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A facile one pot synthesis of 2-arylamino-5-aryloxylalkyl-1,3,4-oxadiazoles and their urease inhibition studies

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Abstract

A one pot method for the synthesis of structural type urease inhibitors, 2-amino-1,3,4-oxadiazoles, was developed. The structures of the compounds were established using spectroanalytical techniques and unambiguously confirmed by single crystal X-ray analysis of compound **3o**. The synthesized compounds were tested against jack beans urease and most of the compounds (**3c**, **3g**, **3j**, **3k**, **3n**, **3r-3v**) were found more active than the standard. The most potent compound **(3u)** had an IC_{50} value of 6.03 ± 0.02 μM as compared to the IC_{50} value of the standard (thiourea; 22.0 ± 1.2 μM). The prominent urease inhibition activity of these compounds may serve as an important finding in the development of less toxic and more potent antiulcer drugs. The compounds were also investigated against four bacterial strains and some of the compounds (**3g** and **3r**) were found more potent than the standard drug (ciprofloxacin) against all the tested strains. The MIC value for compound **3g** was 0.156 μmol/mL against the tested bacterial strains.

Introduction

1,3,4-Oxadiazoles constitute an important class of five membered heterocyclic pharmacophores. They have numerous biological applications such as anti-tumour (1), antibacterial (2), anti-inflammatory (3), anti-HIV (4), anti-convulsant (5), enzyme inhibition (6), NR2B receptors (7) and hypoglycemic (8,9). The insecticidal (10,11), fungicidal (12), herbicidal (13) and antimicrobial (4,14,15,16) activities have also been reported for this class of compounds. Besides, being used as core nucleus in liquid crystalline compounds (17), oxadiazoles are also used as fluorescent materials in organic light emitting diodes (18,19). Different methods for the synthesis of 1,3,4-oxadiazoles have recently been reviewed along

with other five membered heterocycles (20). 2-Amino-1,3,4-oxadiazles are commonly synthesized by cyclization of corresponding semicarbazide or thiosemicarbazide derivatives (21-23). The use of semicarbazides requires harsh reaction conditions such as the use of $SOCl_2$, $POCl_3$ or P_2O_5. The use of phosphonium salts and other reagents result in significant by-products and are thus limited to solid phase synthesis. For the cyclization of thiosemicarbazides, different reagent like DCC or EDC or highly reactive alkylating agents have been used (24). H_2S scavengers such as mercuric or lead oxide are another important approach for the synthesis of these compounds (25). Thiosemicarbazides may easily be purified by precipitation from the reaction mixture or recrystallization, but it is not necessary (26). The use of H_2S scavengers in the cyclization of thiosemicarbazides (25) provoked us to investigate their use in one pot procedure. In the past, we have reported the synthesis of different chiral azoles from thiosemicarbazide intermediates and their biological applications (27,28) and faced the problem of lower yields. To overcome the problem of low yields in step-wise procedure, we investigated a one pot approach for the synthesis of 2-arylamino-1,3,4-oxadiazoles (Scheme 1) and explored the pharmacological applications of the products.

Methods and Materials

General

Melting points of the compounds were measured in open capillaries using Gallenkamp melting point apparatus (MP-D) and are uncorrected. Infrared (IR) spectra were recorded on a Thermo Scientific Nicolet 6700FT-IR spectrophotometer (USA) using the ATR (attenuated total reflectance) facility. Mass spectra were measured on a MAT-112-S spectrometer at 70 eV. The ^{1}H and ^{13}C NMR spectra were run on a Bruker Avance 300 MHz NMR spectrophotometer and signals calibrated to the residual solvent signal (chemical shifts in δ ppm). Elemetal analysis was determined using Leco CHNS-932 Elemental Analyzer, Leco Corporation (USA) and were within ± 0.4 % of the theoretical values. The X-ray crystallography was performed on an Oxford

Diffraction Xcalibur E diffractometer. The isothiocyanates **(2a-e)** were commercial products of Sigma-Aldrich. Compounds **1a-g** were prepared according to a reported procedure (28).

Synthesis of compounds 3a-3u

All the 2-arylamino-5-aryloxyalkyl-1,3,4-oxadiazoles were synthesized by the same general procedure: Aryloxyalkanoic acid hydrazide (**1a-g**) was dissolved in methanol (30 mL) and methanolic solution (10 mL) of arylisothiocyanate (**2a-e**, 1.0 eq) added slowly with stirring. The resulting mixture was refluxed and consumption of arylisothiocyanate, *i.e.,* formation of thiosemicarbazide (3-4 hr) monitored by tlc (petroleum ether-ethyl acetate; 6 : 4). After complete consumption of arylisothiocyanate required quantity of mercuric acetate (1.0 eq) was added and the reflux continued. After another 2-3 hours, tlc indicated the formation of one major product, *i.e.,* the product of the cyclization of thiosemicarbazide to 2-aminooxadiazole. The reaction mixture was cooled down to room temperature, filtered and concentrated *in vacuo*. The resulting solid was either recrystallized from aqueous ethanol or purified by flash column chromatography. The structures of the synthesized compounds (**3a-3u**) were established using spectroscopic techniques.

Urease inhibition assay

In vitro inhibitory studies on urease were determined by indophenols method which measures the liberation of ammonia from the reaction (29). Changes in the absorbance at 625 nm of indophenols blue produced in the Berthelot reaction after incubation for 30 min at 30°C was used to assess the amount of ammonia.

In short, 96-well plates were used for the assay mixture and incubated for 30 minutes at 30°C. The ingredients of the assay mixture were: 40 µL buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA and 0.01 M $LiCl_2$, pH 8.2) along with 10 µL of enzyme (5 U/mL) and 10 µL of test compound. In addition to this, equal volumes (40µL each) of phenol reagents (1% w/v phenol

and 0.005% w/v sodium nitroprusside) and alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. After 30 min, the absorbance was measured with the help of a microplate reader (Bio-TekELx 800TM, Instruments, Inc. USA). The reactions were exercised in triplicate. The standard inhibitor in the current assay was thiourea. The IC_{50} values of the compounds were determined.

Antibacterial activity

The serial dilution method was used to analyze *in vitro* antibacterial activities of the compounds by minimum inhibitory concentration (MIC) (30). Further, two Gram negative bacteria namely, *Escherichia coli* and *Shigella flexneri*, and two Gram positive bacteria namely, *Staphylococcus aureus* and *Bacillus subtilis* were deployed in the present antibacterial assay. These bacterial strains were stored and sub-cultured for compound testing using Muller-Hinton broth (Merck) at 37 °C. Moreover, for the production of cell suspension of about 10^{-5} CFU mL^{-1} (colony-forming units per mL), the cells were suspended in saline solution. Serial dilutions to final concentrations of 2.5, 1.25, 0.625, 0.313 and 0.156 µg/mL were prepared in the test tubes of the compounds under study that were previously dissolved in N,N-dimethylformamide (DMF). Twenty four hour (24 h) old inoculum having 100 µL was added to each test tube. The lowest inhibitory concentration of the test compounds were determined by MIC method that shows the zone of inhibition after 18 h, for incubating 24 h at 37 °C. Ciprofloxacin was used as the standard drug in the assay.

X-Ray Structure Determination of Compound 3o

Crystal data: Triclinic, $P\bar{1}$, a = 5.5706(4), b = 9.7318(6), c = 15.8489(8) Å, α = 95.550(5), β = 93.081(5), γ = 95.573(5)°, V = 849.40 $Å^3$, Z = 2, μ = 2.6 mm^{-1}, D_x = 1.598 Mg/m^3. *Data collection and reduction*: A colourless prism 0.4 × 0.15 × 0.10 mm was mounted on a glass fibre in inert oil and transferred to the cold gas stream of the diffractometer (Oxford Diffraction Xcalibur E). A

total of 23036 intensities were recorded to $2\theta_{max}$ 56.6° using monochromated Mo $K\alpha$ radiation (λ = 0.71073 Å); 3983 of these were independent (R_{int} = 0.027). Absorption corrections were performed on the basis of multi-scans. *Structure refinement*: The structure was refined anisotropically on F^2 using the program SHELXL-97 (31). The NH hydrogen was refined freely but using a distance restraint; the methyl was refined as an idealised rigid group allowed to rotate but not tip; other hydrogen atoms were included using a riding model starting from calculated positions. The final *wR*2 for all reflections was 0.066 for 222 parameters, with *R*1 = 0.026 for reflections with $I > 2\sigma(I)$; S = 1.09, max. $\Delta\rho$ = 0.69 e Å^{-3}.

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-864708. Copies of the data can be obtained free of charge from www.ccdc.cam.ac.uk/data_request/cif.

Results and Discussion

Chemistry

The selected α-aryloxypropanoic/butanoic acid hydrazides **1a-g** were prepared starting from different substituted phenols (32) and α-bromopropanoic acid, followed by esterification and hydrazinolysis (scheme 1). The hydrazides were reacted with arylisothiocyanates in refluxing methanol and consumption of the reactants was monitored by thin layer chromatography (tlc). After the consumption of reactants (3-4 hr), required quantity (1.0 eq) of mercuric acetate ($Hg(OAc)_2$) was added and the reflux continued. The reaction mixture gradually turned black indicating the formation of mercuric sulfide (HgS). After the addition of $Hg(OAc)_2$, it took another 2-3 hr for completion of the reaction (tlc). The product was isolated by suction filtration. Recently, a one pot procedure for the synthesis of 1,3,4-oxadiazoles involving resin bound reagents has also been reported (23) but the procedure involves very long reaction times and the resin bound reagents are expensive too. In contrast, the procedure being reported here has the clear advantage of reaction completion in a much shorter time and the reagents are relatively inexpensive.

Seven different aryloxyalkanoic acid hydrazides and five arylisothiocyanates were utilized in the current procedure. The investigated one pot procedure is clearly advantageous over the step wise procedure (22) in that: no isolation of the intermediate thiosemicarbazides is required, it is less time consuming and gives better purified yields.

The IR spectra of compounds **3a-u** indicated the formation of 1,3,4-oxadiazole ring by the presence of weak CN absorptions in the region of 1620-1580 cm^{-1}. A single absorption for NH was observed in the range 3270-3250 cm^{-1}. In ^{1}H NMR spectra, the cyclization to 1,3,4-oxadiazoles was confirmed by the appearance of only one downfield singlet in the range of δ 11.67-8.47 with one proton integral, assigned to the only NH proton. The integration of aromatic signals also confirmed the cyclization by the presence of two benzene rings with varied substitution pattern. The signals for aromatic protons were observed as two multiplets for *p*-substituted aminophenyl moiety and two multiplets for *p*-substituted phenoxy moiety. The ^{1}H NMR spectra of compounds **3e**, **3i**, **3k**, and **3o** contained three signals for the 3,4-dichlorophenoxy moiety in the aromatic region; a doublet (^{4}J = 3.0 Hz) assigned to proton H-2 residing between the chlorine and oxygen substituents, the second doublet (^{3}J = 9.0 Hz) for proton H-5 *ortho* to the oxygen atom and a doublet of doublet (^{3}J = 9.0 Hz, ^{4}J = 3.0 Hz) for proton H-6 *ortho* to the chlorine atom. In the aliphatic region, the signal for CH was observed downfield from δ 5.23 to 5.86 either as a triplet or a quartet depending on the nature of R group. In ^{13}C NMR spectra, the signals for imine (CN) carbon were observed in the region of 158-161 ppm. The carbon signals for the compounds (**3c-f**) bearing 4-fluorophenoxy moiety were observed as doublets with different $J_{C,F}$ values depending on the number of intervening bonds. The structures of the compounds **3a-u** were further confirmed by the mass spectral analysis. The mass fragmentation patterns were in accordance with the structural features of the molecules (figure 1). The most important fragments for the cleavage of oxadiazole nucleus were observed at *m/z* = 119 + X and *m/z* = 132/133 + R + R´, corresponding to the C–O–C-N and N–N–C–O cleavage. Other fragments containing aminoaryl and aryloxy moieties were also observed. The observed fragments led to the confirmation of the suggested structures for 2,5-disubstituted 1,3,4-oxadiazoles. The purity of the synthesized compounds was ascertained by

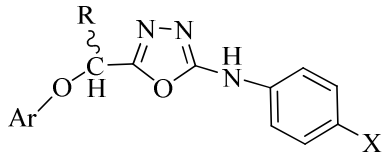
elemental analysis and the calculated values for CHN were found in a very good agreement with the observed data.

The structures of the synthesized compounds were also confirmed by single crystal X-Rays analysis of compound **3o**. Except for the ethyl group, the molecule has an "L"-shape (Figure 2). Molecular dimensions (e.g. of the five-membered ring, see figure caption) may be regarded as normal. The molecular packing involves layers parallel to the *bc* plane (Figure 3) in which molecules are linked by short contacts Cl···Cl 3.43, Cl···O1 3.34 and N3···Br 3.53 Å. The N3-H03 group, a classical hydrogen bond donor, links the layers via the interaction to N2 of a molecule related by *a* axis translation.

Urease Inhibition Studies

All the synthesized 1,3,4-oxidiazole derivatives were investigated for their urease inhibitory activities against Jack beans urease. Among investigated compounds, **3c, 3g, 3j, 3k, 3n, 3r, 3s, 3t** and **3u** were found more potent with higher inhibition as compared to the IC_{50} value of the standard inhibitor (thiourea) as shown in table 1. The presence of ethyl group rather than methyl at the alkoxycarbon, as well as strong electron withdrawing groups (fluoro or nitro) at position 4 of the arylmino group was found more effective in imparting inhibitory characters to these compounds. Compound **3u** has the least IC_{50} value among the most potent compounds in the current study. This may be attributed to the presence of highly electron withdrawing nitro group. Since the fluorine atom has the highest electro-negativity and electron withdrawing capacity, therefore, the second most active compound **3g** possesses a fluorine substituent at position 4 of the arylamino group. The compound **3t** ranked the second least IC_{50} value after **3u** and **3g**. Compounds **3g**, **3t** and **3u** are three times more potent than the standard as can be observed from their IC_{50} values. It appears that greater the electron withdrawing ability of the 4-arylamino part of the molecule, least IC_{50} value is the outcome.

Table 1. Urease inhibition activities of the synthesized compounds **(3a-u)**.



Compd.	Ar	R	X	IC_{50}+SEM (μM)
3a	4-ClC_6H_4	Me	Me	26.2 ± 2.2
3b	4-BrC_6H_4	Me	Me	33.5 ± 1.4
3c	4-MeC_6H_4	Me	Me	9.82 ± 0.08
3d	3,4-$Cl_2C_6H_3$	Me	Me	41.0 ± 1.3
3e	4-BrC_6H_4	Et	Me	47.4 ± 3.4
3f	3,4-$Cl_2C_6H_3$	Et	Me	47.4 ± 2.2
3g	4-BrC_6H_4	Me	F	6.21 ± 0.04
3h	4-ClC_6H_4	Me	F	45.9 ± 5.2
3i	3,4-$Cl_2C_6H_3$	Me	F	45.3 ± 8.1
3j	4-MeC_6H_4	Me	F	12.4 ± 0.06
3k	4-BrC_6H_4	Me	Cl	11.6 ± 0.05
3l	4-ClC_6H_4	Me	Cl	33.2 ± 2.7
3m	3,4-$Cl_2C_6H_3$	Me	Cl	42.8 ± 6.7
3n	4-MeC_6H_4	Me	Cl	11.5 ± 0.07
3o	4-BrC_6H_4	Et	Cl	57.2 ± 6.3
3p	4-BrC_6H_4	Et	Br	45.5 ± 4.2
3q	4-MeC_6H_4	Et	Br	35.8 ± 1.2

3r	4-BrC_6H_4	Et	F	7.65 ± 0.06
3s	3,4-$Cl_2C_6H_3$	Et	F	8.02 ± 0.07
3t	4-MeC_6H_4	Et	F	7.42 ± 0.03
3u	4-BrC_6H_4	Et	NO_2	6.03 ± 0.02
Thiourea				22.0 ± 1.2

Antibacterial activity

The antibacterial activity of the synthesized 1,3,4-oxidiazole derivatives was evaluated using two strains in each category; Gram-positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilis*, and Gram-negative bacteria namely, *Escherichia coli* and *Shigella flexneri*. The reference drug used in the assay was ciprofloxacin. The minimum inhibitory concentrations of the compounds were determined by using Muller-Hinton broth (Merck). The compounds demonstrated excellent antibacterial activity against the tested strains.

Compounds **3g** and **3r** proved to be the most potent antibacterials against all the tested strains in the current study. Compounds **3c**, **3g**, **3k**, **3r**, **3t** and **3u** were equipotent (MIC: 0.156µg/mL) as the standard ciproloxicin against *E. coli*. Some of the compounds (**3c, 3g, 3j, 3k, 3n, 3r, 3t** and **3u**) were four times more active than the standard (ciprofloxacin) against *B. subtilis* as shown in table 2. In addition, the potency of compounds **3c, 3f, 3g, 3j, 3q, 3r** and **3s** against *S. aureus* is comparable to that of ciprofloxacin. Compounds **3b** and **3g** were twice as potent as the standard against *S. flexneri*. The activity of the rest of the compounds, except **3c, 3e, 3l** and **3o**, was comparable to the standard against *S. flexneri*. The two most potent antibacterial compounds **(3g** and **3r)** possess a fluorine atom at position 4 of the arylamino group while a bromine atom at position 4 of the arylkoxy group. Since halogens are electron withdrawing, the electron withdrawing effect of these groups might be the possible reason for higher antibacterial activity of the compounds.

Table 2. The MIC (µmol/mL) values[a] of 1,3,4-oxidiazole derivatives **(3a-u)** against bacterial strains.

Compd.	Bacterial strains and MIC values (µmol/mL)			
	E. coli	*B. subtilis*	*S. aureus*	*S. flexneri*
3a	0.625	0.625	0.625	0.313
3b	0.625	0.625	0.313	0.156
3c	0.156	0.156	0.156	0.625
3d	0.313	0.313	0.313	0.313
3e	1.250	1.250	0.625	0.625
3f	0.625	0.625	0.156	0.313
3g	0.156	0.156	0.156	0.156
3h	0.313	1.250	1.250	0.313
3i	0.625	0.625	1.250	0.313
3j	0.313	0.156	0.156	0.313
3k	0.156	0.156	0.313	0.313
3l	0.313	0.625	0.625	0.625
3m	1.250	0.625	0.625	0.313
3n	0.625	0.156	0.313	0.313
3o	0.625	0.625	0.313	0.625
3p	0.313	0.625	0.156	0.313
3q	1.250	0.625	0.313	0.313
3r	0.156	0.156	0.156	0.313

3s	0.313	0.156	0.156	0.313
3t	0.156	0.156	0.313	0.313
3u	0.156	0.156	0.625	0.313
Ciprofloxacin	0.156	0.625	0.156	0.313

[a]values are the average of three experiments

Conclusions

The current study was directed to the design and synthesis of urease inhibitors. The designed inhibitors were synthesized by a one pot procedure using variety of arloxyalkanoic acids and arylisothiocyanates. The synthesized compounds, in general, were active as urease inhibitors with compounds **3r, 3t** and **3u** exhibiting IC_{50} values almost three times lower than the standard (table 1). Besides urease inhibition the synthesized compounds were investigated against four different bacterial strains and compounds **3g** and **3r** were found active against all the tested strains. Among others, compounds **3c, 3g, 3j, 3k, 3n, 3r, 3t** and **3u** exhibited four times low MIC values than the standard (ciprofloxacin) against *B. Subtilis* (table 2). Overall, as the preliminary investigations suggest, the designed molecules are not only strong urease inhibitors but may also be utilized in the development of new more potent azole antibiotics.

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Conflict of Interest

The authors have declared no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

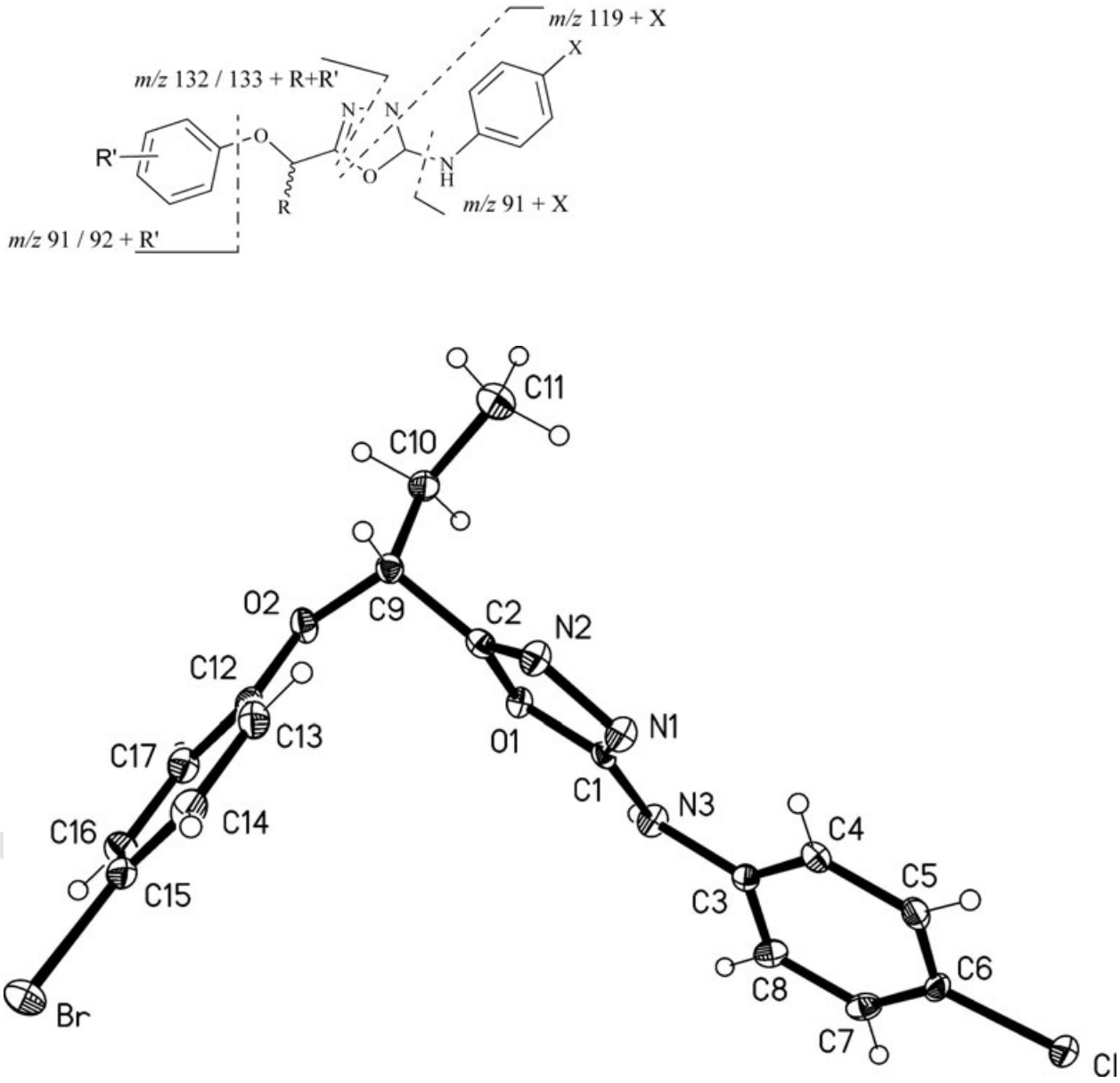
Appendix S1: Physical properties and spectral data of the target compounds is provided.

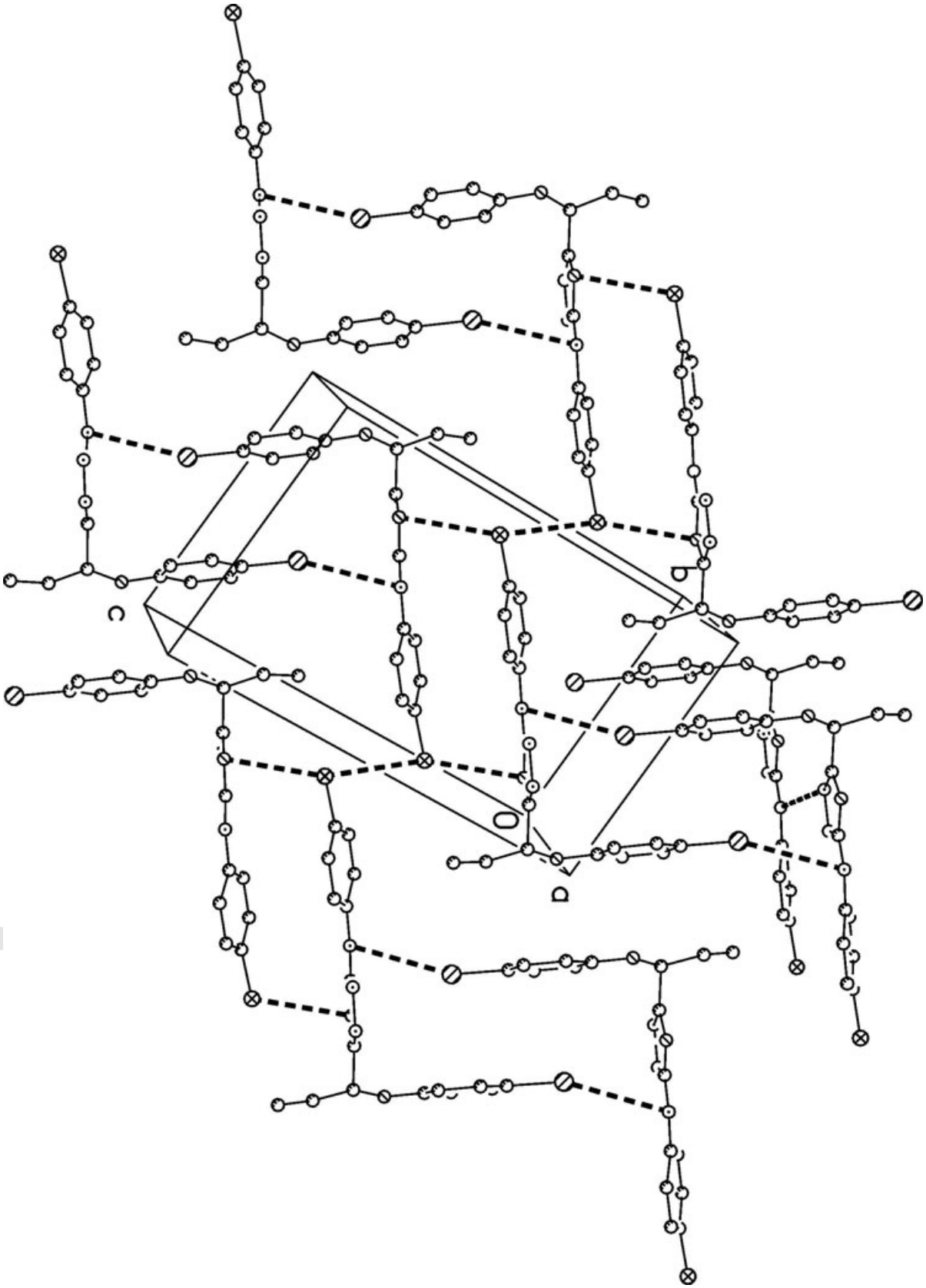
Figure Legends

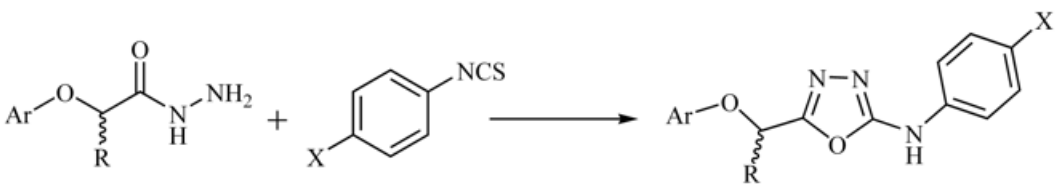
Figure 1: Suggested mass fragmentation pattern.

Figure 2: Molecular structure of compound **3o** in the crystal. Ellipsoids represent 50% probability levels. Selected bond lengths (Å): O1-C1 1.3640(18), O1-C2 1.3709(18), N1-C1 1.299(2), N1-N2 1.4140(18), N2-C2 1.279(2).

Figure 3: Packing diagram of compound **3o** in the crystal, showing the layer structure. Dashed lines indicate short intermolecular contacts (see text). One contact to the next layer is shown at the top of the diagram.







1a-g **2a-e** **3a-u**

Compd	Ar	R	X	Compd	Ar	R	X
3a	4-ClC_6H_4	Me	Me	**3l**	4-ClC_6H_4	Me	Cl
3b	4-BrC_6H_4	Me	Me	**3m**	3,4-$Cl_2C_6H_3$	Me	Cl
3c	4-MeC_6H_4	Me	Me	**3n**	4-MeC_6H_4	Me	Cl
3d	3,4-$Cl_2C_6H_3$	Me	Me	**3o**	4-BrC_6H_4	Et	Cl
3e	4-BrC_6H_4	Et	Me	**3p**	4-BrC_6H_4	Et	Br
3f	3,4-$Cl_2C_6H_3$	Et	Me	**3q**	4-MeC_6H_4	Et	Br
3g	4-BrC_6H_4	Me	F	**3r**	4-BrC_6H_4	Et	F
3h	4-ClC_6H_4	Me	F	**3s**	3,4-$Cl_2C_6H_3$	Et	F
3i	3,4-$Cl_2C_6H_3$	Me	F	**3t**	4-MeC_6H_4	Et	F
3j	4-MeC_6H_4	Me	F	**3u**	4-BrC_6H_4	Et	NO_2
3k	4-BrC_6H_4	Me	Cl				

Scheme 1: One pot synthesis of 1,3,4-oxadiazoles. Reagents and Conditions: (i) MeOH, reflux (ii) $Hg(OAc)_2$, Reflux.