_2	$C_{16}H_{12}N_6SO_4$	384	90	211–212	30.7 ± 4.8
2h	OH	$C_{16}H_{13}N_5SO_3$	355	75	229–230	32.9 ± 3.2
Diclofenac sodium[c]				75.0 ± 5.7		

[a] Percentage of inhibition on carrageenin-induced rat paw edema at the dosis of 25 mg/kg. The result is the mean value ± S.E.M. for each test group.
[b] $P < 0.001$ and $P < 0.01$ represented a significant difference when compared with control group.
[c] At the dosis of 2 mg/kg.

Table 2

Physical and pharmacological data for derivatives 5-[3-(4-substituted-aryl)-1,2,4-oxadiazol-5-yl]-2-(*N*-phenylamino)-1,3,4-oxadiazoles **3a-h**.

Comp.	X	Molecular formula	Molecular weight	Yield (%)[a]	M.p. (°C)	CFE, %inhibn ± S.E.M.[b,c]
3°	H	$C_{16}H_{11}N_5O_2$	305	75 (10)	238–240	49.6 ± 5.8
3b	CH_3	$C_{17}H_{13}N_5O_2$	319	80 (9)	236–237	48.6 ± 2.8
3c	F	$C_{16}H_{10}N_5O_2F$	323	73 (7)	225–227	47.9 ± 2.3
3d	Cl	$C_{16}H_{10}N_5O_2Cl$	339	90 (8)	229–230	49.9 ± 4.9
3e	Br	$C_{16}H_{10}N_5O_2Br$	384	84 (6)	232–233	41.4 ± 5.7
3f	OCH_3	$C_{17}H_{13}N_5O_3$	335	93 (7)	225–227 (dec.)	39.2 ± 0.54
3g	NO_2	$C_{16}H_{10}N_6O_4$	350	85 (2)	259–260 (dec.)	61.3 ± 2.4
3h	OH	$C_{16}H_{11}N_5O_3$	321	89 (8)	218–220 (dec.)	59.2 ± 8.1
Diclofenac sodium[d]	75.0 ± 5.7					

[a] Values in parenthesis report the percentage of the compound obtained as side product after isolation in the reaction to obtain **2a-h**.
[b] Percentage of inhibition on carrageenin-induced rat paw edema at the dosis of 25 mg/kg. The result is the mean value ± S.E.M. for each test group.
[c] $P < 0.001$ and $P < 0.01$ represented a significant difference when compared with control group.
[d] At the dosis of 2 mg/kg.

carbonyl group acts as a good electron-donating group, enhancing the nucleophilicity of the oxygen atom. Thus, the reaction described by pathway **b** can take place in addition to the pathway **a**. Protons migration, elimination of the thiol group, and aromatisation of the five-membered ring should be a specially favoured and rapid process, owing to the direct conjugation between 1,3,4-oxadiazole and the other two aromatic rings.

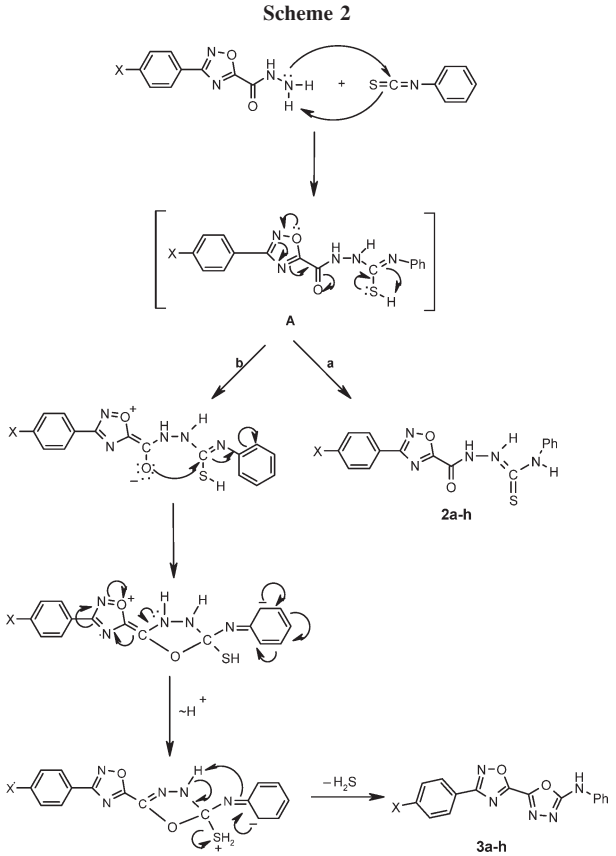
The nature of the para-substituents on the phenyl ring influences the quantity of products formed. The strong electron-withdrawing nitro-substituent doubtless affects the cyclisation reaction, since it decreases the stabilising effect of the aromatic rings on the carbonyl group, leading to only 2% of compound **3g** as a by-product. The other substituents seem to influence the ring closure process to almost the same extent, as suggested by the yields for isolated products (Table 2).

To obtain appropriate amounts of compounds **3a-h**, thiosemicarbazides **2a-h** were treated with DCC in dry THF, leading to the well known oxidative process of ring closure with good yields. The characterization and results of pharmacological tests for compounds **2a-h** and **3a-h** are given in Tables 1 and 2 as well as in the experimental protocols.

The anti-inflammatory activity of synthesized compounds **2a-h** and **3a-h** was evaluated using the carrageenin induced edema method [26]. The compounds were tested at a 25 mg/kg oral dose and compared with the standard drug diclofenac sodium in a dose of 2 mg/kg. Comparing the results in Tables 1 and 2 it can easily be concluded that compounds exhibiting the thiosemicarbazide moiety **2a-h** have a low level of activity. In contrast to these poor results, compounds **3a-h**, possessing the two oxadiazole rings directly linked to each other are more effective in edema inhibition. It was found that the presence of NO_2 and OH groups, in products **3g** and **3h** respectively, promotes better biological responses.

In the light of these results, we envisage introducing structural modifications in compounds **3a-h** to identify

Scheme 2



more potent substances, which can then be submitted to further biological studies.

In summary, a set of 16 new compounds were designed and synthesized to evaluate their anti-inflammatory profiles and to correlate them with molecular properties of these products. Although compounds bearing a thiosemicarbazide moiety **2a-h** are practically inactive, their derivatives **3a-h** showed better biological response to the carrageenin footpad edema (CFE) test, so that they can serve as models for designing a new series of derivatives, expected to be more effective as NSAIDs drugs. Compounds **3g** and **3h** were especially active, suggesting that groups capable of establishing hydrogen bonds should be crucial for the biological response. Studies of GI effects of these products were not carried out because of the limitation of their anti-inflammatory profiles.

The spontaneous ring closure of compounds bearing the thiosemicarbazide group **2a-h** leading to compounds **3a-h** was observed for the first time for this kind of transformation, constituting an interesting subject for further experimental and theoretical studies.

EXPERIMENTAL

Melting points were determined on a Gallenkamp capillary apparatus and are uncorrected. Infrared spectra were recorded using KBr discs on a Perkin-Elmer Paragon 500 FT-IR spectrometer. ^{1}H-NMR spectra were recorded on a Bruker DPX-200 spectrometer, with chemical shifts δ reported in ppm unities relative to the internal standard TMS. Mass spectra were obtained by using a Finnigam MAT 8200 or MAT 95 mass spectrometer. Values for High resolution mass spectrometry (HRMS) lie within the permitted limit intervals with resolution of 10,000. Reactions were generally run under an argon atmosphere. Elemental analysis was performed on a Carlo Erba EA 1110 elemental analyzer. Organic solutions were concentrated at vacuum on a rotary evaporator at room temperature or at 60°C. Column chromatography was performed with ICN Biomedicals silica gel 60, 32–63 μm.

Compounds **3-**(4-substitutedaryl)-1,2,4-oxadiazol-5-yl carbohydrazide (**1a-h**) were prepared by the procedure given in literature [23].

General procedure for the preparation of *N*^{1}-[3-(4-substitutedaryl)-1,2,4-oxadiazol-5-yl carbonyl]-*N*^{4}-phenyl thiosemicarbazides (2a-h). A solution of hydrazides **1a-h** (0.5 mmol) in 2.5 mL THF was gently heated at 50°C, then phenyl isothiocyanate (0.5 mmol, 0.068 g) was added and the reaction was allowed to proceed at reflux under inert atmosphere for 30 min before cooling to room temperature. Solvent was removed in vacuo and the crude products were recrystallized from THF/petroleum ether (30–60°C) to afford, after filtration, the thiosemicarbazides **2a-h** as main products, whose yields, melting points and results of biological evaluation are given in Table 1. The filtrate was concentrated and the residue was eluted on a silica gel column using CH_2Cl_2/MeOH 10:0.2 as eluent to give the secondary ring closed products **3a-h,** and their yields were reported in Table 2.

***N*^{1}-(3-Phenyl-1,2,4-oxadiazol-5-ylcarbonyl)-N^4-phenyl thiosemicarbazide (2a).** IR ($\tilde{\nu}$, cm^{-1}): 3302, 3207 (N—H), 1683 (C=O), 1226 (C=S); ^{1}H-NMR (DMSO-d_6, ppm): 11.5 (s, 1H, CONH), 10.0 (s, 1H, CSNH), 9.85 (s, 1H, NH), 8.10–8.05 (m, 2H, ortho-oxadiazole ArH), 7.63–7.58 (m, 3H, ArH), 7.42–7.29 (m, 4H, ArH), 7.16 (t, 1H, J = 7.1 Hz, para-amino ArH); MS (m/z, %): 339 ($M^{+\bullet}$, 24), 305 (8), 204 (21), 193 (39), 135 (94), 77 (100); HRMS, Calc. (Found) for $C_{16}H_{13}N_5SO_2$: 339.07900 (339.07970); *Anal*. Calcd.: C, 56.62; H, 3.86; N, 20.64. Found: C, 56.69; H, 4.07; N, 20.84.

***N*^{1}-[3-(4-Tolyl)-1,2,4-oxadiazol-5-ylcarbonyl]-N^4-phenyl thiosemicarbazide (2b).** IR ($\tilde{\nu}$, cm^{-1}): 3326, 3220, 3151 (N—H), 1689 (C=O), 1231 (C=S); ^{1}H-NMR (DMSO-d_6, ppm): 11.5 (s, 1H, CONH), 10.0 (s, 1H, CSNH), 9.84 (s, 1H, NH), 7.96 (d, 2H, AB-System, J = 8.0 Hz, ortho-oxadiazole ArH), 7.43–7.33 (m, 6H, ArH), 7.16 (t, 1H, J = 6.8 Hz, para-amino ArH), 2.48 (s, 3H, CH_3); MS (m/z, %): 353 ($M^{+\bullet}$, 21), 218 (12), 193 (27), 132 (81), 77 (100); HRMS, Calc. (Found) for $C_{17}H_{15}N_5SO_2$: 353.09465 (353.09573); *Anal*. Calcd.: C, 57.77; H, 4.28; N, 19.82. Found: C, 57.78; H, 4.29; N, 20.23.

***N*^{1}-[3-(4-Fluorophenyl)-1,2,4-oxadiazol-5-ylcarbonyl]-N^4-phenylthiosemicarbazide (2c).** IR ($\tilde{\nu}$, cm^{-1}): 3306, 3226, 3159 (N—H), 1688 (C=O), 1227 (C=S); ^{1}H-NMR (DMSO-d_6, ppm): 11.6 (s, 1H, CONH), 10.1 (s, 1H, CSNH), 9.84 (s, 1H, NH), 8.12 (dd, 2H, J = 8.4 Hz, ortho-oxadiazole ArH), 7.51–7.29 (m, 6H, ArH), 7.16 (t, 1H, J = 7.1 Hz, para-amino ArH); MS (m/z, %): 357 ($M^{+\bullet}$, 13), 222 (13), 193 (17), 136 (100), 77 (56); HRMS, Calc. (Found) for $C_{16}H_{12}N_5SO_2F$: 357.06957 (357.07017); *Anal*. Calcd.: C, 53.77; H, 3.38; N, 19.60. Found: C, 53.50; H, 3.49; N, 19.46.

***N*^{1}-[3-(4-Chlorophenyl)-1,2,4-oxadiazol-5-ylcarbonyl]-N^4-phenyl thiosemicarbazide (2d).** IR ($\tilde{\nu}$, cm^{-1}): 3325, 3221, 3158 (N—H), 1687 (C=O), 1232 (C=S); ^{1}H-NMR (DMSO-d_6, ppm): 11.6 (s, 1H, CONH), 10.1 (s, 1H, CSNH), 9.82 (s, 1H, NH), 8.08 (d, 2H, AB-System, J = 8.5 Hz, ortho-oxadiazole ArH), 7.70 (d, 2H, AB-System, J = 8.8 Hz, meta-oxadiazole ArH), 7.39–7.29 (m, 4H, ArH), 7.16 (t, 1H, J = 7.0 Hz, para-amino ArH); MS (m/z, %): 375 ($M^{+\bullet}$+2, 11), 373 ($M^{+\bullet}$, 25), 238 (13), 193 (48), 135 (100), 77 (79); HRMS, Calc. (Found) for $C_{16}H_{12}N_5SO_2{}^{35}Cl$: 373.04002 (373.03986); *Anal*. Calcd.: C, 51.41; H, 3.23; N, . Found: C, 51.49; H, 3.40; N, 18.80.

***N*^{1}-[3-(4-Bromophenyl)-1,2,4-oxadiazol-5-ylcarbonyl]-N^4-phenyl thiosemicarbazide (2e).** IR ($\tilde{\nu}$, cm^{-1}): 3319, 3187, 3099 (N—H), 1688 (C=O), 1219 (C=S); ^{1}H-NMR (DMSO-d_6, ppm): 11.6 (s, 1H, CONH), 10.1 (s, 1H, CSNH), 9.83 (s, 1H, NH), 8.00 (d, 2H, AB-System, J = 8.4 Hz, ortho-oxadiazole ArH), 7.84 (d, 2H, AB-System, J = 8.7 Hz, meta-oxadiazole ArH), 7.42–7.29 (m, 4H, ArH), 7.16 (t, 1H, J = 6.9 Hz, para-amino ArH); MS (m/z, %): 419 ($M^{+\bullet}$+2, 11), 417 ($M^{+\bullet}$, 11), 284/282 (13/13), 196 (55), 135 (100), 77 (52); HRMS, Calc. (Found) for $C_{16}H_{12}N_5SO_2{}^{81}Br$: 418.98746 (418.98777); *Anal*. Calcd.: C, 45.94; H, 2.89; N, 16.74. Found: C, 45.90; H, 2.90; N, 16.85.

***N*^{1}-[3-(4-Methoxyphenyl)-1,2,4-oxadiazol-5-ylcarbonyl]-N^4-phenyl thiosemicarbazide (2f).** IR ($\tilde{\nu}$, cm^{-1}): 3301, 3220, 3163 (N—H), 1686 (C=O), 1226 (C=S); ^{1}H-NMR (DMSO-d_6, ppm): 12.3 (s, 1H, CONH), 10.9 (s, 1H, CSNH), 10.7 (s, 1H, NH), 8.84 (d, 2H, AB-System, J = 9.1 Hz, ortho-oxadiazole ArH), 8.23 (m, 4H, ArH), 7.98 (broad m, 3H, ArH), 4.67 (s, 3H, OCH_3); MS (m/z, %): 369 ($M^{+\bullet}$, 4), 234

(13), 193 (6), 148 (100), 135 (67), 77 (48); HRMS, Calc. (Found) for $C_{17}H_{15}N_5SO_3$: 369.08956 (369.08884); *Anal*. Calcd.: C, 55.27; H, 4.09; N, 18.96. Found: C, 55.12; H, 4.05; N,19.05.

N*^{1}*-[3-(4-Nitrophenyl)-1,2,4-oxadiazol-5-ylcarbonyl]-N*^{4}*-phenyl thiosemicarbazide (2g). IR ($\tilde{\nu}$, cm^{-1}): 3329, 3223, 3157 (N—H), 1690 (C=O), 1220 (C=S); ^{1}H-NMR (DMSO-d_6, ppm): 11.6 (s, 1H, CONH), 10.1 (s, 1H, CSNH), 9.83 (s, 1H, NH), 8.46 (d, 2H, AB-System, J = 8.1 Hz, ortho-oxadiazole ArH), 8.33 (d, 2H, AB-System, J = 8.7 Hz, meta-oxadiazole ArH), 7.42–7.13 (m, 4H, ArH), 7.17 (t, 1H, J = 7.0 Hz, para-amino ArH); MS (m/z, %): 384 ($M^{+\bullet}$, 2), 249 (23), 135 (100), 77 (67); HRMS, Calc. (Found) for $C_{16}H_{12}N_6SO_4$: 384.06407 (384.06372); *Anal*. Calcd.: C, 49.99; H, 3.15; N, 21.86. Found: C, 50.02; H, 3.24; N, 21.89.

N*^{1}*-[3-(4-Hydroxyphenyl)-1,2,4-oxadiazol-5-ylcarbonyl]-N*^{4}*-phenyl thiosemicarbazide (2h). IR ($\tilde{\nu}$, cm^{-1}): 3289, 3183 (broad, N—H), 1694 (C=O), 1220 (C=S); ^{1}H-NMR (DMSO-d_6, ppm): 11.5 (s, 1H, CONH), 10.3 (s, 1H, OH), 10.0 (s, 1H, CSNH), 9.84 (s, 1H, NH), 7.90 (d, 2H, AB-System, J = 8.8 Hz, ortho-oxadiazole ArH), 7.42–7.29 (m, 4H, ArH), 7.16 (t, 1H, J = 7.1 Hz, para-amino ArH), 6.94 (d, 2H, AB-System, J = 8.8 Hz, meta-oxadiazole ArH); MS (m/z, %): 355 ($M^{+\bullet}$, 0.4), 220 (13), 134 (100), 77 (37); HRMS, Calc. (Found) for $C_{16}H_{13}N_5SO_3$: 355.07391 (355.07361); *Anal*. Calcd.: C, 54.07; H, 3.69; N, 19.71. Found: C, 53.91; H, 3.82; N, 19.67.

General procedure for the preparation of 5-[3-(4-substitutedaryl)-1,2,4-oxadiazol-5-yl]-2-(*N*-phenylamino)-1,3,4-oxadiazoles (3a-h). Thiosemicarbazides **2a-h** (0.5 mmol) were dissolved in 5 mL of dry THF together with DCC (0.75 mmol, 1.5 eq.), and the solution was refluxed under inert atmosphere for 2 h. After solvent evaporation under vacuum, crude products were digested in CH_2Cl_2/petroleum ether (30–60°C) 1:1, cooled in ice bath, filtered and washed with cold CH_2Cl_2/ petroleum ether 1:1. All products were purified by column chromatography on silica gel using CH_2Cl_2/MeOH 10:0.2 as eluent.

5-(3-Phenyl-1,2,4-oxadiazol-5-yl)-2-(*N*-phenylamino)-1,3,4-oxadiazole (3a). IR ($\tilde{\nu}$, cm^{-1}): 3172 (N—H), 1672 (C=C), 1638 (C=N); ^{1}H-NMR (DMSO-d_6, ppm): 11.3 (s, 1H, NH), 8.07 (broad s, 2H, ortho-oxadiazole ArH), 7.61 (broad d, 5H, J = 6.4 Hz, ArH), 7.39 (t, 2H, J = 7.6 Hz, meta-oxadiazole ArH), 7.07 (t, 1H, J = 7.1 Hz, para-amino ArH); MS (m/z, %): 305 ($M^{+\bullet}$, 100), 145 (18), 120 (46), 92 (15), 77 (71); HRMS, Calc. (Found) for $C_{16}H_{11}N_5O_2$: 305.09127 (305.09170); *Anal*. Calcd.: C, 62.95; H, 3.63; N, 22.94. Found: C, 62.80; H, 3.66; N, 23.15.

5-[3-(4-Tolyl)-1,2,4-oxadiazol-5-yl]-2-(N-phenylamino)-1,3,4-oxadiazole (3b). IR ($\tilde{\nu}$, cm^{-1}): 3258 (N—H), 1636 (C=C), 1621 (C=N); ^{1}H-NMR (DMSO-d_6, ppm): 11.3 (s, 1H, NH), 8.15 (dd, 2H, J = 5.2 Hz, ortho-oxadiazole ArH), 7.60 (d, 2H, AB-System, J = 7.6 Hz, ArH), 7.45 (m, 4H, ArH), 7.09 (t, 1H, J = 7.3 Hz, para-amino ArH), 2.39 (s, 3H, CH_3); MS (m/z, %): 319 ($M^{+\bullet}$, 100), 160 (13), 120 (13), 92 (6), 77 (13); HRMS, Calc. (Found) for $C_{17}H_{13}N_5O_2$: 319.10692 (319.10594); *Anal*. Calcd.: C, 63.94; H, 4.10; N, 21.93. Found: C, 63.96; H, 4.17; N, 21.93.

5-[3-(4-Fluorophenyl)-1,2,4-oxadiazol-5-yl]-2-(*N*-phenylamino)-1,3,4-oxadiazole (3c). IR ($\tilde{\nu}$, cm^{-1}): 3286 (N—H), 1639 (C=C), 1614 (C=N); ^{1}H-NMR (DMSO-d_6, ppm): 11.3 (s, 1H, NH), 8.15 (dd, 2H, J = 5.2 Hz, ortho-oxadiazole ArH), 7.63 (d, 2H, AB-System, J = 7.6 Hz, ArH), 7.45 (m, 4H, ArH), 7.09 (t, 1H, J = 7.3 Hz, para-amino ArH); MS (m/z, %): 323 ($M^{+\bullet}$, 100), 163 (8), 120 (32), 92 (9), 77 (24); HRMS, Calc. (Found) for $C_{16}H_{10}N_5O_2F$: 323.08185 (323.08104); *Anal*. Calcd.: C, 59.44; H, 3.12; N, 21.66. Found: C, 59.32; H, 3.23; N, 21.79.

5-[3-(4-Chlorophenyl)-1,2,4-oxadiazol-5-yl]-2-(*N*-phenylamino)-1,3,4-oxadiazole (3d). IR ($\tilde{\nu}$, cm^{-1}): 3290 (N—H), 1640 (C=C), 1613 (C=N); ^{1}H-NMR (DMSO-d_6, ppm): 11.3 (s, 1H, NH), 8.09 (d, 2H, AB-System, J = 8.5 Hz, ortho-oxadiazole ArH), 7.69 (d, 2H, AB-System, J = 8.8 Hz, meta-oxadiazole ArH), 7.62 (d, 2H, AB-System, J = 7.9 Hz, ortho-amino ArH), 7.40 (t, 2H, J = 7.6 Hz, meta-amino ArH), 7.08 (t, 1H, J = 7.3 Hz, para-amino ArH); MS (m/z, %): 341 ($M^{+\bullet}$+2, 35), 339 ($M^{+\bullet}$, 100), 181/179 (3/8), 120 (56), 92 (30), 77 (76); HRMS, Calc. (Found) for $C_{16}H_{10}N_5O_2{}^{35}Cl$: 339.05230 (339.05128); *Anal*. Calcd.: C, 56.56; H, 2.97; N, 20.61. Found: C, 56.70; H, 3.18; N, 20.70.

5-[3-(4-Bromophenyl)-1,2,4-oxadiazol-5-yl]-2-(*N*-phenylamino)-1,3,4-oxadiazole (3e). IR ($\tilde{\nu}$, cm^{-1}): 3292 (N—H), 1621 (C=C), 1584 (C=N); ^{1}H-NMR (DMSO-d_6, ppm): 11.3 (s, 1H, NH), 8.01 (d, 2H, AB-System, J = 8.3 Hz, ortho-oxadiazole ArH), 7.82 (d, 2H, AB-System, J = 8.6 Hz, meta-oxadiazole ArH), 7.61 (d, 2H, AB-System, J = 7.7 Hz, ortho-amino ArH), 7.40 (t, 2H, J = 7.5 Hz, meta-amino ArH), 7.07 (t, 1H, J = 7.4 Hz, para-amino ArH); MS (m/z, %): 385 ($M^{+\bullet}$+2, 99), 383 ($M^{+\bullet}$, 100), 227/225 (5/5), 120 (42), 92 (9), 77 (36); HRMS, Calc. (Found) for $C_{16}H_{10}N_5O_2{}^{79}Br$: 383.00179 (383.00193); *Anal*. Calcd.: C, 50.02; H, 2.62; N, 18.23. Found: C, 50.23; H, 2.80; N, 18.32.

5-[3-(4-Methoxyphenyl)-1,2,4-oxadiazol-5-yl]-2-(*N*-phenylamino)-1,3,4-oxadiazole (3f). IR ($\tilde{\nu}$, cm^{-1}): 3286 (N—H), 1639 (C=C), 1614 (C=N); ^{1}H-NMR (DMSO-d_6, ppm): 11.3 (s, 1H, NH), 8.03 (d, 2H, AB-System, J = 8.3 Hz, ortho-oxadiazole ArH), 7.60 (d, 2H, AB-System, J = 7.7 Hz, ortho-amino ArH), 7.40 (t, 2H, J = 7.5 Hz, meta-amino ArH), 7.14 (d, 2H, AB-System, J = 8.7 Hz, meta-oxadiazole ArH), 7.07 (t, 1H, para-amino ArH), 3.84 (s, 3H, OCH_3); MS (m/z, %): 335 ($M^{+\bullet}$, 100), 175 (4), 120 (10), 92 (6), 77 (15); HRMS, Calc. (Found) for $C_{17}H_{13}N_5O_3$: 335.10184 (335.10141); *Anal*. Calcd.: C, 60.89; H, 3.91; N, 20.88. Found: C, 60.64; H, 3.97; N, 20.89.

5-[3-(4-Nitrophenyl)-1,2,4-oxadiazol-5-yl]-2-(*N*-phenylamino)-1,3,4-oxadiazole (3g). IR ($\tilde{\nu}$, cm^{-1}): 3372 (N—H), 1611 (C=C), 1580 (C=N); ^{1}H-NMR (DMSO-d_6, ppm): 11.6 (s, 1H, NH), 8.45 (d, 2H, AB-System, J = 8.6 Hz, ortho-oxadiazole ArH), 8.34 (d, 2H, AB-System, J = 9.0 Hz, meta-oxadiazole ArH), 7.63 (d, 2H, AB-System, J = 7.9 Hz, ortho-amino ArH), 7.41 (t, 2H, J = 8.2 Hz, meta-amino ArH), 7.09 (t, 1H, J = 7.6 Hz, para-amino ArH); MS (m/z, %): 350 ($M^{+\bullet}$, 100), 190 (3), 120 (26), 92 (8), 77 (17); HRMS, Calc. (Found) for $C_{16}H_{10}N_6O_4$: 350.07635 (350.07591); *Anal*. Calcd.: C, 54.85; H, 2.88; N, 23.99. Found: C, 54.53; H, 2.99; N, 23.69.

5-[3-(4-Hydroxyphenyl)-1,2,4-oxadiazol-5-yl]-2-(*N*-phenylamino)-1,3,4-oxadiazole (3h). IR ($\tilde{\nu}$, cm^{-1}): 3298 (broad, N—H), 1626 (C=C); ^{1}H-NMR (DMSO-d_6, ppm): 11.2 (s, 1H, NH), 10.3 (s, 1H, OH), 7.90 (d, 2H, AB-System, J = 9.3 Hz, ortho-oxadiazole ArH), 7.61 (d, 2H, AB-System, J = 8.2 Hz, ortho-amino ArH), 7.39 (t, 2H, J = 7.4 Hz, meta-amino ArH), 7.07 (t, 1H, J = 7.3 Hz, para-amino ArH), 6.94 (d, 2H, AB-System, J = 8.8 Hz, meta-oxadiazole ArH); MS (m/z, %): 321 ($M^{+\bullet}$, 100), 161 (12), 120 (13), 92 (10), 77 (26); HRMS, Calc. (Found) for $C_{16}H_{11}N_5O_3$: 321.08619 (321.08603); *Anal*. Calcd.: C, 59.81; H, 3.45; N, 21.80. Found: C, 59.56; H, 3.63; N, 21.66.

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