(3+2) Annulation of Amidinothioureas with Binucleophile: Synthesis and Antimicrobial Activity of 3-Phenylamino-5-aryl/alkyl-1,2,4-oxadiazole Derivatives

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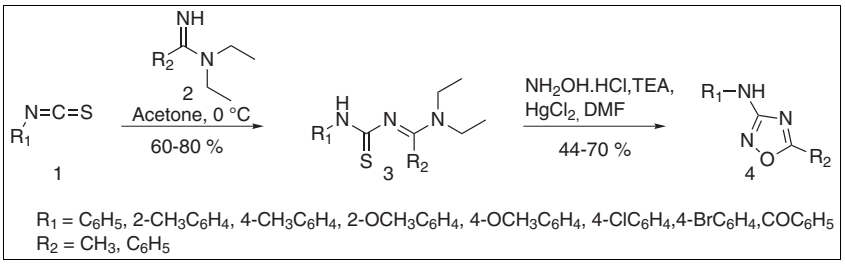
Received June 29, 2012
DOI 10.1002/jhet.1873
Published online 12 May 2014 in Wiley Online Library (wileyonlinelibrary.com).



Herein, we report an efficient method for preparation of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole by (3+2) annulation of amidinothioureas with binucleophilic hydroxylamine hydrochloride in the presence of mercury (II) chloride. Desired 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole was prepared in good to moderate yields. On the basis of the literature precedence, the mechanism for the formation of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole is proposed. The synthesized compounds were tested for their antimicrobial activity and showed promising inhibition of Gram-positive bacteria (*Staphylococcus aureus)* and fungi (*Candida albicans*).

J. Heterocyclic Chem., **51**, 1752 (2014).

INTRODUCTION

Oxadiazoles represent an important class of heterocyclic compounds, and their rich chemistry has been widely reviewed over the years [1,2]. 3,5-Disubstituted 1,2,4-oxadiazole is a versatile template and has wide utility in the medicinal chemistry. These classes of compound have been reported to be β-tryptase inhibitors [3], apoptosis inducers [4–6], reverse transcriptase inhibitors [7], gamma-aminobutyric acid modulators [8], $\alpha_v\beta_3$ receptor antagonists [9], and cannabinoid receptor 2 agonists [10]. 1,2,4-Oxadiazole ring system is widely used as an amide, ester, and isothiourea bioisostere because of its hydrolytic stability. Such a kind of bioisosteric replacement of amide group of peptide is an extensively explored area in the field of peptidomimetics [11–14]. 5-Amino-1,2,4-oxadiazole scaffold has been a part of widely investigated vasodilating drug imolamine [15], which is used in the treatment of angina pectoris, and fulvinazole [16], which has shown antischistosomal activity (Fig. 1).

Different synthetic methods have been reported for the synthesis of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole derivatives. The displacement of amidoxime-derived 5-chloro-1,2,4-oxadiazole with primary or secondary amine gave access to 5-amino-1,2,4-oxadiazole derivatives [17–19]. Reaction of hydroximoyl chloride with guanidine derivatives leads to the formation of 5-amino (primary or secondary)-1,2,4-oxadiazole derivatives [20]. The most common method for the preparation of 3-aryl/alkylamino 5-aryl/alkyl 1,2,4-oxadiazoles not only involve the use of toxic and corrosive cyanogen bromide but are also inefficient, and poor yields are being generally obtained [21a]. Recently, the synthesis of 3-aryl/alkylamino 5-aryl/alkyl-1,2,4-oxadiazoles has been reported from *N*-acyl thiourea derivatives [21b].

Despite numerous reports on their syntheses, to date, a highly efficient route to these 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole derivatives remains a challenge. In this context, a general and concise route for their synthesis would be a valuable tool for the exploration of chemistry and biology of these heterocycles.

RESULTS AND DISCUSSION

With the aforementioned background and keeping in mind some recent reports [22–24], we hypothesized that amidinothioureas **2**, which are prepared by reaction of corresponding isothiocyanate and *N*,*N*-diethyl-benza/acetamidine, would react with binucleophilic hydroxylamine hydrochloride in the presence of mercury (II) chloride catalyzed desulfurization/elimination reaction to give 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole derivatives

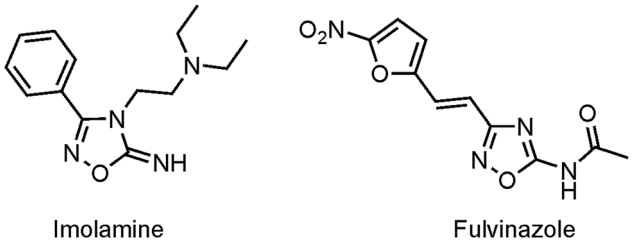


Figure 1. Drugs containing 1,2,4 oxadiazole scaffold.

4 (Scheme 1). This proposed reaction will allow us to perform this (3+2) annulation and would give access to a wide range of substitution patterns in oxadiazole derivatives.

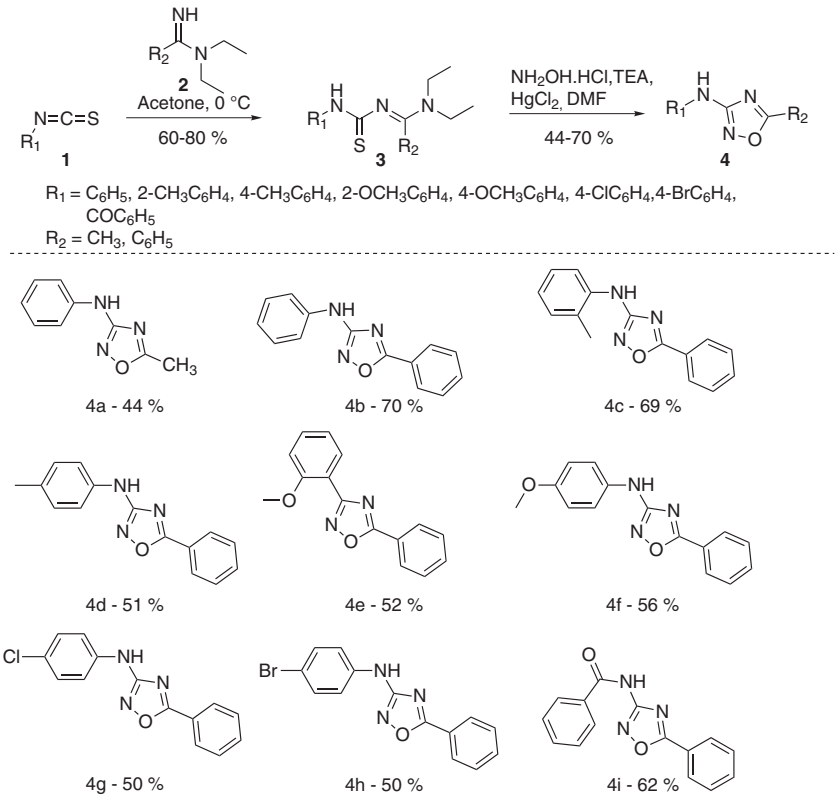
Towards this aim, an initial attempt was made, by refluxing the reaction mixture containing amidinothiourea **2** and hydoxylamine hydrochloride in methanol, but we did not see any progress in the reaction. Even the addition of TEA (2 equiv) does not lead to a formation of a product. Having obtained disappointing results with the aforementioned method, we turned our attention towards the polar aprotic solvent DMF. Reactions of amidinothiourea **2** and hydoxylamine hydrochloride when carried out in DMF using TEA as a base at room temperature and at 80°C did not gave target **4**. Then, we carried out the same reaction in the presence of strong thiophile mercury (II) chloride. Here, the desulfurization of amidinothiourea **2** was observed visually on the basis of the formation of a black precipitate of mercury sulfite. This reaction after careful filtration of mercury sulfite precipitate and conventional workup gave a mere 10% yield of the desired **4**.

After careful experimentation, 1.0 equiv of amidinothiourea **2** with 1.1 equiv of hydroxylamine hydrochloride in the presence of 1.1 equiv of mercury (II) chloride and 3.2 equiv TEA in DMF gave the desired 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole derivatives in moderate to good yields. The generality of this new synthetic method was investigated by employing the aforementioned optimized conditions. The results are summarized in Scheme 1.

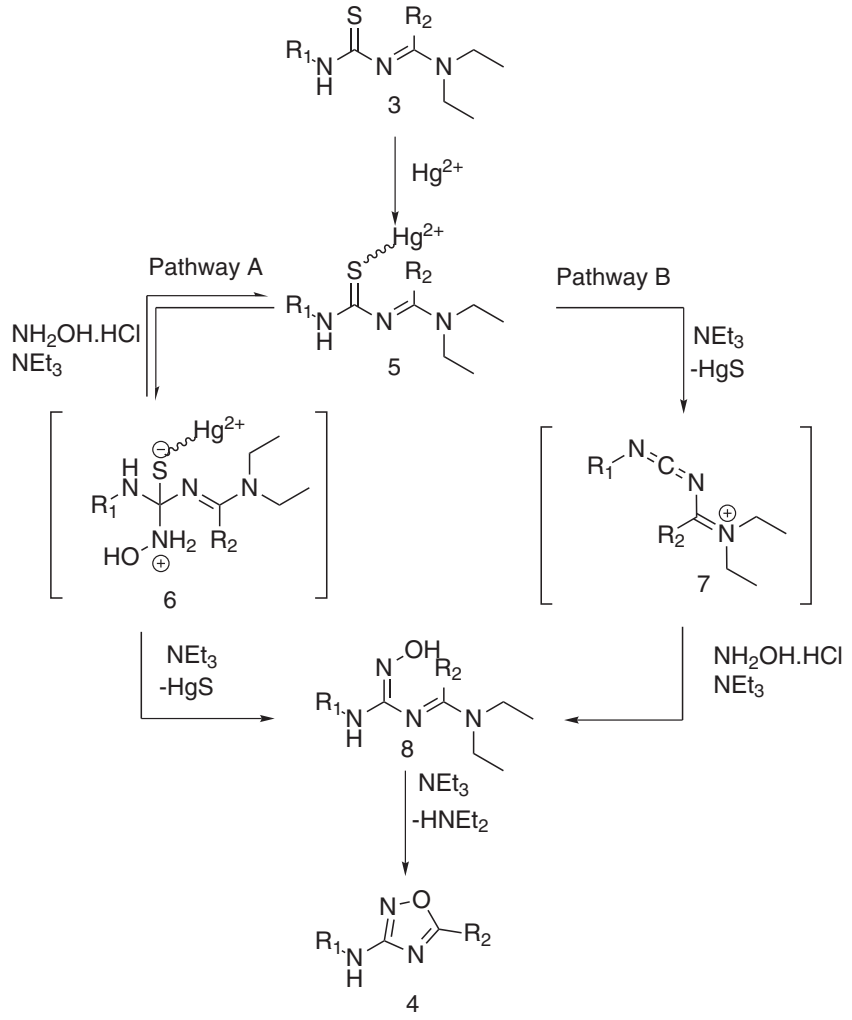
The isolated 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole derivatives (**4a–4i**) were characterized by spectroscopic methods. Disappearance of diethylamine peak in ^{1}H nmr spectrum present in amidinothiourea **2** indicated the formation of **4a**. Further, observation of a $[M]^+$ ion peak of 175.0740 in HRMS, corresponding to the molecular formula $C_{14}H_{11}ON_3$, and seven-line ^{13}C nmr spectrum with the characteristic carbon resonances at δ 165.22 and 174.86 confirmed the structure of **4a**.

On the basis of the literature [25,26] precedence for the formation of guanidine from thiourea, two mechanistic pathways can be postulated for the formation of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole derivatives (Scheme 2).

Scheme 1. Synthesis of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole derivatives from several functionalized amidinothioureas.



Scheme 2. Plausible mechanism for the formation of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole.



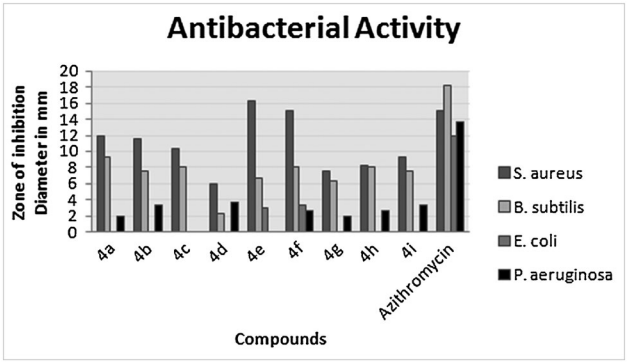


Figure 2. Antimicrobial activity of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole.

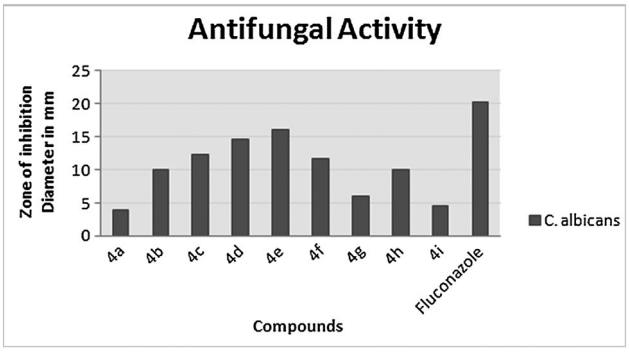


Figure 3. Antifungal activity of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole.

Both of which should involve an initial activated species **5** formed by complexion of amidinothiourea **2** with Hg^{2+}. In the absence of mercury chloride, the desired product did not form. In pathway A, amidinothiourea **2** may undergo nucleophilic attack from hydroxylamine hydrochloride in the presence of TEA to form intermediate **6**, followed by elimination of diethylamine to afford target product **4**. Alternatively, in pathway B, **5** undergoes desulfurization to

form a carbodiimide intermediate **7**, which is trapped by hydroxylamine hydrochloride in the presence of TEA, and subsequent elimination of diethylamine by nucleophilic oxygen will give the target product **4**.

All the newly synthesized 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazoles were tested for their antibacterial and antifungal activity [27–29]. The antimicrobial activity was compared with standard drug azithromycin, and the antifungal activity was compared with standard drug fluconazole (Figs 2 and 3). Overall in a series, activity against fungus was more compared with bacteria. Electron-donating substitution in the phenylamino ring at the third position of 1,2,4-oxadiazole is favorable for antifungal activity. The series showed minimum or no activity against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). When compared with standard azithromycin, compound **4e** showed better antimicrobial activity against Gram-positive *Staphylococcus aureus*.

CONCLUSION

In conclusion, we have developed a convenient synthesis of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazoles from readily available starting materials under mild conditions. The reaction is applicable to a wide range of substituted isothiocyanates and *N*,*N*-diethylamidines. Because of the importance of these compounds in medicinal chemistry, the present reaction may prove to be an efficient means for library construction. Some of the compounds from this series have shown promising antifungal and antimicrobial activities. Further extension of this chemistry towards the synthesis of other heterocycles can be easily envisioned and currently is being explored in our laboratory.

EXPERIMENTAL

General methods. All reactions were performed under air atmosphere. All other reagents and solvents are purchased from Avara chemicals Hyderabad, India and used without further purification. TLC was performed on precoated aluminum plate with silica gel 60 F_{254} available from Merck. Flash column chromatography was performed using SiO_2 60 (particle size 0.040–0.055 mm, 230–400 mesh) Proton and carbon nmr spectra were obtained on Bruker Avance 600 MHz nmr spectrometer. Chemical shifts are reported as δ values in parts per million (ppm) as referenced to a residual solvent. ^{1}H nmr spectra are tabulated as follows: chemical shift, multiplicity (s, singlet; m, multiplet); HRMS were obtained at the University of Pittsburgh Mass Spectrometry facility. The antimicrobial and antifungal activities were tested as per the reported protocol [27–29].

General procedure for the synthesis of 3-phenylamino-5-aryl/alkyl-1,2,4-oxadiazole (4). Amidinothiourea **3** (1.0 equiv) was taken in DMF in a round bottom flask. To it, hydroxylamine hydrochloride (1.1 equiv) was added followed by TEA (3.2 equiv). The reaction mixture was then cooled between 0°C and 5°C, and mercury (II) chloride (1.1 equiv) was added to it. Reaction mixture was maintained at the same temperature for 20 min. The reaction was then monitored by TLC, and after completion, precipitate of mercury sulfite was filtered through celite bed. The filtrate was then poured in cold water and stirred for 10 min. The precipitated product was filtered and was purified by column chromatography using suitable combination of hexane and ethyl acetate.

CAUTION: $HgCl_2$ is very toxic, and both the reagent and the crude product must be manipulated carefully. Although the byproduct of the reaction, HgS, is highly water insoluble, the solid residue retained in the short celite column must be disposed of in a suitable flask. The appropriate disposal of celite and SiO_2 used in the purification process must also be considered.

Characterization. ***5-Methyl-N-phenyl-1,2,4-oxadiazol-3-amine (4a).*** White solid, mp 124–126°C, Rf: 0.52 (hexane/ethyl acetate 1.5:0.35), IR (KBr, cm^{-1}: 3413, 3346, 3010, 2952, 2882, 1863, 1577, 1509, 1467, 1266.), ^{1}H nmr (600 MHz, $CDCl_3$) δ [ppm]: 2.5 (s, 3H, $–CH_3$); 6.75 (s, 1H, –NH–); 7–7.5 (m, 5H, ArH). ^{13}C nmr (150 MHz, $CDCl_3$) δ [ppm]: 12.43, 117.28, 122.11, 129.27, 138.91, 165.22, and 174.86. MS [M^+]: 175, HRMS (TOF MS ES+) calcd. for $C_9H_9ON_3$ [M]$^+$ 175.0759, found 175.0740.

N,5-Diphenyl-1,2,4-oxadiazol-3-amine (4b). White solid, mp 132–134°C, Rf: 0.64 (hexane/ethyl acetate 1.5:0.35), ir (KBr, cm^{-1}: 3411, 3073, 2921, 2551, 1723, 1566, 1447, 900, 733, 691.), ^{1}H nmr (600 MHz, $CDCl_3$) δ [ppm]: 6.9 (s, 1H, –NH–); 7.1–8.1 (m, 10H, ArH). ^{13}C nmr (150 MHz, $CDCl_3$) δ [ppm]: 117.29, 122.23, 124.09, 128.03, 129.07, 132.79, 138.95, 165.66, and 173.66. MS [M^+]: 237, HRMS (TOF MS ES+) calcd. for $C_{14}H_{11}ON_3$ [M]$^+$ 237.0902, found 237.0911.

5-Phenyl-N-o-tolyl-1,2,4-oxadiazol-3-amine (4c). White solid, mp 80–81°C, Rf: 0.68 (hexane/ethyl acetate 1.5:0.35), ir (KBr, cm^{-1}: 3448, 3041, 2999, 1905, 1785, 1572, 1585, 1462, 1343, 1254, 1118.), ^{1}H nmr (600 MHz, $CDCl_3$) δ [ppm]: 2.36 (s, 3H, $–CH_3$); 6.55 (s, 1H, –NH–); 7–8.2 (m, 9H, ArH). ^{13}C nmr (150 MHz, $CDCl_3$) δ [ppm]: 17.71, 117.99, 122.40, 124.14, 124.77, 128.00, 129.08, 130.46, 133.132.77, 137.23, 165.89, and 173.66. MS [M^+]: 251. HRMS (TOF MS ES+) calcd. for $C_{15}H_{13}ON_3$ [M]$^+$ 251.1072, found 251.1055.

5-Phenyl-N-p-tolyl-1,2,4-oxadiazol-3-amine (4d). White solid, mp 116–118°C, Rf: 0.6 (hexane/ethyl acetate 1.5:0.35), ir (KBr, cm^{-1}: 3286, 3208, 2921, 2364, 1978, 1608, 1566, 1405, 1118, 936, 811, 743, 691, 582.), ^{1}H nmr (600 MHz, $CDCl_3$) δ [ppm]: 2.32 (s, 3H, $–CH_3$); 6.75 (s, 1H, –NH–); 7.2–8.2 (m, 9H, ArH). ^{13}C nmr (150 MHz, $CDCl_3$) δ [ppm]: 20.70, 117.37, 124.16, 128.01, 129.05, 129.76, 131.64, 132.73, and 136.47. MS [M^+]: 251, HRMS (TOF MS ES+) calcd. for $C_{15}H_{13}ON_3$ [M]$^+$ 251.1018, found 251.1049.

3-(2-Methoxyphenyl)-5-phenyl-1,2,4-oxadiazole (4e). White solid, mp 88–90°C, Rf: 0.69 (hexane/ethyl acetate 1.5:0.35), ir (KBr, cm^{-1}: 3448, 3020, 2848, 2364, 2035, 1865, 1572, 1457, 1363, 1249, 1108, 1020, 910, 733, 619, 504.), ^{1}H nmr (600 MHz, $CDCl_3$) δ [ppm]: 3.9 (s, 3H, $–OCH_3$); 7.3 (s, 1H, –NH–); 6.9–7.1 and 7.5–8.2 (m, 9H, ArH). ^{13}C nmr (150 MHz, $CDCl_3$) δ [ppm]: 55.72, 109.83, 117.12, 121.26, 121.64, 124.24, 128.00, 128.75, 129.05, 132.69, 147.10, 165.57, and 173.47. MS [M^+]: 267, HRMS (TOF MS ES+) calcd. for $C_{15}H_{13}O_2N_3$ [M]$^+$ 267.1048, found 267.1015.

N-(4-Methoxyphenyl)-5-phenyl-1,2,4-oxadiazol-3-amine (4f). White solid, mp 106–108°C, Rf: 0.46 (hexane/ethyl acetate 1.5:0.35), ir (KBr, cm^{-1}: 3291, 3223, 3129, 2780, 1726, 1384, 1294, 1254, 1091, 978, 906, 759, 655.), ^{1}H nmr (600 MHz, $CDCl_3$) δ [ppm]: 3.8 (s, 3H, $–OCH_3$); 6.7 (s, 1H, –NH–); 6.9–8.1

(m, 9H, ArH). ^{13}C nmr (150 MHz, $CDCl_3$) δ [ppm]: 55.58, 114.52, 119.05, 124.18, 127.99, 129.03, 132.41, 132.70, 155.03, 165.96, and 173.58. MS [M^+]: 267, HRMS (TOF MS ES+) calcd. for $C_{15}H_{13}O_2N_3$ [M]$^+$ 267.1048, found 267.0997.

N-(4-Chlorophenyl)-5-phenyl-1,2,4-oxadiazol-3-amine (4g). White solid, mp 180–182°C, Rf: 0.53 (hexane/ethyl acetate 1.5:0.35), ir (KBr, cm^{-1}: 3416, 3093, 1889, 1514, 1410, 1098, 1009, 936, 821, 780, 701.), ^{1}H nmr (600 MHz, $CDCl_3$) δ [ppm]: 6.75 (s, 1H, –NH–); 7.2–8.2 (m, 9H, ArH). ^{13}C nmr (150 MHz, $CDCl_3$) δ [ppm]: 118.53, 123.99, 127.13, 128.04, 139.11, 129.25, 132.90, 137.54, 165.46, and 173.84. MS [M^+]: 271, 273, HRMS (TOF MS ES+) calcd. for $C_{14}H_{10}ON_3Cl$ [M]$^+$ 271.0472, found 271.0502.

N-(4-Bromophenyl)-5-phenyl-1,2,4-oxadiazol-3-amine (4h). White solid, mp 194–196°C, Rf: 0.4 (hexane/ethyl acetate 1.5:0.2), ir (KBr, cm^{-1}: 3911, 3416, 3093, 2884, 2593, 2364, 1889, 1554, 1405, 1077, 1009, 736, 816, 775, 665.), ^{1}H nmr (600 MHz, $CDCl_3$) δ [ppm]: 6.75 (s, 1H, –NH–); 7.2–8.2 (m, 9H, ArH). ^{13}C nmr (150 MHz, $CDCl_3$) δ [ppm]: 114.53, 118.92, 123.97, 128.05, 129.12, 132.18, 132.92, 138.03, 165.43, and 173.86. MS [M^+]: 315, 317, HRMS (TOF MS ES+) calcd. for $C_{14}H_{10}ON_3Br$ [M]$^+$ 314.9945, found 315.0001.

N-(5-Phenyl-1,2,4-oxadiazol-3-yl)benzamide (4i). White solid, mp118–120°C, Rf: 0.7 (hexane/ethyl acetate 1:1), ir (KBr, cm^{-1}: 3244, 2776, 2364, 2182, 1686, 1630, 1488, 1291, 1159, 1098, 926, 712, 660.), ^{1}H nmr (600 MHz, $CDCl_3$) δ [ppm]: 7.2–8.2 (m, 10H, ArH); 8.7 (s, 1H, –NH–). ^{13}C nmr (150 MHz, $CDCl_3$) δ [ppm]: 123.62, 127.57, 128.12, 129.00, 129.16, 132.51, 132.97, 133.17, 163.21, and 174.97. HRMS (TOF MS ES+) calcd. for $C_{15}H_{11}O_2N_3$ [M]$^+$ 288.0749, found 288.0760.

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